

Assessing the responses of wheat roots and shoots to variations in soil water, temperature and CO_2 concentration

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. In order to meet the food requirements of the current trend in population growth, enhancement in wheat production and yield are urgently needed. Water and temperature stress are major constraints to wheat production and subsequently food security in the context of climate change. Plant growth is affected by both above- and belowground environmental conditions and increasing atmospheric CO_2 concentrations have been reported to enhance growth and yield of most agricultural crops. The responses of wheat roots and shoots to variations in soil water, temperature and CO_2 concentration were studied in this research. X-ray Computed Tomography (X-ray CT) was used to visualise and quantify the behaviour of roots grown in soil under contrasting conditions for water content, temperature and CO_2 concentration. Photosynthesis, stomatal conductance and transpiration were also measured to examine the shoot behaviour under the same environmental conditions. The results showed that total root volume (after 14 days) under the combined effect of elevated CO_2 and temperature and mean root diameter for all experiments increased significantly with increasing water stress. However, total root volume decreased significantly under the effect of water stress independently and in combination with elevated CO_2 and temperature. Mean root diameter also decreased significantly under repeated soil wetting and drying cycles. Total root volume and mean

root diameter at 400 ppm CO_2 growth was significantly greater than at 800 ppm CO_2 growth at 14 days, while it was significantly lower at 30 days. Photosynthesis, stomatal conductance and transpiration decreased significantly by increasing water stress. Photosynthesis at 800 ppm CO_2 was significantly greater than at 400 ppm CO_2 while stomatal conductance and transpiration at 400 ppm CO_2 were significantly greater. The elevated CO_2 enhanced root architecture system and promoted photosynthesis. This research has demonstrated how the interacting variables of water, temperature and CO_2 impact on the growth of wheat plants roots and shoots supporting the development of new management strategies for wheat to assist with the food security challenge under a changing environment.

Declaration

I hereby declare the work presented in this thesis entitled '' **Assessing the responses of wheat roots and shoots to variations in soil water, temperature and CO_2 concentration**'' was carried by me for the degree of Ph.D. in Soil Science under the supervision of Professor Sacha Mooney and Dr. Erik Murchie, School of Bioscience, and University of Nottingham, UK.

The contents of this thesis, in full or in parts, have not been submitted to anywhere for any award. Any sources of information have been used, they have been acknowledged.

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List of abbreviations

PAR	Photosynthetic active radiation
E	Germination to emergence
GS	Growth stage
VPDs	Vapour pressure deficits
WUE	Water-use efficiency
ROS	Reactive oxygen species
LOI	Loss of Ignition
ICPMS	Inductively coupled plasma mass spectrometer
TEG	Trace element grade
FC	Field capacity
µCT	Micro-computed tomography
Amax	Photosynthesis
Gs	Stomatal conductance
T	Transpiration
inWUE	Intrinsic water use efficiency
iWUE	Instantaneous water use efficiency
Plantdwt	Total dry weight
Psynth_14	Photosynthesis at 14 days
Psynth_21	Photosynthesis at 21 days
RSRatio	Root to shoot ratio
Root_dwt	Root dry weight
Shoot_dwt	Shoot dry weight
STomC_14	Stomatal conductance at 14 days
Transp	Transpiration
VWC	Volumetric water content
WRDiam	Mean root diameter by using WinRHZIO
WRRootvol	Total root volume by using WinRHZIO
WUE_R	Water use efficiency (based on root dry weight)
WUE_SH	Water use efficiency (based on shoot dry weight)
WUE_T	Water use efficiency (based on total dry weight of plant)
X_rayAvgDiam	Mean root diameter by using X-ray µCT
X_ray_RootVol	Total root volume by using X-ray µCT
iwuepc	Instantaneous water use efficiency (photosynthesis/ stomatal conductance)
iwuept	Instantaneous water use efficiency (photosynthesis/transpiration)
porosity_sp	Air filled porosity
FACE	Free air carbon dioxide enrichment (FACE)
reg_p_ht_14	Shoot length by mm
ANOVA	Analysis of variance
ROI	Region of interest
DRW	Drying and re-wetting cycles
FACE	Free air carbon dioxide enrichment
RDI	Regulated deficit irrigation
Ψ_{leaf}	Leaf water potential

1.0 Introduction

1.1 Wheat

Wheat (*Triticum aestivum* L.) is the main food for more than 35% of the world's population and is considered as a very important grain crop in the majority of developing countries (Eid et al., 2009, Braun et al., 2010, Metwali et al., 2011). About 20% of the globally produced food depends on wheat and it is classified as a main source of protein and a second source of calories just after rice (Braun et al., 2010). Wheat is considered a "staff of life" for around 1.2 billion "wheat-dependent" to 2.5 billion "wheat-consuming" poor-men, women and children-who live on less than $US 2/day (FAOSTAT, 2010).

During the last twenty years, more than 207 million hectares of land has been used for wheat. It is estimated that this area is around 1.4 times greater than that used for maize (*Zea mays* L.) and paddy rice (*Oryza sativa* L.) (FAO, 2011a). It has been reported that the total value of trade for wheat in 2008 was about 1.5 and 3 times greater than that of maize and rice respectively (FAO, 2011b). In addition it has been noted that about 19% calories and 21% protein is produced by wheat for the global populations (FAO, 2011b). It is also estimated that the world population is likely to reach 9 billion in 2050 from more than 7 billion at the current time. The world demand for food for the next four decades is likely to increase significantly (Godfray et al., 2010). The global demand for cereals is likely to increase by 56% by 2050 compared to the base year 2000.

It is assumed that 26% of this increase will be from wheat (Hubert et al., 2010). The International Food Policy Research Institute (IFPRI) states the global need for wheat will be increased to 775 million tonnes by 2020 compared to 552 million tonnes in 1993, and 60% in a total by 2050 (Rosegrant et al., 1997, Rosegrant and Agcaoili, 2010). In addition, world demand for wheat in 2050 may be increased by 31% over the 683 million tonnes used in 2008 due to changing dietary patterns and a great increase in the world population (Dixon et al., 2009, FAO, 2011b).

Different factors have been reported to affect wheat yield including biotic factors (diseases, insect pests, and weeds) and abiotic factors (drought, high temperature, salinity, flooding, freezing, high irradiation, and nutrient deficiency or toxicities) (Pradhan, 2011). High temperature and water shortage are considered as the most significant environmental factors decreasing global yield (Curtis et al., 2002).

Recently, the Intergovernmental Panel on Climate Change (IPCC) predicted that most land areas will be subjected to hotter and more frequent episodes of warm days and nights and more un-predictable precipitation in forthcoming years (IPCC, 2007). It is assumed that North America will be exposed to more intense, more frequent and longer heat waves within the second half of this century which will be higher in Midwest of USA (Meehl and Tebaldi, 2004). Easterling et al. (1997) reported the universal increase in daily minimum temperature was double that of daily maximum temperature over the past century. It has been reported that more than of two third of

world's wheat harvested area was under rain-fed conditions (Portmann et al., 2010).

Abiotic stresses have a serious effect on global crop production (Pan et al., 2002). Here we focus on water shortages or deficits. Water shortage is considered as a main cause for decreasing crop production by more than 50% for many crop plants (Bayoumi et al., 2008). Plants might be affected by water stress at any time of their life, however; germination and seedling establishment are considered as the critical growth stages (Pessarakli, 2010).

In the majority of undeveloped countries around 37% of land area consists of semiarid environments. In these countries wheat is mainly developed on rain-fed lands without additional irrigation and the available water is regarded as a main constraint to wheat yield (Qayyum et al., 2013). For 87% of wheat production in US about 94% of yield loss is due to environmental factors and the remaining 6% is due to biotic factors (Boyer, 1982). FAO (2011c) reported around 5.16 million hectares of winter wheat was affected by water shortage in China. In addition, more than 10.3 million hectares was damaged by drought in Russia (FAO, 2010).

An air temperature of 20–25°C is considered as optimal for wheat growth and development (Acevedo et al., 2002). It has been suggested that high temperature at anthesis (around 40 °C) has been an important factor in reducing yields in European wheat (Semenov and Shewry, 2011). During the past 8000 years, wheat was regarded as a main food for Europe, West and Central Asia and

North African regions (Curtis et al., 2002). High temperature stress has been regarded as a main abiotic factor with the biggest threat at heading and the grain filling stage (Abdalla et al., 2010). A considerable reduction in wheat production may occur due to a simultaneous effect of drought and high temperature (Smith and Katz, 2013, Mittler, 2006). Some studies have shown the combined impacts of drought and high temperature on physiology, growth, water relations and yield were significantly higher than independent effects (Shah and Paulsen, 2003, Sharma and Kaur, 2009, Grigorova et al., 2011). The negative effects of these stress factors on wheat production can be decreased by avoiding stress mainly at the sensitive stages of plant development such as the reproductive and grain filling stages (Saini et al., 1983, Saini and Westgate, 1999).

The growth of the wheat plant can be classified into three comprehensive phases including seed germination and seedling establishment phase, vegetative phase, and reproductive phase followed by maturity and ripening. Each of these phases can be sub-classified into various growth stages. There are several different methods (scales) are used to describe them, such as, Zadoks, Huan, and Feekes staging systems. In this study, the Zadoks staging system has been used (Zadoks et al., 1974). The growth stages are also usually categorized into E (from the germination to the seedling emergence stage), GS1 (Growth Stage 1: from the emergence to the double ridge stage), GS2 (Growth Stage 2: from the double

ridge to the anthesis stage), and GS3 (Growth Stage 3: from the anthesis to the maturity stage) (Acevedo et al., 2002). According to Zadoks scale, wheat growth is classified as in Table 1.1.

Table 1.1: Classification of wheat according to Zadoks scale (Zadoks et al., 1974).

Zadoks Scale	Description
Germination	
0	Dry Seed
1	Start of imbibition
3	Imbibition complete
5	Radicle emerged from seed
7	Coleoptile emerged from seed
9	Leaf just at coleoptile tip
Seedling Growth	
10	First leaf through coleoptile
11	First leaf extended
12	Second leaf extending
13	Third leaf extending
14	Fourth leaf extending
15	Fifth leaf extending
16	Sixth leaf extending
17	Seventh leaf extending
18	Eighth leaf extending
19	Nine or more leaves extended
Tillering	
20	Main shoot only
21	Main shoot and one tiller
22	Main shoot and two tillers
23	Main shoot and three tillers
24	Main shoot and four tillers
25	Main shoot and five tillers
26	Main shoot and six tillers
27	Main shoot and seven tillers
28	Main shoot and eight tillers
29	Main shoot and nine tillers
Stem Elongation	
30	Psuedo-stem elongation
31	First node detectable
32	Second node detectable
33	Third node detectable
34	Fourth node detectable
35	Fifth node detectable
36	Sixth node detectable
37	Flag leaf just visible
39	Flag leaf ligule/collar just visible
Booting	
40	-----
41	Flag leaf sheath extending
45	Boot just swollen
47	Flag leaf sheath opening
49	First awns visible
Inflorescence Emergence	
50	First spikelet of inflorescence visible
53	1/4 of inflorescence emerged
55	1/2 of inflorescence emerged
57	3/4 of inflorescence emerged
59	Emergence of inflorescence completed
Anthesis	
60	Beginning of anthesis
65	Anthesis 1/2 completed
69	Anthesis completed
Milk Development	
70	-----
71	Kernel watery-ripe
73	Early milk
75	Medium milk
77	Late milk
Dough Development	
80	-----
83	Early dough
85	Soft dough
87	Hard dough
Ripening	
90	-----
91	Kernel hard (difficult to divide by thumbnail)
92	Kernel hard (can no longer be dented by thumbnail)
93	Kernel loosening in daytime
94	Overripe, straw dead and collapsing
95	Seed dormant
96	Viable seed giving 50% germination
97	Seed not dormant
98	Secondary dormancy induced
99	Secondary dormancy lost

1.2 Water stress

Taiz and Zeiger (2006) defined stress as "an external factor with negative impacts on the plant and is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes, which are related to overall growth". Both stress and plant characteristics play important roles in the survival and growth of the plant. Survival and growth of a plant relies on several stress factors and plant characteristics such as period and number of exposures and severity of stress, and genotype and growth stage of a given plant (Farooq et al., 2009).

Water shortage can be a meteorological term referring to a period without considerable precipitation (Jaleel et al., 2009). Drought is a severe water shortage that negatively affects plant growth and greatly reduces plant output (Pan et al., 2002). The reduction of the water in soil and environmental conditions are regarded as continuous causes of water loss by the process of transpiration or evaporation (Jaleel et al., 2009). Thus, water shortage is regarded as a global problem affecting the survival of agricultural crops and the maintenance of food production (Jaleel et al., 2007).

1.2.1 Influence of water stress on root growth

Root activity is significantly affected by the physiochemical and biological status of the surrounding soil, especially the relationship between the soil pore structure and the root development. Roots are well-structured morphologically and functionally for capturing of water, minerals and nutrients by apoplastic and/or symplastic traits

from soil. The absorbed resources are moved through the xylem and stem to the leaves to be used for entire plant growth and development (Tester and Leigh, 2001). Failure within the root functions leads to a decrease in plant output with a great range of morphological, physiological, biochemical and molecular symptoms which also include a lack of nutrients (Leucci et al., 2008). This has a significant effect on plant growth (McDonald and Davies, 1996), respiration (Ribas-Carbo et al., 2005) and photosynthesis (Flexas et al., 2004), especially when plants are exposed to water shortage for a long duration.

The roots sense the water shortage firstly through their tips (Shimazaki et al., 2005). The root tips also respond to different stimuli such as ions, electricity, touch and other soluble molecules to regulate the rate, direction and magnitude of root growth and movement through generating biological impulses. Hawes et al. (2000) reported that roots move to the direction of water and nutrient and escape from toxic substances efficiently.

Generally, plants respond to water shortage through decreasing leaf and stem growth with a simultaneous increase in root growth to explore the a wider soil volume for water (Leucci et al., 2008). These differential development responses are considered as a compensatory mechanism utilized by terrestrial plants in drought condition (Spollen et al., 1993). In winter wheat, water stress happens at the grain-filling stage and leads to a greater portion of dry matter for roots allowing greater water adsorption (Leport et al.,

1998, Huang and Fu, 2000, Bryla et al., 2001). However, it has been reported that larger root systems are more efficient than smaller root systems (Baozhong et al., 1999, Zhang and Zhang, 2000).

Numerous studies have shown the effect of water shortage on root growth in the plants, such as wheat (Pritchard et al., 1991), maize (Wu et al., 1994, Wu and Cosgrove, 2000, Shimazaki et al., 2005, Spollen et al., 1993), mung bean (Itoh et al., 1987), rice (Pérez-Molphe-Balch et al., 1996), pea (Bracale et al., 1997) and cotton (Zhong and Lauchli, 1993). It has been shown that the root growth maintenance at low water potential relies on the modification of the cell wall yielding properties of plants. The importance of root growth is of increasing interest to plant breeders (Gewin, 2010). When plants are grown in soils containing sufficient supplies of water or nutrients, large-scale root systems can grow vigorously.

1.2.2 Influence of water stress on shoot growth

Water availability primarily affects shoots and roots, photosynthesis and dry mater accumulation (Blum, 1996). The decrease of leaf elongation rate and closing stomata is considered as an initial response of plants to water stress to reduce water consumption via transpiration (Tatar and Gevrek, 2008). The roots and shoots of a plant have an interdependent and competing relation for their demand for water, mineral and organic nutrition, because of their respective different functions and exposed environment (Shangguan et al., 2004, Shangguan, 2003).

Shoot growth is very sensitive to water stress conditions. Much of the evidence over the last 20 years has shown that the inhibition of growth is metabolically regulated, rather than being a direct consequence of altered plant water status arising from soil drying (Sharp, 2002). Moreover, shoot growth can be so sensitive to soil drying that a considerable inhibition can occur before the development of decreased water potentials in the aerial plant parts (Gowing et al., 1990), and such observations have led to much interest in the involvement of non-hydraulic regulatory signals from the roots (Davies et al., 2000).

1.2.3 Influence of water stress on the physiology of wheat

Wilting is considered as a first noticeable symptom of water stress which highlights how transpirational water loss can lead to the rate of water absorption being exceeded (Buchanan et al., 2001). However, several important physiological processes are significantly affected by drought.

1.2.3.1 Photosynthesis

Photosynthesis has an important role in determining the growth of plant and yield and along with respiration is regarded as a primary physiological process (Ali et al., 2010). Arfan et al. (2007) reported a positive correlation between wheat photosynthesis and grain yield. The rate of photosynthesis frequently restricts plant growth when soil water availability is reduced (Amede et al., 1999, Huang and Fu, 2000). Photosynthesis is reduced by water shortage as a result of

lowering of stomatal and mesophyll conductance (Flexas et al., 2004), or by oxidative damage of the chloroplast (Zhou et al., 2007).

However, it is often difficult to ascertain whether photosynthesis reduces through stomatal conductance (Sharkey, 1990, Chaves, 1991, Ort et al., 1994, Cornic and Massacci, 1996) or by metabolic impairment (Lawlor, 1995). It has been established that photosynthesis can be reduced by a combined effect of water stress and high temperature through decreasing stomatal conductance to control water loss by transpiration (Ahmed et al., 2012). High temperature alone can reduce photosynthesis via non-stomatal effects (e.g. Rubisco inactivation and electron transport). The rate of photosynthesis is affected by the intensity and duration of water shortage, prevalent temperature, type of species and cultivars (Athar and Ashraf, 2009). As mentioned, a decrease in photosynthesis due to water stress occurs as a result of stomatal closure and impairments in photochemical and/or biochemical reactions (Tang et al., 2002, Yu et al., 2009). Thus, a strong interdependent can be found between water stress and photosynthesis and their impacts on crop yield.

1.2.3.2 Stomatal conductance

Stomata are small structures found on the outer layer of leaves. They are composed of two guard cells and a small variable aperture in between (Farquhar, 1977, Hetherington and Woodward, 2003). The opening and closing of the stomata are regulated by the

turgidity of the guard cells (Hetherington and Woodward, 2003) which is in turn controlled by a complex signalling process. Stomata play an important role in the physiological processes of the plant via the plant's water balance (Ahmed et al., 2012). Control of the exchange of gasses such as CO_2, water vapour, and O_2 between inner part of leaves and the atmosphere are considered as a main function of stomata (Pradhan, 2011). Stomatal conductance (or its reciprocal resistance) is a physical property of the leaf and refers to the ease with which gasses can pass between the environment and the inter-cellular spaces. It is controlled largely by stomatal aperture and morphology. Reduced stomatal conductance affects not only plant water loss (transpiration), but also reduces CO_2 uptake by the plant (Mitra, 2014). Therefore, there is a trade-off between water loss and carbon gain, mediated by stomatal conductance. In the presence of a water deficit plants take up less CO_2 compared to their counterpart grown under optimal water supply (Williams et al., 1998, Schwarz et al., 2004). However under experimental conditions stomatal conductance develops in the presence of water stress as a compensatory mechanism during plant growth (Ashraf and Harris, 2004, Parida and Das, 2005, Ashraf and Foolad, 2007, Suriyan and Chalermpol, 2009).

1.2.3.3 Transpiration

Transpiration can be defined as a physical and physiological process through which water vapour is discharged from the leaves of plants, mainly through stomata. Rates of transpiration are determined by

interactions between leaf stomatal conductance and the environmental conditions, notably humidity and temperature. Transpiration plays an important role in cooling plant leaves (Hetherington and Woodward, 2003). Plants respond to water stress by reducing water loss via transpiration process through decreasing leaf elongation rate and closing of stomata (Johari-Pireivatlou and Maralian, 2013). As an example of offsetting, water shortage can lead to closing of the stomata which in turn, decreases the transpiration rate affecting heat dissipation. The result of this is an increase in the foliar temperature (Hsiao, 2000) which can in turn lead to stomatal opening. Temperature also influences the stability of photosystem II, as has been previously reported (Ortiz and Cardemil, 2001). A negative correlation was reported between transpiration and crop yield under drought conditions (Ahmed et al., 2012) however a positive relationship can be seen in favourable environments. A close relationship can be observed between water stress and high temperature. Overall therefore, it is not easy to distinguish/separate the influences caused by these two environmental factors in plants (Kriedmann and Dowton, 1981, Anderson et al., 2000).

1.2.4 Influence of water stress on crop production

Generally, the productivity of wheat is directly related to the acquisition and use of resources. In Mediterranean and rain-fed regions productivity is often limited by water shortage (Mastrangelo et al., 2000). It is reported that crop production (wheat, maize and

rice) could decline by 30% by the end of this century in South Asia as a result of climate change-induced temperature increases (Hossain and da Silva, 2013).

Water stress can affect yield at all stages of plant growth. However, it has been reported that the effect of the environmental conditions such as variation in air temperature and moisture is greater on crop yield during critical growth stages of plant particularly at anthesis. During this stage, a relative higher temperature with water stress reduces stomatal conductance and photosynthetic efficiency raising plant temperature and reducing carbon gain (Ahmed et al., 2012). Increases or decreases in temperature and water availability can also affect wheat productivity and photosynthetic efficiency (Wang et al., 2008).

In wheat, grain yield is the function of the number of plants ha^{-1}, the number of fertile tillers $plant^{-1}$, the number of grains $spike^{-1}$, and individual grain weight. Grain yield is affected directly or indirectly by water shortage through the components described above. Water shortage negatively affects these components and results in a reduction in grain yield (Fábián et al., 2011). Drought at the last two stages (GS2 and GS3) is a frequent phenomenon resulting in wheat yield loss in Kansas (USA) (Pradhan, 2011).

End-of-season drought or terminal drought is characteristic of regions with Mediterranean-type climates, where rainfall decreases and evaporation and temperature increase in spring, when wheat enters its reproductive stage (Fitzpatrick, 1970). Terminal drought is

the major cause of grain yield variability in wheat. It enhances leaf senescence and reduces photosynthesis after anthesis, thus reducing final biomass, grain yield and harvest index (Passioura, 1983, Kobata et al., 1992, Palta, 1996, Yang et al., 2001). The reductions in grain yield and harvest index are mostly consequences of floret abortion and reduced grain size, depending on the time and intensity of the terminal drought (Passioura, 1983). Thus, the crop yield and water stress are interdependent, it is crucial to understand the effect of water shortage on the plant growth at specific growth stages to help to develop varieties that tolerate water deficiency.

Simultaneous stresses of water shortage and high air temperature cause severe yield loss especially during the grain filling period of wheat and at anthesis (Smith and Katz, 2013, Altenbach et al., 2003). However, only limited studies have considered the effect of combined stress on crop yield, however it is assumed that the simultaneous effects of two stress factors on crop productivity may be different to the combined effect of individual stresses (Rizhsky et al., 2002, Mittler, 2006).

1.2.5 Influence of water stress on development stages of wheat

In general, the success of modern agriculture is severely affected by water stress (Hasanuzzaman et al., 2012, Hossain et al., 2012). Water shortage is a universal phenomenon that negatively affects plants according to the growth stage at the time of occurrence

(Lopez et al., 2003, Martiniello and Teixeira da Silva, 2011, Hossain et al., 2012).

1.2.5.1 Germination to emergence (E)

Generally, the water content of wheat seed is estimated about 12% by weight. Seeds undergo germination when the water content is increased to more than 35% of the dry weight (Evans et al., 1975). Therefore, water shortage at early wheat growth inversely effects seed germination and crop establishment.

1.2.5.2 Emergence to double ridge (GS1)

Water stress decreases leaf area during GS1 (Giunta et al., 1995, Royo et al., 2004). Turgor-dependent activities, such as leaf expansion and root elongation reduce as a result of cells losing turgidity and shrinkage (Taiz and Zeiger, 2006). Phyllochron also increases in wheat by water stress at this stage (Simane et al., 1993). Tillering is another trait affected by water shortage (Blum et al., 1990). Thus, water stress reduces relative moisture content, leaf area and biomass production during this stage (Giunta et al., 1995, El Hafid et al., 1998a, Dulai et al., 2006, Liu et al., 2006b).

1.2.5.3 Double ridge to anthesis (GS2)

Water stress also inversely affects the growth of leaves, stem, and roots during their development at GS2 as mentioned above. Reallocating stem reserves under advantageous conditions in wheat drives up to 20% grain-weight (Gebbing et al., 1999). Up to 50% of grain weight under post anthesis water stress might be from stem

reserves (Van Herwaarden et al., 1998). Therefore, a reduction in accumulation of stem reserves (water soluble carbohydrates) at this stage decreases grain yield by unfavorably influencing photosynthesis through water stress at pre-anthesis (Ehdaie et al., 2006). It has been reported that water stress causes a reduction in the main stem weight up to 23% (Abdoli and Saeidi, 2012, Ehdaie et al., 2006). Spikelet number spike^{-1} is the main trait susceptible to water stress at this stage (Oosterhuis and Cartwright, 1983, Moustafa et al., 1996, Sangtarash, 2010). Consequently, grain number reduces greatly when water stress occurs at this stage.

1.2.5.4 Anthesis to maturity (GS3)

Water stress decreases grain number when it occurs at or directly after anthesis (Sangtarash, 2010). While water shortage post anthesis decreased grain yield by reducing individual grain weight (Ahmadi and Baker, 2001, Ji et al., 2010). The decrease in individual grain weight is probably due to lower grain filling duration (Wardlaw and Willenbrink, 2000, Prasad et al., 2008a). Wardlaw and Willenbrink (2000) recorded that water stress during anthesis reduced individual grain weight of the wheat cultivar Lyallpur 73 by about 38%. Ahmadi and Baker (2001) reported a 43% decrease in cultivar Cadenza exposed to severe drought from 15 days post anthesis.

1.2.6 Influence of water stress on vegetative growth stages of wheat

Crops are generally more sensitive to drought and/or heat stress during the reproductive stages of development, which mainly influences seed numbers. Recently, the importance of the effect of drought and/or heat stress during early growth stages are gaining attention (Saeidi et al., 2015, Madhu and Hatfield, 2013). Water stress during the vegetative stage decreased some agronomic traits such as grain yield, biomass and the number of grains per spike. Likewise, water stress at the vegetative growth stage significantly decreased some values for physiological parameters such as chlorophyll a, b and total chlorophyll, performance index (a chlorophyll fluorescence parameter) and relative water content and significantly increased stress associated parameters such as carotenoid concentration in the leaves (Saeidi et al., 2015).

Recently, there have been many studies on impacts of soil water content on the photosynthetic parameters and yields of wheat (Cui et al., 2015), and it is generally recognized that improving soil water content helps to increase the photosynthetic characteristics of leaves, which in turn, contributes to an increase in the grain filling rate and yield of wheat (Xue et al., 2006, Lan-Ping et al., 2011). Determining the effects of a water deficit during periods of vegetative growth on photosynthetic traits and yield will provide a reasonable strategy for water-saving management of wheat (Triticum aestivum L.) and exploited photosynthetic traits for the selection of drought tolerant wheat genotypes (Liu et al., 2016).

They have found that the leaves of different wheat genotypes exhibited a lower net photosynthetic rate, stomatal conductance and transpiration under water stress. Therefore, it is important to investigate whether water deficits during vegetative growth increase or decrease shoot and root growth in wheat, to provide guidance toward water-saving cultivation.

Water limitations are commonly thought to trigger an adaptive response in plants to avoid water stress by promoting greater relative carbon allocation to root growth, which ultimately would result in higher root to shoot ratio and greater capacity to absorb water relative to shoots that must be supported (Kozlowski and Pallardy, 2002). In response to drought or decreased soil water availability over extended periods, Thornley (1996) proposed that plant water status was more strongly affected by root to shoot partitioning and root density changes than by the interaction of soil water content with stomatal conductance. Timing of drought also has great effect on partitioning of carbohydrates and nitrogen. A change of partitioning is towards roots rather than shoots when drought occurs during early vegetative growth stages has been reported, increasing the root-to-shoot ratio.This increase is due mainly to decreased shoot weight rather than increased root weight (Prasad et al., 2008a).

The response of plant to short term of water deficit during vegetative stages under prevailing soil and climatic conditions has been evaluated. Short-duration water deficits during the rapid

vegetative reported to reduce 28–32% of final dry matter weight (Cakir, 2004). NeSmith and Ritchie (1992) have also showed that short-term effects of water deficits lead to delay leaf tip emergence and leaf area reduction. In addition, it has been reported that plant at about the 10 visible leaves stage begins a rapid, steady increase in nutrient and dry weight accumulation, which continues into the reproductive stage (Ritchie et al., 1992) and they also concluded that water deficit at this stage may reduce the size of the leaves (the photosynthetic factory). It is though that plant life cycle consists of two clearly distinct independent (vegetative and reproductive) phases, roots in vegetative growth stage continue to grow after pollen shed and fertilisation have taken place, provided surplus nutrient assimilate is available (Salvador and Pearce, 1995). It has been studied that the effect of moisture stress during early growth stages of a plant and found out that a delay in the irrigation during this stage reduced plant dry weight (Jama and Ottman, 1993).

1.3 High temperature

1.3.1 Influence of high temperature on root growth

Most studies on the effect of high temperature on wheat are from experiments that stressed the whole plant in growth chambers or the field. Only a few studies have considered the effect of high temperature on roots. The whole plant and grain yield can be affected by temperature stress; however, the effect of high temperature on roots occurs generally only at a depth of a few

centimetres from the soil surface (Acevedo et al., 2002, Guedira and Paulsen, 2002). This is because the effect of high temperature on roots not only reduces root growth (Nielsen, 1974), but also decreases the partitioning of photosynthates to shoots and thus affects growth of the whole plant (Li et al., 1994).

Compared to the other plant parts, wheat roots are less adapted to intense temperature variation and the roots have the lowest optimum temperature for growth (Guedira and Paulsen, 2002). It has also been reported that about 60% of kernel weight decreased when the whole plant or root at anthesis stage was temperature-stressed at 30°C (Guedira and Paulsen, 2002). When the shoot was only temperature-stressed at the same stage of the plant growth, the reduction of the weight was less than (43%) (Guedira and Paulsen, 2002). These authors concluded that dry matter was affected more by root than by shoot temperatures. It has also been shown that the yield of wheat decreased significantly by high root temperature during maturation, regardless of the shoot temperature (Kuroyanagi and Paulsen, 1988).

1.3.2 Influence of high temperature on development stages of wheat

The growth and development rate of wheat increases in high temperature in all development phases, but it reduces when stress intensity and duration become higher. High temperature, however, reduces the period of each growth stage that has an inverse impact on crop performance and productivity (Pradhan, 2011). A considerable reduction in the duration of all growth stages, GS1,

GS2 and GS3 was reported when 20 wheat species were sown in the summer at higher average temperature (12°C) (Shpiler and Blum, 1986, Wollenweber et al., 2003). Prasad et al. (2008a) reported that growth at high temperature in spring reduces time for flowering, grain set, and physiological maturity. In Asia, Europe and Great Plains of USA, wheat is generally sown in the autumn season. Thus, the crop does not experience high temperature during germination to emergence stage (E). However, high temperature may stress all wheat growth stages in tropical regions.

1.3.2.1 Germination to emergence (E)

High air temperature along with high radiation may increase soil temperature to 10° to 15°C more than the air temperature. In such conditions, seedlings may die and number of plants ha^{-1} will be affected (Acevedo et al., 2002). Plant population below 100 m^{-2} are been considered yield limiting situation in wheat (Acevedo and Nachit, 1990).

1.3.2.2 Emergence to double ridge (GS1)

Acevedo and Nachit (1990) reported that an increase in mean seasonal temperature from 12°C to 20°C at GS1 resulted in reduction of GS1 period by 33 d, plant height by 25 cm, and leaf area index by 2.3 units. It has also been observed that high temperature stress at this stage resulted in a reduction in leaf number and fertile tiller numbers $plant^{-1}$ in wheat (Midmore et al., 1984). In addition, a significant reduction was observed in seedling

shoots length and shoots dry weight of eight wheat varieties by high temperature of 35°C (Tripathi et al., 2009).

1.3.2.3 Double ridge to anthesis (GS2)

GS2 is highly susceptible to stress conditions including high temperature. It has been reported that the reduction in period of GS2 by 25 d, fertile tiller number m^{-2} by 54, and the grain number m^{-2} by 36 occurred as a result of an increase in mean seasonal temperature from 12°C to 21°C during GS2. Furthermore, the effect of temperature stress is exacerbated on all of above mentioned traits when mean seasonal temperature increase to above 24°C (Acevedo and Nachit, 1990). The high temperature at anthesis decreases grain number $spike^{-1}$. This leads to a decrease in the grain yield mainly due to the effects of temperature stress on floral organs (Yang et al., 2002, Prasad et al., 2008a).

1.3.2.4 Anthesis to maturity (GS3)

During GS3, the reduction in grain weight has been well reported due to high temperature accelerating leaf senescence and reducing the individual grain weight (Gibson and Paulsen, 1999, Khanna-Chopra and Viswanathan, 1999, Pradhan, 2011, Uçan et al., 2007, Tester and Leigh, 2001). High temperature also reduces the period of grain filling and results in an increase in grain filling rate (Prasad et al., 2006, Prasad et al., 2008b). The decrease in grain weight under heat stress leads to a decrease in wheat production (Guedira and Paulsen, 2002). It has been reported that a 50% decrease in average grain weight of 30 synthetic hexaploid wheat was due to a

temperature of 10°C higher than the ambient (20/15°C) at 10 days after anthesis (Yang et al., 2002).

1.3.3 Influence of high temperature on the physiology of wheat

1.3.3.1 Photosynthesis

Photosynthesis in wheat (a C3 plant) is reduced by excessively high temperatures (Yang et al., 2002, Fokar et al., 1998, Al-Khatib and Paulsen, 1990, Reynolds et al., 2000). Air temperature during growth has a direct effect on important physiological processes such as photosynthesis and nutrient and water uptake which change overall growth and development (Ahmed et al., 2012). It is important to note that the rates of photosynthesis are reduced by high and low temperatures, whereas the maximum (optimal) photosynthesis rate is achieved at intermediate temperature. It is important to define the optimal temperature for photosynthesis which is lower for C3 plants in comparison to C4 plants. At higher temperatures (above the optimum) in C3 plants, photosynthesis often declines as a result of increased photorespiration due to the increased oxygenation rate of the Rubisco reaction. At even higher temperatures (above 35 or 40 °C) Rubisco activation often declines and the risk of photooxidative stress increases. Fufezan et al. (2007) reported that leaf temperature has a significant effect on the photosynthetic rates at light saturation. Plant photosynthesis is directly affected by interactions between increases in temperature and declining water availability through leaf water potential changes and oxidative stress resistance (Berry and Bjorkman, 1980, Lee et

al., 2000). Excessive temperatures (above 35 °C for a C3 plant) severely affect plant leaf photosynthesis (Taub et al., 2000, Wollenweber et al., 2003).

Under high temperature and moisture stress, plants face multiple physiological problems like an inability to take up CO_2 and reduced leaf cooling capacity (due to stomatal closure). The minimum transpiration that elevate temperature (5-6^{o}C) near leaves can result in producing reactive oxygen species (ROS) (Athar and Ashraf, 2009).

Water shortage and high temperature are important environmental factors limiting plant growth and photosynthesis in many areas of the world, and while the two stresses often occur simultaneously (Shah and Paulsen, 2003), less is known about how their combination impacts on plants (Rizhsky et al., 2004). An additional concern is global climate change that will probably alter the distribution of precipitation, and exaggerate drought in arid and semiarid areas (Wigley and Raper, 2001, Chaves et al., 2003). Water shortage and above optimal temperatures have opposing effects on photosynthesis and interact strongly. Few studies have focused on the combination of these two factors, although the effects of drought or high temperature have been studied intensively as separate factors (Xu and Zhou, 2006).

1.3.3.2 Stomatal conductance

Temperature alone (with no change in water relations) can affect stomatal conductance positively up to an optimum (normally defined by the photosynthetic optimum) where after it declines. As discussed already a combined increase in atmospheric temperature and decrease in soil moisture can lead to a decrease in stomatal aperture and, eventually, decrease plant productivity which can be improved by selecting a suitable genotype with better physiological performance under unstable environmental conditions (Ahmed et al., 2010, Medlyn et al., 2001). Li et al. (2003) suggested that reduced transpiration rate itself could decrease stomatal conductance. Ahmed et al. (2012) reported that the combined effect of high temperature and water stress at anthesis stage reduce stomatal conductance and photosynthetic efficiency therefore affecting crop yield. Kimball et al. (2002) concluded that climatic variation can change the microclimate resulting in a decrease of 33% to 50% in stomatal conductance.

1.3.3.3 Transpiration

Transpiration is closely related to stomatal conductance and is a crucial process for maintaining leaf temperature by providing a cooling effect (Ahmed et al., 2012), see above. It has also been reported that an increase in temperature reduces water availability for plant photosynthesis through increasing the transpiration rate depleting soil water and leading yield to decrease (Ahmed et al., 2012). Li et al. (2003) suggested that decreased transpiration rate

can lower stomatal conductance. In addition a negative correlation was observed between grain yield and transpiration rate (Ahmed et al., 2012). Furthermore, transpiration is directly affected by heat and moisture and it is negatively linked to yield. Therefore, an increase in the transpiration rate can lead to yield decrease.

1.3.4 Influence of high temperature on yield

Increasing crop productivity under stresses within unstable environmental conditions is regarded as a main challenge for the future. It has been reported that heat stress even for a short period reduces crop productivity and can itself induce water stress (Siebert et al., 2014). The annual average rise of 0.3°C in world temperature will alter water availability and usage, therefore, will impact on cereals production to a greater extent (McCarthy, 2001). It has been reported that high temperature results in a significant reduction in wheat yield (up to 70%) (Fokar et al., 1998, Gibson and Paulsen, 1999, Khanna-Chopra and Viswanathan, 1999, Prasad et al., 2008a). High temperatures have a significant effect on the grain yield by accelerating phenology and reducing biomass production (Slafer and Rawson, 1995), increasing the rates of grain filling, but reduce the duration of grain filling and ultimately grain yield (Wardlaw and Moncur, 1995, Zahedi and Jenner, 2003, Asseng et al., 2011). It has been reported that heat stress at early flowering results in a reduction in grain number and decreasing crop yield (Stratonovitch and Semenov, 2015). During meiosis, temperatures exceeding 30 °C are reported to cause abnormal development of

both ovary and anthers which reduces floret fertility and, consequently, the number of developing grains (Saini et al., 1984, Grant et al., 2011). Then, at the beginning of grain filling, temperatures above 35 °C affect the development of the endosperm which limits maximum grain weight (Hawker and Jenner, 1993). It is ultimately important to understand the relationship between climate change and farming due to the impact of climate on agricultural production processes (Akdemir et al., 2012).

1.3.5 Influence of high temperature on vegetative growth stage of wheat

Responses to temperature differ among crop species throughout their life cycle and are primarily the phenological responses, i.e., stages of plant development. For each species, a defined range of maximum and minimum temperatures form the boundaries of observable growth (Hatfield and Prueger, 2015). It has been found that vegetative development (node and leaf appearance rate) are directly related to temperature and that increases as temperatures increase to the species optimum level. For most plant species, vegetative development usually has a higher optimum temperature than for reproductive development. Increased temperature above the optimum level has been seen to be harmful for plant growth (Hatfield and Prueger, 2015).

Longevity of leaves is maximised by low shoot and root temperatures, and antagonized by either high shoot or high root temperature. It has been reported that leaves senesced slowly on

plants grown at 15/15°C, and a considerable viable area remained after the grain reached physiological maturity (Guedira and Paulsen, 2002). It has also been reported that high shoot temperature (30/15°C) accelerated senescence, but high root temperature (15/30°C) was even more detrimental. The combined high temperature treatments (30/30°C) caused leaves to senesce within 2 weeks (seedling growth stages) (Guedira and Paulsen, 2002). However, the development rates were faster in the vegetative development stage and the shortening of this period was not detrimental to yield (Hatfield and Prueger, 2015). The effect of high temperature on the early stage of wheat growth needs to be evaluated to better understand the impact of temperature stress on shoot and root growth of wheat plants. The influence of higher temperatures during the vegetative stage may have adverse effects on above- and belowground biomass of the plant and adversely affect plant growth.

Photosynthesis is the most thermo-sensitive part of plant function (Kim and Portis, 2005) and hence is adversely affected at high temperatures. It has been reported that photosynthesis of wheat tolerates temperatures of 30–35°C, but is adversely affected above 40°C (Schuster and Monson, 1990). It has been showed at the beginning of early growth stage of plants that high temperature decreased means photosynthetic rates 32% and means total biomass 32%. Besides, wheat genotypes that most tolerant of high temperature had stable rates and/or long durations of

photosynthetic activity (Al-Khatib and Paulsen, 1999). Timlin et al. (2006) have suggested that temperature impacts on photosynthesis, respiration, and C partitioning are important processes and mainly affect plant growth and development rates. Various physiological injuries have been reported to occur under elevated temperatures, such as scorching of leaves and stems, leaf senescence, shoot and root growth inhibition, which consequently lead to decreased plant productivity (Vollenweider and Günthardt-Goerg, 2005). In many cases, plant architecture changes and hypocotyls and petioles elongate resembling the morphological responses of shade avoidance (Hua, 2009). However, plant growth is reduced by high temperatures through affecting the shoot net assimilation rates and thus the total dry weight of the plant (Wahid et al., 2007).

1.4 Water use efficiency

Recently, it has been emphasised the importance of improving efficiency and performance of irrigation systems for better water use productivity (Car et al., 2007). Understanding water use is an important to evaluate the potential of new crops in areas where water is a limiting factor (Johnson and Henderson, 2002). Crop water use efficiency is an especially important consideration where irrigation water resources are reduced or declining (Gao et al., 2007). Efficiency can be defined in different ways, depending on the nature of the inputs and outputs to be considered. Water use efficiency is defined as the ratio of economic yield to total water use

or evapotranspiration (Zaffaroni and Schneiter, 1989). It can be expressed mathematically as illustrated by (Micheal, 1978):

$$\mathbf{WUE} = \frac{\mathbf{Yield\ (usually\ the\ economic\ yield)}}{\mathbf{water\ used\ to\ produce\ yield}}$$

If the crop yield is expressed in kg m^{-2} and the water use is expressed in m^3 m^{-2}, then WUE has units of kg m^{-3} on a unit water volume basis or g kg^{-1} when expressed on a unit water mass basis (Fatah, 2009). Further, Micheal (1978) explained that the water use efficiency can be defined in another way as follows:

$$\mathbf{Field\ water\ use\ efficiency} = \frac{\mathbf{Y}}{\mathbf{WR}}$$

Where:

Y= crop yield (kg).

WR= the total amount of water used in the field.

Of the two indices for water use efficiency, the concept of crop water use efficiency is generally more thought of from a fundamental interest perspective, while field water use efficiency is of greater practical importance (Micheal, 1978). Palacios-Velez (2010) revealed the efficiency in the use of water for irrigation consists of various components and takes into account losses during storage, conveyance and application to irrigation plots. Determining the different components and understanding what improvements can be made are essential in making the most effective use of this resource.

Recently, interesting field studies have focused on improving soil water use efficiency (WUE) (defined as a harvested yield per unit of soil water used in the field) and saving irrigation water through the

plant physiological attributes regulation has become an important and exciting research field in recent years (Gao et al., 2007). It has also been stated that crop WUE mainly depends on the transpiration rate per unit leaf area and the capability of roots for water absorption (Gao et al., 2007). Thus, effective use of water by crops should result from optimisation of root and shoot systems whose structure and function are closely linked.

Plants may have developed a strategy for acquiring higher resource use efficiency by coordinating root and shoot relations so as to keep a balance between the total shoot and root structure and function (Chen et al., 2005, Equiza et al., 2001). Variance in water availability affects plant photosynthesis, growth, productivity, and community composition (Guenni et al., 2004).

Irrigation management will change by considering the production per unit of water consumed, i.e. the water productivity (Zhang et al., 2008). It is important to maximise irrigation scheduling to produce the maximum plant yield (Uçan et al., 2007). Such schedules need to be developed for different ecological regions, as plant water consumption during the vegetation period depends mostly on plant growth, soil and climatic conditions.

It has been found in the Mediterranean that WUE values for wheat differed from 0.5 to 2.5 g l^{-1} and for barley between 1.46 and 2.78 g l^{-1} (Katerji et al., 2008). Moreover, for field grown barley cv. Rum and durum wheat cv. Hourani in Jordan, a WUE 70% higher for barley than for durum wheat was measured (Ebrahim, 2008). Blum (2005) reported that under stress conditions WUE is frequently

equated with drought resistance and the improvement of crop productivity, which is not necessarily the case. Drought resistance is sometimes considered as a penalty towards yield potential, which is not necessarily the case (Blum, 2005). Yield potential is defined as the highest yield realized under non-stress conditions (Blum, 2005). Li et al. (2001) stated that conserving soil water and water use efficiency are the main production factors in modern rain-fed and irrigated agriculture.

1.5 Carbon dioxide (CO_2)

1.5.1 Effect of elevated CO_2 on wheat physiology

1.5.1.1 Photosynthesis

An increased level of atmospheric CO_2 during growth raises biomass production in field grown wheat as a result of greater rates of net leaf photosynthesis and reduced photorespiration (Manderscheid et al., 2003, Högy et al., 2009). It has been reported that wheat growth, development and grain yield are directly affected by combined increases in atmospheric CO_2 concentration and temperature by rising photosynthesis rates and phenological development (Semenov et al., 2012). Prior et al. (2011) emphasised that carbon dioxide links the atmosphere to the biosphere and is a fundamental substrate for photosynthesis. Elevated CO_2 stimulates photosynthesis which leads to increased carbon (C) uptake and assimilation, thereby increasing plant growth.

However, as a result of CO_2 use differences during photosynthesis, plants with a C3 photosynthetic pathway often exhibit greater

growth response relative to those with a C4 pathway (Amthor, 1995, Amthor and Loomis, 1996, Bowes, 1993, Rogers et al., 1997, Poorter, 1993). Although increased photosynthesis under elevated CO_2 enhances growth for most plants research has consistently shown that this increase varies for plants with a C3 (33% to 40% increase) versus a C4 (10% to 15% increase) photosynthetic pathway (Prior et al., 2003). Many reviews have discussed the positive effects of CO_2-enriched air on plants including increased photosynthetic capacity, water-use efficiency, and growth (Amthor, 1995, Wittwer, 1995). Elevated atmospheric CO_2 concentrations have been shown to increase plant productivity and are attributed to direct stimulation of photosynthesis and increased water-use efficiency due to reduced evapotranspiration (Kimball et al., 2002).

1.5.1.2 Stomatal conductance and transpiration

Stomatal density is defined as the number of stomata per unit leaf area, and was theoretically revealed to be correlated with stomatal conductance (Gs) (Franks and Beerling, 2009). Prasad et al. (2005) found that a partial stomatal closure is one of the most important phenomena responsive to increasing CO_2 levels. At increased CO_2, stomatal conductance decreased significantly (Ainsworth et al., 2002). Increased intercellular concentrations of CO_2 in the leaves is thought to result in a decreased stomatal conductance (Cure and Acock, 1986) and thus improve water use efficiency. It has been shown that the positive impact of elevated CO_2 on shoot and root biomass and root length was reduced under high temperature is

likely to be associated with high temperature or with the reduction in stomatal conductance and transpiration that occurs under elevated CO_2 (Long et al., 2004). Lower transpiration will lead to increased leaf (Idso et al., 1987) and canopy temperatures (Kimball et al., 1995), decreasing growth and biomass partitioning (Farooq et al., 2009).

1.5.2 Effect of elevated CO_2 on shoot growth

Increasing atmospheric CO_2 significantly increased shoot biomass and leaf area (Obrist and Arnone Iii, 2003). Aboveground traits in different crops have been shown to be affected by increasing CO_2 and temperature (Reynolds-Henne et al., 2010, Wertin et al., 2010), growth and development of the root systems may also be influenced (Clair and Lynch, 2010).

The combined effect of elevated CO_2 and water deficits during the vegetative growth of wheat has also been studied. Schütz and Fangmeier (2001) found that shoot biomass and grain yield were 60% and 57%, respectively, greater under elevated CO_2 combined with drought than under ambient CO_2 plus drought, indicating that elevated CO_2 ameliorated the effect of drought on biomass and grain yield.

Benlloch-Gonzalez et al. (2014) reported that there were significant effects of temperature and CO_2 and their interaction on above-ground biomass. It has also been shown that increasing CO_2 level was associated with increases in above-ground biomass and it was

higher in plants grown at elevated CO_2 in ambient and high temperature treatments.

1.5.3 Effect of elevated CO_2 on root growth

Root biomass, shoot biomass and leaf area are significantly increased by increasing atmospheric CO_2 (Obrist and Arnone Iii, 2003). In many plant species, increasing atmospheric CO_2 motivates root biomass and root length production, even more than shoot biomass or leaf area production (Rogers et al., 1999, Pritchard and Rogers, 2000). Increased root growth contributes to root biomass and root dry weight under elevated CO_2 regardless of species or study conditions (Rogers et al., 1994, Rogers et al., 1995). Roots often exhibit the greatest relative dry weight gain among plant organs (Hocking and Meyer, 1991, Norby et al., 1992), even more than shoot biomass or the production of leaf area (Pritchard and Rogers, 2000, Bernacchi et al., 2000).

The most commonly reported root measurement is the root dry weight (Rogers et al., 1994), which increased with an elevation of CO_2 for such crops as sorghum (Chaudhuri et al., 1986), soybean (Rogers et al., 1992) and winter wheat (Chaudhuri et al., 1990). These data suggest that root systems more thoroughly exploit a given volume of soil in the presence of higher CO_2. Roots of plants grown under elevated CO_2 reach deeper soil layers (Rogers et al., 1992) or attain maximum rooting depth ahead of plants grown under ambient CO_2 (Chaudhuri et al., 1990).

It has been revealed that at elevated CO_2 the distribution of photosynthate below-ground can be preferentially induced by increased C supply (Ceulemans and Mousseau, 1994, Lekkerkerk et al., 1990, Prior et al., 1997, Rogers et al., 1994). In many cases, it is the largest proportion of below-ground biomass that is produced under elevated CO_2 (Rogers et al., 1994, Wittwer, 1995), and results in increased root-to-shoot ratio (Rogers et al., 1995). This is not surprising in that plants tend to allocate photosynthate to tissues needed to acquire the most limiting resource (Chapin et al., 1987); the most limiting resource becomes water or nutrients under elevated CO_2.

1.5.4 Effect of elevated CO_2 on yield

Pilumwonga et al. (2007) reported that there is substantial concern for increasing CO_2 concentration in the atmosphere, largely associated with increases in temperature and their effects on crop production. Increasing CO_2 concentrations and other greenhouse gases, comprising methane, and nitrous oxide, will potentially raise the global average temperatures by 1.4-5.8 ºC (Houghton et al., 2001). Over the next 50 years, the climate conditions around the wheat-growing regions of the world are expected to alter with potentially severe effects on wheat production (Tubiello et al., 2007, Forster et al., 2007). It is suggested that plants will be subjected to a combined effect of higher CO_2 and temperature under future climate change scenarios (Rosenzweig and Hillel, 1998).

It has been shown that elevated CO_2 improves grain yield by increasing grain size or the number of spikes (Högy and Fangmeier, 2008). The combined effect of elevated CO_2 and water deficits during the vegetative growth of wheat has also been studied (de Oliveira et al., 2013). It has been reported that under the optimal growth conditions and in the presence of increased CO_2 concentration, plant photosynthesis and growth are increased. However, at higher temperatures the elevated concentration of CO_2 negatively affects the yield and yield components (Prasad et al., 2005). Therefore, it is very important to understand in detail the effect of elevated CO_2 on plant growth under stress condition. In the context of the effect of high temperature with elevated CO_2 on crop yield under water stress, it is unknown to what extend this will impact on root and shoot growth. Therefore, it is crucial to understand the combined effects of elevated CO_2, temperature and water stress to identify the management of crop and to sustain a sufficient crop output in a future climate.

1.5.3 Effect of elevated CO_2 on vegetative growth stage

Atmospheric CO_2 concentrations have steadily increased from approximately 315 ppm in 1959 to a current atmospheric concentration of approximately 385 ppm which equates to an average annual increase rate of nearly 2 ppm (Keeling and Whorf, 2005). It is predicted that CO_2 concentrations will reach levels between 500 and 1000 ppm by 2100 (IPCC, 2007). Carbon dioxide (CO_2) is not only a major greenhouse gas, but also important to

plant growth (Kimball, 2010). It has been demonstrated that increases in atmospheric CO_2 concentration will have direct and indirect effects on crop plants (IPCC, 2007).

Exposure of crop plants to high CO_2 concentration often stimulates growth of shoots and roots (Rogers et al., 1995). Increased CO_2 stimulates photosynthesis and C allocation to plant roots which leads to an increase in above and below biomass (Rogers et al., 1994). Root growth of crop plants is often stimulated to a greater extent than other plant parts by increased CO_2 concentration (Heinemann et al., 2006, Vanaja et al., 2007) because of increased C translocation to the roots. Observations from a spring wheat a free air carbon dioxide enrichment (FACE) study reported a 37% increase in root dry mass under elevated CO_2 during early vegetative growth and continued root growth rates until anthesis (Wechsung et al., 1999). Elevated CO_2 increases biomass production in wheat as a result of greater rates of net leaf photosynthesis and reduced photorespiration (Manderscheid et al., 2003, Högy et al., 2009).

Root growth and development is also affected by the combined effect of temperature and elevated CO_2 concentration. It has been reported in groundnut (Arachis hypogaea L.) that fibrous root dry weight increased with increased CO_2 when plants were grown at 25/15°C, but decreased with increasing CO_2 at 35/25°C. At 35/25°C, there was greater root dry weight by 34% at 400 ppm, 14% at 600 ppm, and 7% at 800 ppm compared with 25/15°C. Root

biomass was higher under the effect of elevated CO_2 during vegetative than during reproductive growth for non-limited soil water treatments (Pilumwonga et al., 2007). It has been shown that during vegetative growth a lower value of potential transpiration accounted for only 30–50% of the higher CO_2 response of the assimilation rate under limited soil water, when soil water contents were not very low and transpiration demands were not very high. The remaining 50–70% of the response must therefore be attributed to the higher root biomass of the FACE plants (Clark et al., 2003). It has also been shown that higher root biomass under elevated CO_2 led to a higher water uptake capacity during vegetative growth, when soil water contents were still not very low. During that time under soil water limitation the higher root biomass of the FACE plants accounted for up to 50% of the higher CO_2 response of the assimilation rate. The direct effect of increasing CO_2 on root /shoot growth has no clearly defined conclusion (Vanuytrecht et al., 2012).

The stimulation of photosynthesis is generally increased strongly and predictably by the combined effect of elevated CO_2 and temperature (Long, 1991). It has been reported that both the vegetative and reproductive growth of crop plants decrease at above optimal temperatures but this may be partially offset by greater rates of net photosynthesis due to CO_2 enrichment (Boote et al., 2005). However, at excessively high temperatures, the CO_2-dependent stimulation of photosynthesis may be negated by low rates of Ribulose-1, 5-bisphosphate (Rubp-regeneration). When this

situation occurs, the stimulation of photosynthesis by elevated CO_2 is highly insensitive to measurement temperatures (Ziska, 2001, Yamori et al., 2005). Additionally, acclimation of photosynthesis to seasonal changes in temperature can result in the stimulation of photosynthesis by elevated CO_2 being nearly constant at different times of the year despite seasonal variations in temperature. This phenomenon has been attributed to thermal acclimation of the photosynthesis system (Bunce, 1998, Bunce and Norman, 2000).

The combined effects of elevated CO_2 and water stress on wheat shoot and root growth at early growth stages are not well understood and neither is the combined effect of elevated CO_2, high temperatures and water stress (de Oliveira et al., 2013). It has been claimed that the effect of CO_2 concentration on root and shoot ratio is difficult to determine due to complexity in accurate root biomass estimation under diverse crops and conditions (Madhu and Hatfield, 2013). Roots become more numerous, longer, thicker, and faster growing in crops exposed to high CO_2 with increased root length in many plant species. It has been suggested that branching and extension of roots under elevated CO_2 may lead to altered root architecture and the ability of roots to acquire water and nutrients from the soil profile with exploration of the soil volume (Madhu and Hatfield, 2013).To determine the effect of elevated CO_2 on early shoot and root growth and the importance of detecting root and shoot growth characteristics in undisturbed soil, sections of this study focused on studying the elevated CO_2 on the interactive

effects of elevated CO_2, high temperature and drought on shoot and root growth.

1.6 Irrigation deficit

Available water resources for agriculture have been rapidly decreasing in recent years due to increased competition for freshwater between agriculture and other sectors (Gan et al., 2013). Regulated deficit irrigation (RDI) is considered a key water-saving practice for efficient use of the limited water resources (Chai et al., 2014). It has been recognised that RDI can save irrigation water up to 20 to 30% and increase WUE up to 30% in favourable situations. In some extreme cases, the RDI approach can save water up to 50 % with a minimal impact on crop yield (Xie et al., 2012, Li et al., 2010).

Mild water deficit applied in the early stages of growth has been shown to enhance the level of drought resistance later in the life cycle and consequently maintain (Liu et al., 2006a) or even increase plant yields (Cui et al., 2009b, Xue et al., 2006). It has also been reported that plants grew under mild water stress may lead to optimized stomatal control over gas exchange (Wang et al., 2010), improving the ratio of photosynthesis to transpiration or to stomatal conductance of CO_2 (Cui et al., 2009a). Water deficit at appropriate growth stages has been successfully applied previously to regulate physiological–biochemical processes, vegetative growth and plant reproduction, resulting in higher grain yields and water use efficiency (Zhang et al., 2006, Fereres and Soriano, 2007, Du et al.,

2010). It has been reported that RDI at early-growth stage can subsequently improve plants physiological properties in the late growth stage, such as photosynthesis (Meng et al., 2003). Ma et al. (2016) have also showed that RDI at early growth stages enhanced leaf photosynthetic rate during anthesis that led to increased water use efficiency. Silva et al. (2012) found on the day of maximum water stress that the stomatal conductance of plants exposed to long wetting and drying cycles (LC, 7 days) was less than that of short wetting and drying cycles (SC, 2 days). However, stomatal conductance of LC plants 3 days after irrigation was the same as that of plants 2 days after irrigation SC. This shows that water was available for normal transpiration of LC plants for at least the first 3 days of a cycle but not the entire period (Silva et al., 2012). Liang et al. (2002) found that at the beginning of early growth stage (three weeks) stomatal conductance of wheat steadily decreased with decreases in the days of drying. Besides, they reported that the drying-rewatering alternation had a significant effect on wheat stomata that led to decreased wheat transpiration. The decreased wheat water consumption mainly resulted from the decreased stomata conductance and transpiration rate (Liang et al., 2002). Thus, the drying and wetting alternation had after-effects on wheat growth. Meanwhile, the drying and re-wetting alternation also increased root to shoot ratio (dry weight) (Silva et al., 2012, Liang et al., 2002).

As the soil dries, plants initiate adaptive responses that regulate yield determining processes such as leaf expansion and leaf gas exchange (including photosynthesis). One of the most enduring controversies in plant water relations (Kramer, 1988, Christmann et al., 2007) is whether leaf growth and gas exchange are regulated hydraulically [via changes in leaf water potential (Ψ_{leaf}) or turgor] or chemically [via changes in ionic and phytohormonal signals originating from the roots or shoots]. Several lines of evidence suggest that trying to distinguish the effects of each in the shoot is somewhat artificial, as the effect of the plant hormone abscisic acid (ABA) on stomatal aperture is mediated by Ψ_{leaf} (Tardieu and Davies, 1992), and ABA affects stomatal conductance (gs) both directly by acting on the stomatal guard cells and also indirectly by decreasing leaf hydraulic conductance (Pantin et al., 2013). Nevertheless, it is useful to consider that both forms of regulation can alter crop responses to soil drying and rewetting.

It has been reported that repeated soil drying and rewetting (DRW) cycles affect both plant water uptake and the activity of soil microbes involved in nutrient cycling, thereby affecting soil nutrient availability (Dodd et al., 2015). Understanding of how plants and soils respond to DRW cycles seems critical to optimize resource use efficiency in agriculture, especially given global concerns such as decreasing supplies of fresh water for irrigation (Elliott et al., 2014), and the limited availability and high cost of synthetic fertilizers to increase crop yields (Cordell et al., 2009). It has been demonstrated

that wetting and drying cycles have generally improved crop WUE, and sometimes either actual and/ or economic yields (Davies et al., 2011). However, many soil drying experiments reported in the plant physiological literature expose plants to a single drying cycle (often until loss of plant turgor), usually to evaluate relationships between soil and plant water status and physiological responses such as leaf growth and gas exchange (Dodd et al., 2015). No single study, as yet, has been performed on repeated soil drying and re-wetting (DRW) cycles mainly at early shoot and root growth in undisturbed soils. Therefore, the present study for the first time aimed to investigate the effect of DRW cycles on wheat shoot and root characteristics during vegetative growth stage and in undisturbed soils. Thus, this will be helpful to understand the agronomic responses that integrate crop performance over the entire growing season, and of physiological responses that may be restricted to specific tissues at specific times of the growing season.

1.7 Aims and hypothesis

The overall aim of this work was to investigate the responses of shoot and root growth of wheat to water stress and temperature. It also aimed to study the combined effect of elevated CO_2 and temperature on shoot and root growth under water stress. X-ray Computed Tomography (X-ray CT) was used to visualise the root behaviour in undisturbed soil following exposure to aforementioned stress conditions. Photosynthesis, stomatal conductance and

transpiration were also measured to examine the shoot behaviour under the same environmental conditions.

The main hypothesis was:

The combined effect of water stress and temperature has a negative impact on shoot and root growth of wheat while elevated CO_2 enhances both photosynthesis and root architecture development.

To address this hypothesis, the following sub hypotheses were considered:

1. The null hypothesis was that different levels of water stress have a consistent negative effect on root growth and development, photosynthesis, stomatal conductance and transpiration. It was also hypothesized that the root and shoot growth under water deficiency would be better in the clay than in the sandy soil due to differences in soil pore size.
2. It was hypothesized water shortage would have a varying impact on root and shoot growth depending on different drought tolerant and resistant cultivars chosen for experimentation.
3. The null hypothesis was that elevated CO_2 and temperature has a significant positive effect on shoot and root growth and could mitigate the negative impact of drought on growth. It was also hypothesised that elevated CO_2 would enhance photosynthesis and growth of root systems architecture.

4. It was hypothesized that repeated soil wetting and drying cycles would have a more positive impact on root and shoot growth of wheat as opposed to a more consistent water stress treatment.

To study the main hypothesis, the following sub aims have been developed.

1. To study the effect of water stress and temperature on wheat growth, photosynthesis, stomatal conductance and transpiration and the interaction between the root growth and photosynthesis (chapter 3).
2. To test for the consistency of this effect by studying the impact of soil water content on different varieties of wheat including those with drought tolerance and resistance. (chapter 4).
3. To study the effect of elevated CO_2 and temperature on shoot and root growth under water stress (chapter 5).
4. To study the effect of repeated soil wetting and drying cycles on wheat growth, photosynthesis, stomatal conductance and transpiration in different soils under high temperature (chapter 6).
5. To examine and visualise the 3D root architecture responses to different water stress, temperature and CO_2 concentration by using X-ray Computed Tomography (chapter 3, 4, 5, 6).

2.0 Materials and Methods

The information presented in this chapter is not been presented elsewhere in the thesis. Methods specific to particular experiments are included in each chapter where relevant.

2.1 Soil physical properties

2.1.1 Soil types

Two soil types were used extensively in this research. A Newport series, sandy loam / loamy sand soil (FAO Class brown soil) and a Worcester series clay / clay loam soil (FAO Class argillic pelosol) from the University of Nottingham farm collected from Bunny, Nottinghamshire, UK (52.52 °N, 1.07 °W). The soils were air-dried and sieved to <2 mm for packing into the columns for the use in the relevant experimental chapters.

2.1.2 Soil hydraulic properties

2.1.2.1 Water release curve

The soil water release curve is a plot of the soil water content against the soil matric potential which can be used to derive the available water capacity (Rowell, 1994). Using a combination of pressure plate and membrane apparatus, water release curves were obtained for both soil types at a range of reductive matric potentials from 0 to -1500 kPa. Due to the variability in the data by this method, all data were subsequently fitted to the Van Genuchten-Mualem model using RETC software, (http://www.scisoftware.com/) as is routinely undertaken.

2.1.2.2 Pressure plate apparatus

The pressure plate method was used to measure matric potential from 0 to -500 kPa. The ceramic pressure plate cell consisted of a porous ceramic plate, an internal screen, and an outlet stem. The porous ceramic plate is covered on one side by a thin neoprene diaphragm which is attached to the edges of the ceramic plate. The internal screen between the plate and diaphragm provides a passage for lateral movement of water to the outlet stem. This later connects the passage to an outflow tube fitting which is exposed to the atmosphere outside of the extractor.

The pressure supply and control system consists of an air compressor and a manifold. The air compressor, which is connected to the manifold by using a connecting hose, provides the required pressure to increase the atmospheric pressure inside them.

In this study, the soil columns were weighed twice a week to check the soil moisture content equilibrated under each of these pressures (-10, -30, -60, -100, -300, and -480 kPa). It took approximately 12 weeks to complete this process for each soil.

2.1.2.3 Pressure membrane apparatus

A blade was used to cut the pressure membranes into the correct sizes and placed on the plates, with the rubber “O“ rings placed at the top and bottom of the container. The mass of soil was carefully placed into the container and saturated with air-free water. The container was then sealed and bolted down to ensure a tight fit. Plastic collection tubes were filled with approximately 1 ml of oil to

prevent evaporation of collected water. Individual tubes were then weighed and placed under the pressure plate outlets. Nitrogen gas was added and the pressure set to the isolator. Gas leakages were tested for using washing up liquid and observing bubbles; if any leaks were detected the individual container was cleaned and re-packed with soil. The gas pressure was set to the lowest pressure of -200 kPa when no gas leakages were present. The collection tubes were weighed frequently and once equilibrated a higher pressure was set. This was repeated for pressures of -600, -1000 and -1400 kPa. Soil samples were oven dried at 105 ºC for 24 hr then weighed after the final measurement.

The water release characteristic curves for both soil types are displayed in Figures 2.1 (experimental derived) and 2.2 (modelled).

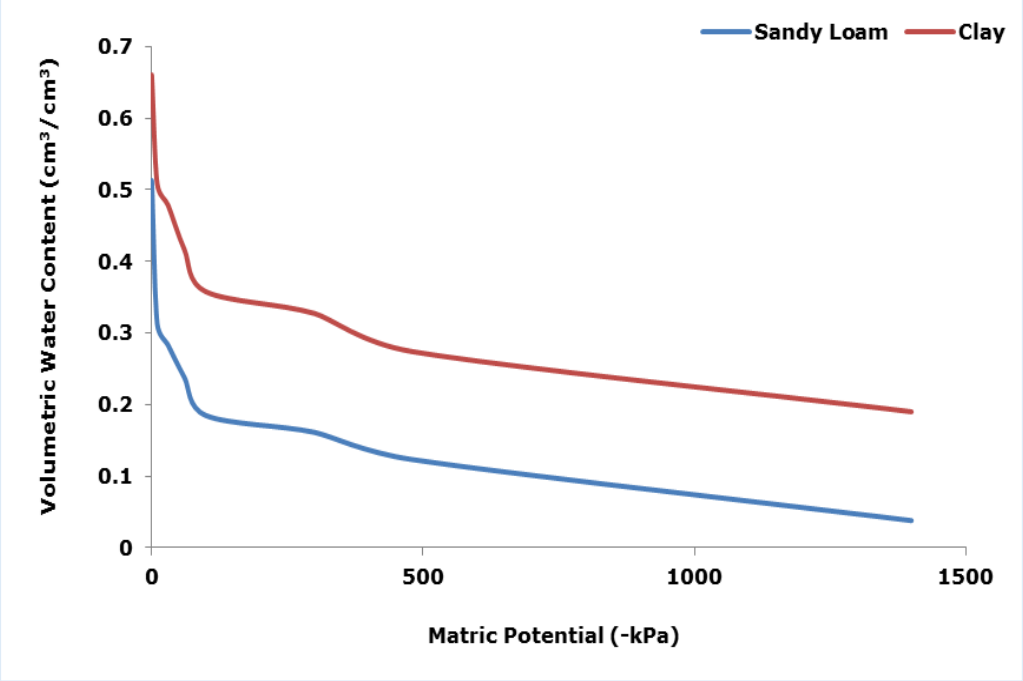


Figure 2.1: Water release curve of the sandy loam and the clay soils of experimental values obtained.

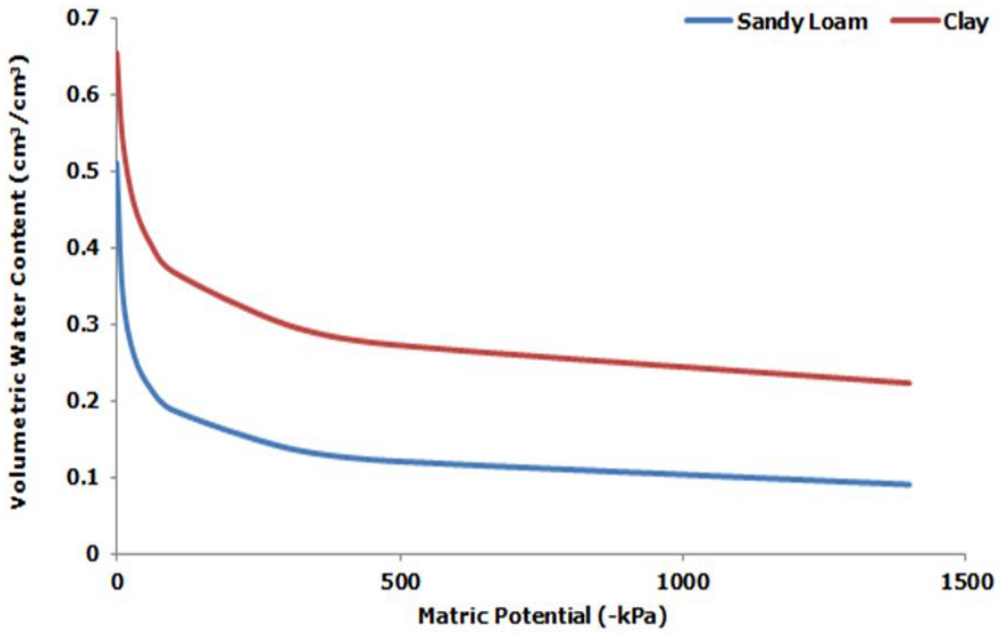


Figure 2.2: Water release curve of the sandy loam and the clay soils of values fitted using the Van Genuchten-Mualem model.

2.1.3 Field capacity

The term field capacity originates from the work of Israelsen and West (1922) and Veihmeyer and Hendrickson (1931) is typically considered as the soil moisture content held in the soil following two days of drainage from a saturated state. At this point it is usually hypothesised that the rate of downward movement has stopped or significantly decreased i.e. gravitational flow has stopped. The matric potential value to express this state varies internationally, in the UK a value of -5 kPa is used, in many European countries the value is -10 kPa and in the US it is -33 kPa. As one might expect the moisture content of soil at field capacity varies significantly with differences in soil. Typical values for volumetric soil moisture content at field capacity are 15 to 25% for sandy soils, 35 to 45%

for loam soils, and 45 to 55% for clay soils. In this research the term field capacity is used to express the moisture status of a soil column following two days of drainage after being held at a saturated state. By calculating the volumetric water content at this state (with a prior knowledge of bulk density) it is also possible to dry the columns further and express this as a % of field capacity such as 50% or 25%. This is not to these values are directly comparable to the moisture content at similar states in the field or that the samples are held at a specific matric potential, more that it provides a means of applying a measurable moisture stress treatment.

2.2 Soil chemical properties

2.2.1 pH

A combined electrode was used to measure soil pH, which includes "reference" and pH-sensitive glass electrodes incorporated into a single electrode stick. The pH meter was calibrated with pH 4.01 containing 10.211 g L^{-1} $KHPO_4$ and pH 7.00 consisting of 3.9 g L^{-1} $NaHPO_4$ and 2.72 g L^{-1} $KH2PO_4$ buffers using the "buffer" and "slope" controls. Approximately, 5 g of air dried < 2 mm soil was measured into a centrifuge tube and 12.5 ml of de-ionised water (suspensions 1:2.5 ratio) was added. The tube was shaken on an orbital shaker (model SLM-INC-OS-16) at 40 rpm for 30 min to attain equilibrium. After rinsing with de-ionised water the tip of the pH electrode was immersed into the soil suspension. A Hanna pH-209 pH meter and a combined glass electrode (Ag/AgCl; PHE 1004)

were used for measuring a pH. The result was recorded after waiting 5 min for the pH reading to stabilise. The average pH value of the two soil types was 7.4 and 7.3 for the sand and the clay soils types, respectively.

2.2.2 Loss of ignition

Organic matter content of soils was determined by igniting samples at 550^{o}C under controlled conditions to burn off the organic material and determine the loss of weight. Approximately 5 g oven-dried soil was placed into the silica crucibles in triplicate, before being placed in a muffle furnace (Gallenkamp size 3, Weiss-Gallenkamp, UK). The oven dried soil samples were ignited overnight in a carefully controlled (timed) heating process. The following day, the crucibles were removed and re-weighed to determine loss on ignition.

2.2.3 Samples preparation for Inductively Coupled Plasma Mass Spectrometer (ICPMS) and CNS Analyser

2.2.3.1 Inductively Coupled Plasma Mass Spectrometer (ICPMS)

A small portion of each soil type was finely ground using an Agate ball mill (Retsch, Model PM400), and then stored in polyethylene bags for elemental analysis. Finely ground soil (250 mg) was digested using 70% hydrofluoric acid, nitric acid and perchloric acid (Trace Element Grade (TEG); Fisher Scientific, UK) in a teflon-coated graphite Block Digestor (Analysco, UK) containing places for 48 PFA digestion vessels. The digested samples were diluted to 50 mL using Milli-Q water (18.3 MΩcm) and stored un-refrigerated in

universal sample bottles (5% HNO3) pending elemental analysis. All digests were diluted to 1 in 10 with Milli-Q water immediately prior to analysis. Multi-element analysis was undertaken by ICP-MS (Model X-SeriesII, Thermo-Fisher Scientific, Bremen, Germany) in 'collision cell mode' (7% hydrogen in helium) to reduce polyatomic interferences. Samples were introduced from an autosampler (Cetac ASX-520 with 4 x 60-place sample racks) through a concentric glass venturi nebuliser (Thermo-Fisher Scientific; 1 mL min^{-1}). Internal standards were introduced to the sample stream via a T-piece and included Sc (100 ng mL^{-1}), Rh (20 ng mL^{-1}) and Ir (10 ng mL^{-1}) in 2% TEG HNO_3. External multi-element calibration standards (Claritas-PPT grade CLMS-2, Certiprep/Fisher) included P, K, Mg, Ca, Na, S all in the preferred range of 0-100 µg L^{-1}. Sample processing was undertaken using Plasma lab software (version 2.5.4; Thermo-Fisher Scientific) set to employ separate calibration blocks and internal cross-calibration where required.

2.2.3.2 Soil total carbon and nitrogen content

Analysis of total carbon and nitrogen was undertaken using a CNS analyser (Flash EA1112; CE Instruments). Approximately 8 - 13 mg of dry finely ground soil was weighed into tin capsules and 5 mg of vanadium pentoxide was then added to each capsule. Capsules were carefully crimped to avoid spillage. A blank (capsule containing only vanadium pentoxide) and certified soil reference standards were used for calibration.

2.3 X-ray CT scanning procedure

2.3.1 Scanning procedure

A Pheonix Nanotom 180NF and a Phoenix V|tome|x m CT scanner were used for scanning root in this study. The overall procedure of the scanning is as follows, however the X-ray power settings, projections' number and detector exposure timing used were different depending on the sample and the experiment (this can be shown in each results chapter). In general a soil column was placed on the stage of sample and the position was adjusted. This is important to ensure that the sample was within the field and was fitted firmly to prevent sample from any movement during the scanning. The suitable power (kV) and current (μA) were then chosen to ensure that X-ray penetrated throughout the sample. In order to check the response of detector the offset (X-ray was switched off) and gain (X-ray was switched on) calibrations were undertaken.

2.3.2 Reconstruction procedure

Reconstruction of images is the mathematical process of producing images from projection data by CT scanning. Recon | x software was used to reconstruct all of the scans after scanning sample. The image optimiser was used to check the sample from any movement during the scanning. This was done by comparing the first and the last image and adjusting when needed. Thereafter, the beam hardening correction tool was utilised by relying on the composition of the sample, 1 – 10 (10 being for high beam hardening).

2.3.3 Image analysis

VG Studio Max® 2.0 was used for all image analysis. There were specific image analysis methods which varied between experiments but the basic protocols remained the same. VG Studio MAX was used to separate points of interest from the scan data; this is referred to as segmentation. VG Studio MAX has the capacity to maintain the whole greyscale histogram rather than compressing it, which is necessary when using 32bit reconstructed scans.

2.4 WinRHIZO

WinRHIZO is a popular and widely used tool in plant root studies (Bouma et al., 2000, Himmelbauer, 2004). It is an image analysis system specially designed for automatic root measurements in different forms and provides measures such as root volume, diameter, length, area Etc. Essentially the roots of plant are washed free from soil and analysed with WinRHIZO (version 2002c) by using EPSON Twain program. The roots are placed on a water tray and scanned with a flatbed scanner at 400dpi. The images were subsequently analysed using WinRHIZO's automatic thresholding for normal roots, ignoring speckles that had an area less than 2mm². From these data total and mean root volume and diameter were collected from each plant.

3.0 Quantifying the influence of water deficit on root and shoot growth of wheat

3.1 Introduction

Water stress has a negative effect on plant growth and development and sharply decreases plant productivity (Pan et al., 2002). Roots play a key role in water and nutrient supply for plants. However, the physiochemical and biological status of the surrounding soil can have a significant effect on their activity, particularly the relationship between root development and soil pore structure, which is both complex and relatively unexplored (Mooney et al., 2012).

Roots are morphologically and functionally structured for water, mineral and nutrient absorption from the soil by apoplastic and/or symplastic traits. Reduction in plant output is mainly observed when crop plants are exposed to water deficiency during long periods, which affects almost all physiological processes, involving growth (McDonald and Davies, 1996) and photosynthesis (Flexas et al., 2004). In general during drought, the aerial tissues (leaves and stems) are significantly inhibited in growth while the roots continuously grow to explore the soil volume for water (Sharp and Davies, 1989).

New developments in imaging technologies such as X-ray micro-Computed Tomography (μCT), to visualize the root of the plants grown in soil have shown much promise (Tracy et al., 2010). Whilst a number of studies have examined the impact of water stress on

shoot and root growth and photosynthesis, no previous study has visualized root systems of wheat under drought conditions to measure undisturbed root characteristics in 3D in soil. This study aimed to investigate the impact of water stress on root and shoot growth of wheat. The null hypothesis was that different levels of water stress have a consistent negative effect on root growth and development, photosynthesis, stomatal conductance and transpiration. It was also hypothesized that the root and shoot growth under water deficiency would be better in the clay than in the sandy soil.

3.2 Materials and methods

3.2.1 Sample preparation and X-ray μCT

A Newport series sandy loam (FAO Class brown soil) and a Worcester series clay loam soil (FAO Class argillic pelosol) were air-dried and sieved to < 2mm. Columns (15cm height × 5cm diameter) were uniformly packed to a representative field bulk density of 1.24 g cm^{-3}. Four moisture treatments were chosen to represent a range of saturated, field and dry conditions namely 100%, 75%, 50% and 25% of a notional field capacity (FC) as explained in chapter two. Four replicates were prepared for each soil type and treatment combination to give a total of 64 columns of which 32 were μCT scanned twice. An equal number of columns in the treatment structure were prepared but not scanned to assess for any potential effects by exposure to X-rays during scanning process. The columns were then water-saturated and drained to reach to different levels of field capacity (100% i.e. 2 days drainage) and left to reach these levels (75%, 50%, and 25% FC). Seeds of Zebedee (Redigo Deter) were germinated for 48 hrs before being planted 5 mm below the soil surface. Thereafter, they were placed in a growth room under conditions of 28/22 °C day/night with a 16 hrs photoperiod, at 50% relative humidity. All columns were placed in a transpired propagator to maintain high relative humidity levels during germination and seedling growth. They were weighed daily and sufficient water was added to maintain soil moisture content close to the four pre-determined moisture contents (100%, 75%, 50%, and 25% FC). Details of the exact water content, matric

potentials and other relevant soil properties are given in Table 3.1. The columns were scanned at day 14 and 21 after germination using a Phoenix Nanotom® (GE Measurement & Control Solutions, Wunstorf, Germany) X-ray µCT scanner set at 110 kV and 130 µA, with a 0.15 mm copper filter and an image averaging of 3. Pixel/voxel resolution was set at 55 µm and each scan took 32.5 min to complete. For each column, 1300 image projections were captured on all sampling dates and each volume/sample had a file size of approx. 2GB. Thereafter, these images were reconstructed using VG StudioMAX (Volume Graphics) image processing software.

3.2.2 Image processing and analysis

Root systems were non-destructively segmented from the grey-scale µCT images using the Region Growing selection tool in VG StudioMAXw2.0 software as described by Tracy et al. (2012). The root system models segmented from the µCT image data were used for quantitative determination of total root volume and mean root diameter. Region Growing classifies voxels in a certain grey-value range from a starting seed point. Tolerance values were adjusted to ensure that only root material was included in the growing region of interest (ROI) from the original seed points. The mean root diameter was measured by distance measurement tool. As the study was concerned with the impact and the interaction between roots and soil, the soil pore characteristics were also measured in addition to root measurements by using calibration wizard to set a threshold to segment the pores and the volume analyser in VGStudioMAX®.

3.2.3 Plant measurements

Photosynthesis (A), stomatal conductance (Gs) and transpiration (T) were measured the day before and after scanning by infrared gas analysis (LI-6400XT Portable Photosynthesis System, Licor, Illinois). The settings were cuvette temperature 30°C, CO_2 level 400 $\mu mol.mol^{-1}$, pressure 100 kPa, ambient RH, flow rate 500 $\mu mol.s^{-1}$, and 1500 $\mu mol.m^{-2}.s^{-1}$ saturating photosynthetically active radiation (PAR). Leaves were placed in the cuvette and approximately 3 minutes allowed for stability. The columns were scanned during the light part of the photoperiod in randomized order to ensure that all four treatment combinations were equally exposed to any diurnal variation in root growth that may have occurred. After CT scanning, the roots were washed from the soil and analysed using WinRHIZO®2002c scanning equipment and software (see more information in chapter two) to calculate total root volume and mean root diameter. The images obtained were collected to compare with the X-ray µCT images. Root and shoot dry weights were also measured by placing in an oven at 75°C temperature for 24 hrs.

3.2.4 Statistical analysis

The results were analysed by general analysis of variance (ANOVA) including water, soil types and their interaction as explanatory variables using Genstat 15.1. In addition, regression analysis was used to test the relationship between two root system visualisation methods (WinRHIZO and X-ray CT). A correlation matrix was used

to test the relationship between all variables by using summary statistics using Genstat 17 for analysing data.

Table 3.1: The values of volumetric water content, matric potential (ψ)(-kPa) at different water content (100%, 75%, 50% and 25% FC), the percentage of the sand, silt, the clay, Organic Matter by Loss on Ignition, pH and nutrient content for the sandy loam and the clay loam soils in Newport and Worcester. Note that matric potential values (ψ) were obtained from the water release curve and not directly measured.

Soil Properties	Sandy Loam	Clay Loam
VWC ($g.g^{-1}$) at 100%FC	0.42	0.45
VWC ($g.g^{-1}$) at 75%FC	0.32	0.34
VWC ($g.g^{-1}$) at 50%FC	0.21	0.23
VWC ($g.g^{-1}$) at 25%FC	0.11	0.11
Ψ (-kPa) at 100%FC	5	30
Ψ (-kPa) at 75%FC	10	150
Ψ (-kPa) at 50%FC	90	1200
Ψ (-kPa) at 25%FC	800	1500
% sand	79	38
% silt	4	31
% clay	17	31
Organic Matter by Loss on Ignition (%)	3.9	4.8
pH	6.9	7.7
Carbon (mg/kg soil)	17355	26768
Nitrogen (mg/kg soil)	1486	1505
Phosphorus (mg/kg soil)	778	797
Potassium (mg/kg soil)	10384	20659
Magnesium (mg/kg soil)	2352	14150
Sulphur (mg/kg soil)	2258	2058
Sodium (mg/kg soil)	1479	3423
Calcium (mg/kg soil)	2071	13150

3.3 Results

3.3.1 Root characteristics

3.3.1.1 Total root volume

To study the effect of water stress on important root characteristics such as total root volume and mean root diameter of Zebedee wheat variety, X-ray CT (14 and 21 days after germination) and WinRHIZO (21 days after germination) were used. The data showed total root volume by X-ray CT 14 days post germination reduced significantly with reducing soil moisture content in both soil types (sandy loam and clay loam) (P_{water}< 0.01, P_{soil}> 0.05, $P_{soil*water}$> 0.05) (Figure 3.1A). The p-values for all variables at 14 and 21 days after germination are shown in Table 3.4.

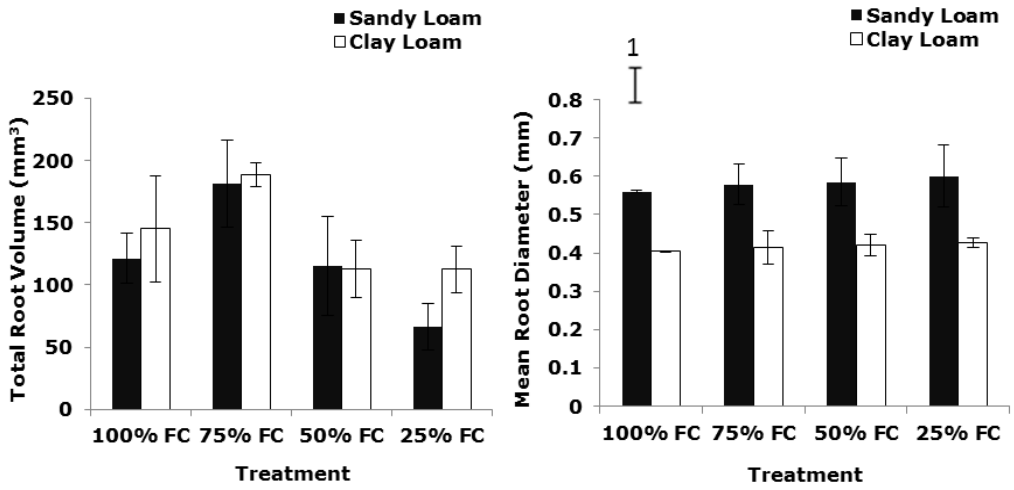
At 21 days post germination, total root volume by X-ray CT also dropped dramatically with declining soil moisture content in the sandy loam and clay loam (P_{soil}< 0.01, P_{water}< 0.001) (Figure 3.1B). The soil*water interaction was significant ($P_{soil*water}$< 0.01) (Figure 3.1B). Similar trends were found by WinRHIZO® analysis as total root volume decreased greatly with decreasing soil water content in the sandy loam and clay loam (P_{water}< 0.05, $P_{soil*water}$ < 0.05) (Figure 3.2). Total root volume in the sandy loam was significantly greater than in the clay loam soil (P_{soil}< 0.01) (Figure 3.1B & 3.2). The lowest total root volume of Zebedee was recorded at 100% FC rather than 75% FC.

3.3.1.2 Mean root diameter

The results found that mean root diameter in the sandy soil was significantly greater than in clay soil. Mean root diameter by X-ray CT increased with decreasing soil moisture content in the sandy loam and clay loam (P_{soil}< 0.001, P_{water}> 0.05, $P_{soil*water}$< 0.05) (Figure 3.1A).

At 21 days post germination, mean root diameter by X-ray CT increased significantly in the sandy loam and clay loam (P_{soil}< 0.001, P_{water}< 0.05, $P_{soil*water}$< 0.05) (Figure 3.1B). Identical trends were found by WinRHIZO® analysis as mean root diameter increased significantly with decreasing soil water content in the sandy loam and clay loam (P_{soil}< 0.001, P_{water}< 0.05, $P_{soil*water}$< 0.05) (Figure 3.2). Mean root diameter in the sandy loam soil was significantly greater than in the clay loam (P_{soil}< 0.001) (Figure 3.1 & 3.2).

A



B

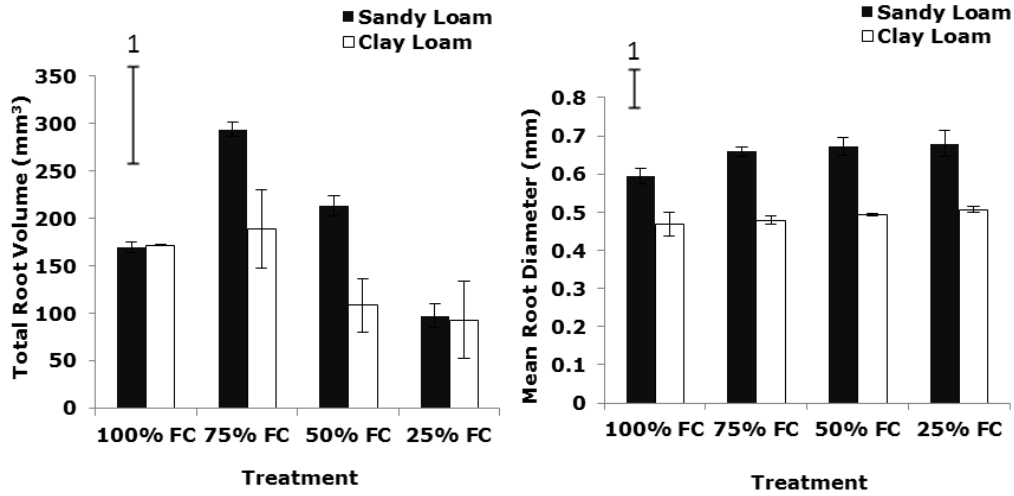


Figure 3.1: The effect of water stress on total volume and mean diameter of root by X-ray CT in wheat variety Zebedee grown in the sandy loam and clay loam soils types 14 (A) and 21 (B) days after germination. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on total root volume at 21 days (B) at $P< 0.01$ and mean root diameter at both dates at $P< 0.05$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

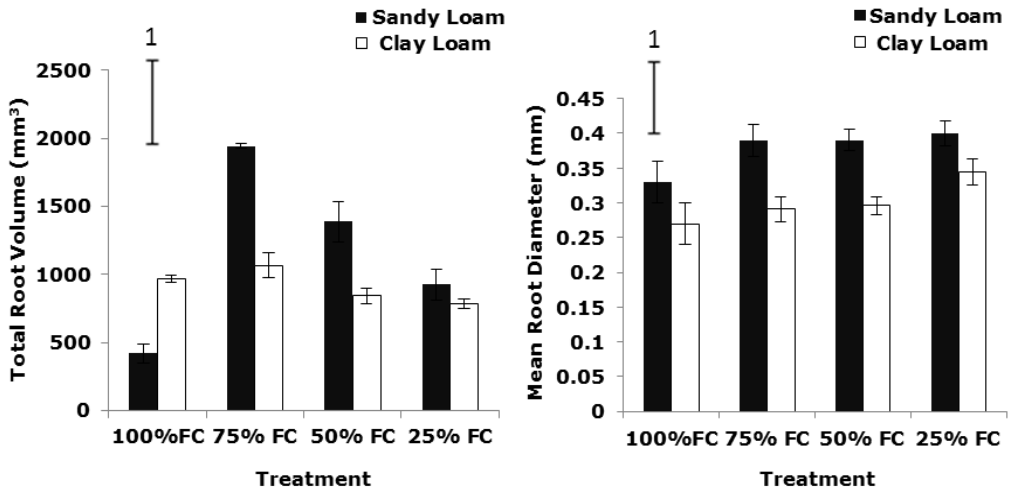
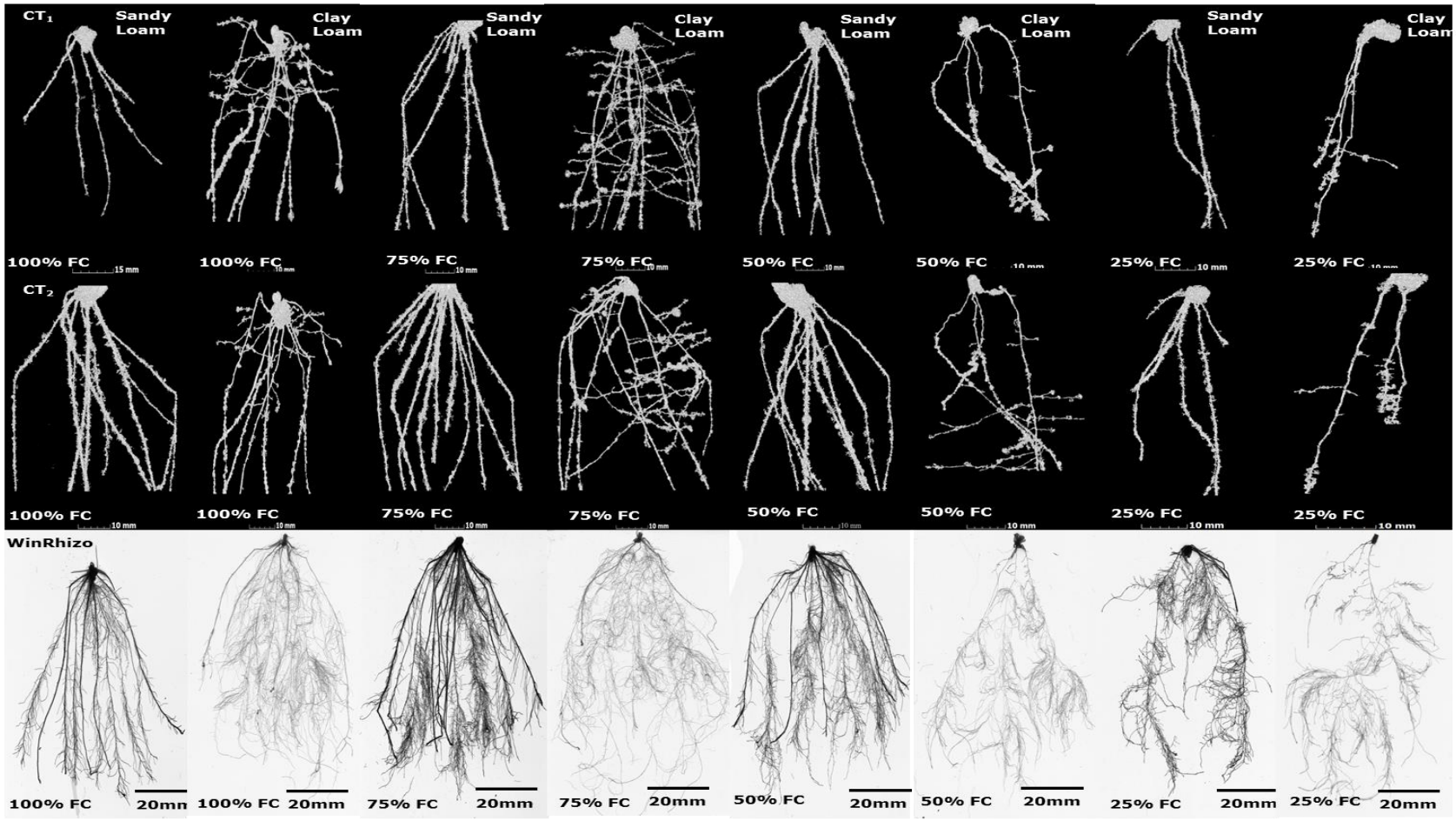


Figure 3.2: The effect of water stress on total volume and mean diameter of root by WinRHIZO in wheat variety Zebedee grown in the sandy loam and the clay loam soils types 21 days after germination. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on total root volume and mean root diameter at $P< 0.05$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

3.3.1.3 Comparison of root imaging methods

An example of images of root architecture for both methods (X-ray CT and WinRHIZO) is shown in Figure 3.3. In general the advantage of the μCT method is that the 3-D root system architecture (RSA) is revealed in addition to soil pore characteristics however the disadvantage is that considerably less of the lateral roots in comparison to the WinRHIZO images were revealed using the resolution chosen for this study. Whilst it is possible to undertake imaging at a higher resolution this usually results in the compromise of examining a smaller sample volume. In contrast WinRHIZO provided more information on the fine roots although some of these are lost in washing, in addition no geometrical information concerning how the roots and soil interact is given. When the two root imaging methods were compared, typical R^2 values were 0.7 for

total root volume in sandy loam soil while typical R^2 values of 0.3 for total root volume in clay and mean root diameter in both soil types (Figure 3.4). X-ray CT was undertaken on undisturbed plant and soil samples while WinRHIZO required roots to be washed from soil first (chapter two). Furthermore, WinRHIZO® takes less time to scan root because is rapid, therefore several samples can be analysed during short time. X-ray CT provides repeated, non-destructive three-dimensional (3-D) images of RSA, although with a reduced sample throughput. The higher values of important root characteristics (total root volume and mean root diameter) by WinRHIZO® were attributed to the resolution issue associated with X-ray CT scanning mentioned earlier.



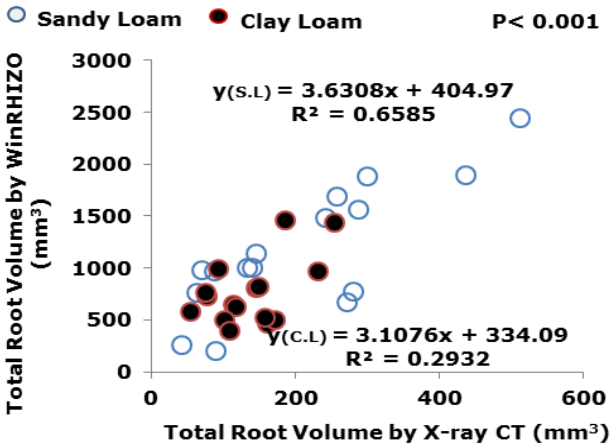
CT$_1$

CT$_2$

WinRHIZO

Figure 3.3: CT images of the roots of Zebedee 14 (CT$_1$) and 21 (CT$_2$) days after germination grown under water deficit (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soils and root systems (at 21 days) of the same plants after extraction and analysis using WinRHIZO® equipment compared to CT$_2$ images. Scale bars represent 10mm and 20mm.

A



B

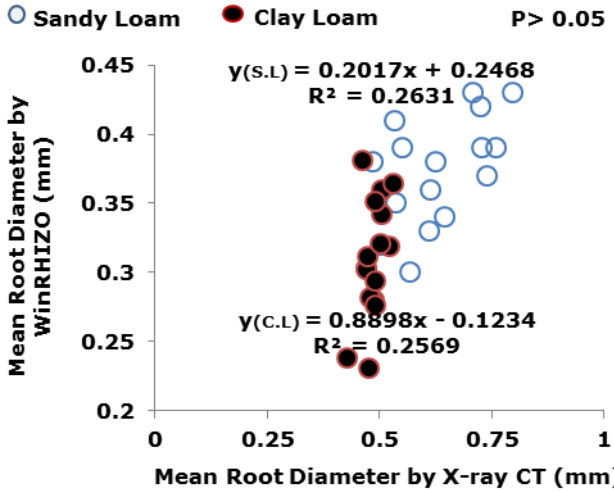


Figure 3.4: The scatter diagram between total root volume (A) and mean root diameter (B) estimated by X-ray CT and WinRHIZO for both soils types (the sandy loam (S.L) and the clay loam (C.L)). P-values refer to linear regression analysis for these two measurements between two different methods (X-ray CT and WinRHIZO).

3.3.2 Photosynthesis, stomatal conductance and transpiration

3.3.2.1 Photosynthesis

To determine the effect of water stress on photosynthesis parameters such as photosynthesis, stomatal conductance and transpiration and the interaction between photosynthesis and root of Zebedee 14 and 21 days post germination, infrared gas analysis (Licor) was used for measuring these parameters. The data revealed

that photosynthesis (light saturated rate) 14 days post germination in the clay loam was significantly greater than in the sandy loam (P_{soil}< 0.05, $P_{soil*water}$> 0.05) (Figure 3.5A). Photosynthesis decreased with decreasing soil moisture content in the sandy loam and clay loam (P_{water}> 0.05).

At 21 days post germination, photosynthesis also reduced dramatically with reducing soil moisture content in the sandy loam and clay loam (P_{water}< 0.001, P_{soil}> 0.05, $P_{soil*water}$> 0.05) (Figure 3.5B).

3.3.2.2 Stomatal conductance

Stomatal conductance 14 days after germination also reduced significantly with decreasing soil moisture content in both soil types (P_{water}< 0.001). Stomatal conductance in the clay loam was significantly greater than in the sandy loam (P_{soil}< 0.001, $P_{soil*water}$> 0.05) (Figure 3.5A).

The effect of the water stress 21 days after germination on stomatal conductance was similar to that at 14 day post germination. Stomatal conductance in both soil types reduced dramatically from 100% FC to 25% FC (P_{water}< 0.001). Stomatal conductance in the clay loam was significantly greater than in the sandy loam (P_{soil}< 0.01, $P_{soil*water}$> 0.05) (Figure 3.5B).

3.3.2.3 Transpiration

The results showed that transpiration 14 and 21 days post germination decreased significantly with decreasing soil moisture

content in both the sandy loam and clay loam (P_{water}< 0.001) (Figure 3.5). Transpiration 14 days in the clay loam was significantly greater than in the sandy loam (P_{soil}< 0.001, $P_{soil*water}$< 0.05) (Figure 3.5A). Whereas, at 21 days, no significant difference between transpiration in both soil types was found, however, in the clay loam it was greater than in the sandy loam (P_{soil}> 0.05, $P_{soil*water}$> 0.05) (Figure 3.5B).

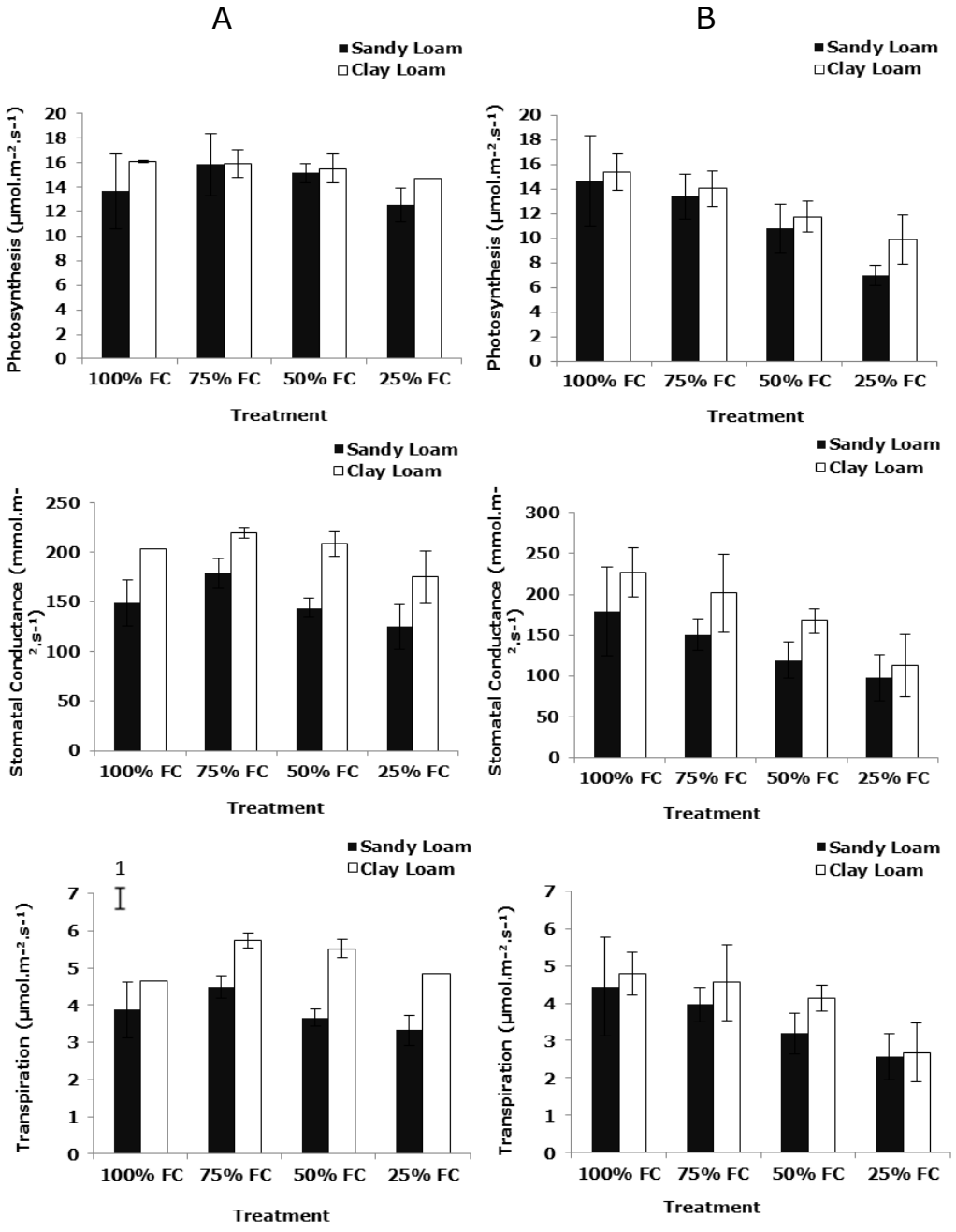


Figure 3.5: The effect of water stress on photosynthesis (A), stomatal conductance (Gs) and transpiration (T) of wheat variety Zebedee 14 (column A) and 21 (column B) days post germination and after scanning in the sandy loam and the clay loam soils types. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on photosynthesis, stomatal conductance and transpiration at $P< 0.05$ at 14 days in (column A). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

3.3.2.4 Intrinsic water use efficiency (photosynthesis/ stomatal conductance)

To investigate the impact of different levels of water stress on water use efficiency (photosynthesis/ stomatal conductance) of wheat plant 14 and 21 days after germination, intrinsic water use efficiency (inWUE) was calculated. The data found that inWUE 14 days after germination in the sandy loam was significantly greater than in the clay loam (P_{soil}< 0.001, $P_{soil*water}$> 0.05). inWUE slightly decreased with decreasing soil moisture content in the sandy loam 100% FC to 75% FC then it increased to 50% FC then decreased to 25% FC. Similarly, it decreased with decreasing soil moisture content in the clay loam from 100% FC to 75% FC then it increased to 25% FC (P_{water}< 0.05) (Figure 3.6A).

inWUE 21 days after germination in the sandy loam was significantly greater than in the clay loam, except at 25% FC was lower (P_{soil}< 0.05, $P_{soil*water}$< 0.05) (Figure 3.6B).

3.3.2.5 Instantaneous water use efficiency (photosynthesis/ transpiration)

To study the effect of water stress on water use efficiency (photosynthesis/transpiration) of wheat 14 and 21 days post germination, instantaneous water use efficiency (iWUE) was calculated. The results showed that iWUE 14 days post germination increased significantly with decreasing soil moisture content in the sandy loam from 100% FC to 50% FC then it decreased to 25% FC. In the clay loam, it reduced significantly with reducing soil moisture content from 100% FC to 75% FC then it increased to 25% FC

(P_{water}= 0.001). iWUE in the sandy loam was significantly greater than in the clay loam (P_{soil}< 0.001, $P_{soil*water}$< 0.001) (Figure 3.6A).

The soil*water interaction was significant for iWUE 21 days post germination ($P_{soil*water}$< 0.01). There was no significant difference between both soil types and water stress levels (P_{water}> 0.05, P_{soil}> 0.05) (Figure 3.6B).

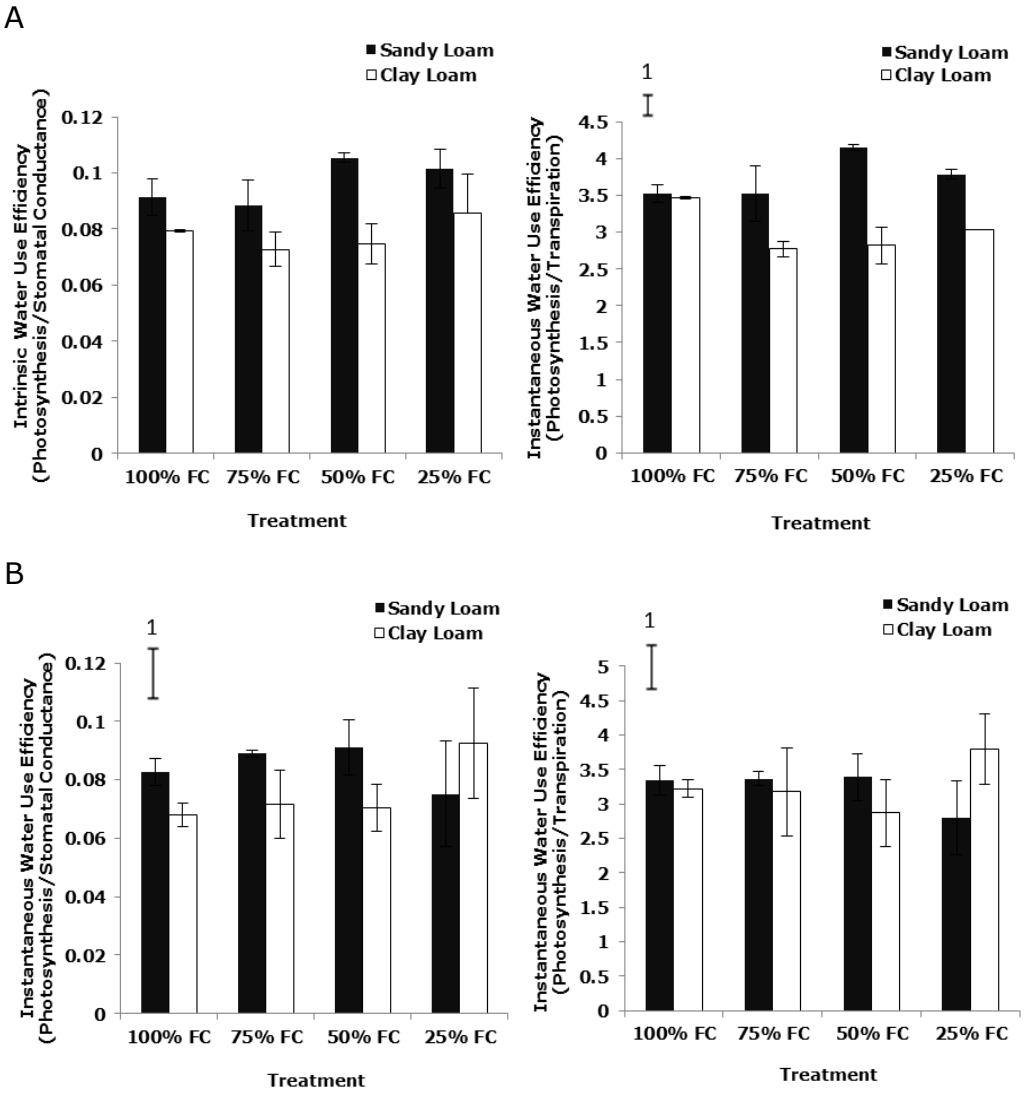


Figure 3.6: inWUE and iWUE of wheat variety Zebedee 14 (A) and 21 (B) days post germination and after scanning grown under different moisture content (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soils types. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on inWUE at P< 0.05 in (B) and iWUE at P< 0.001 in (A) and at P< 0.01 in (B). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

3.3.3. Combined shoot and root characteristics

3.3.3.1 Shoot dry weight

To study the influence of water stress on shoot growth 21 days post germination, shoot dry weight was measured. The data found that shoot dry weight 21 days post germination decreased significantly

with decreasing soil moisture content in the sandy loam, while it decreased slightly in the clay loam (P_{water}< 0.05). Shoot dry weight in the sandy loam was significantly greater than in the clay loam (P_{soil}< 0.001, $P_{soil*water}$< 0.05) (Figure 3.8B). Examples of images of leaves of Zebedee wheat variety grew under different moisture content (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soils 21 days after germination are shown in Figure 3.7.

3.3.3.2 Root dry weight

To investigate the impact of water stress on root growth 21 days post germination, root dry weight was measured. The results revealed that root dry weight decreased greatly with decreasing soil moisture content in the sandy loam, while it decreased slightly in the clay loam from 75% FC as to 25% FC (P_{water}> 0.05, P_{soil}> 0.05, $P_{soil*water}$> 0.05) (Figure 3.8B).

3.3.3.3 Total dry weight

To examine the effect of water stress on plant growth 21 days after germination, total dry weight was calculated. The results showed that total dry weight decreased significantly with decreasing soil moisture content in the sandy loam, while it increased slightly in the clay loam from 100% FC to 50% FC then it decreased to 25% FC (P_{water}< 0.05). Total dry weight in the sandy loam was significantly greater than that of the clay loam (P_{soil}< 0.001, $P_{soil*water}$> 0.05) (Figure 3.8B).

3.3.3.4 Shoot length

To estimate the impact of water stress shoot growth at 14 and 21 days after germination, the length of shoots was measured. The results showed that shoot length 14 days after germination in both soil types declined significantly with decreasing soil moisture content (P_{water}< 0.001). Shoot length in the sandy loam was significantly greater than in the clay loam (P_{soil}< 0.001, $P_{soil*water}$> 0.05) (Figure 3.8A).

Shoot length 21 days post germination in the sandy loam was significantly greater than in the clay loam (P_{soil}< 0.001, $P_{soil*water}$< 0.001). Shoot length also decreased significantly with decreasing soil moisture content in both soil types (P_{water}< 0.001) (Figure 3.8B).

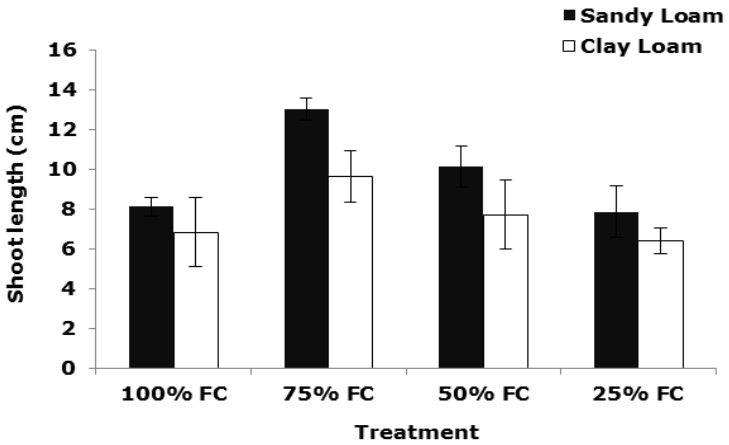
3.3.3.5 Root to shoot ratio

To study the responses of root and shoot growth to water stress, root to shoot ratio (dry weight) was calculated. The data found that root to shoot ratio 21 days post germination in the clay loam was significantly greater than in the sandy loam (P_{soil}< 0.001, P_{water}> 0.05, $P_{soil*water}$> 0.05) (Figure 3.8B).



Figure 3.7: Leaves of wheat under different moisture content (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soils 21 days after germination. Scale bar represents 20mm.

A



B

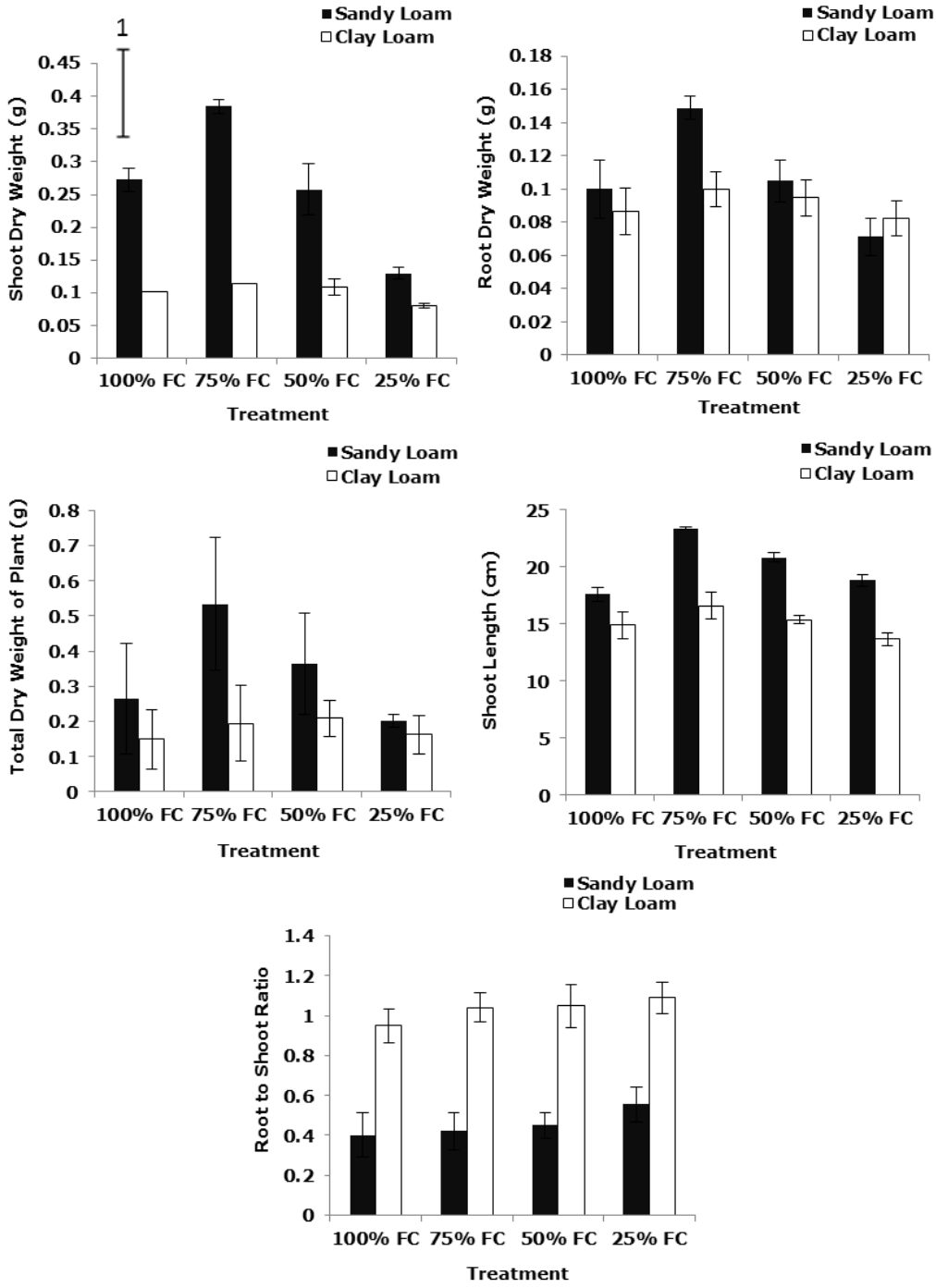


Figure 3.8: The effect water stress on shoot, root and total dry weight, shoot length and root to shoot ratio (dry weight) of wheat variety Zebedee 14 (shoot length in A) and 21 (B) days post germination grown under different moisture content (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soil. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on shoot, root and total dry weight, shoot length and root to shoot ratio (dry weight) at $P < 0.05$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

3.3.4 Water use efficiency

3.3.4.1 Water use efficiency (based on shoot and root dry weight)

Water use efficiency increased significantly with decreasing soil moisture content in both soil types ($P_{water} < 0.001$, $P_{soil*water} > 0.05$). Water use efficiency in the sandy loam was significantly greater than in the clay loam ($P_{soil} < 0.001$, $P_{soil} < 0.01$ for water use efficiency (based on shoot and root dry weight respectively) (Figure 3.9).

3.3.4.2 Water use efficiency (based on total dry weight)

Water use efficiency based on total dry weight at 21 days post germination increased significantly with decreasing soil moisture content in both soil types. It increased in the sandy loam from 100% FC to 75% FC then it slightly reduced to 50% FC then it increased to 25% FC. Similarly, in the clay loam it increased significantly from 100% FC to 25% FC ($P_{water} < 0.001$). Water use efficiency in the sandy loam was significantly greater than in the clay loam ($P_{soil} < 0.001$, $P_{soil*water} > 0.05$) (Figure 3.9).

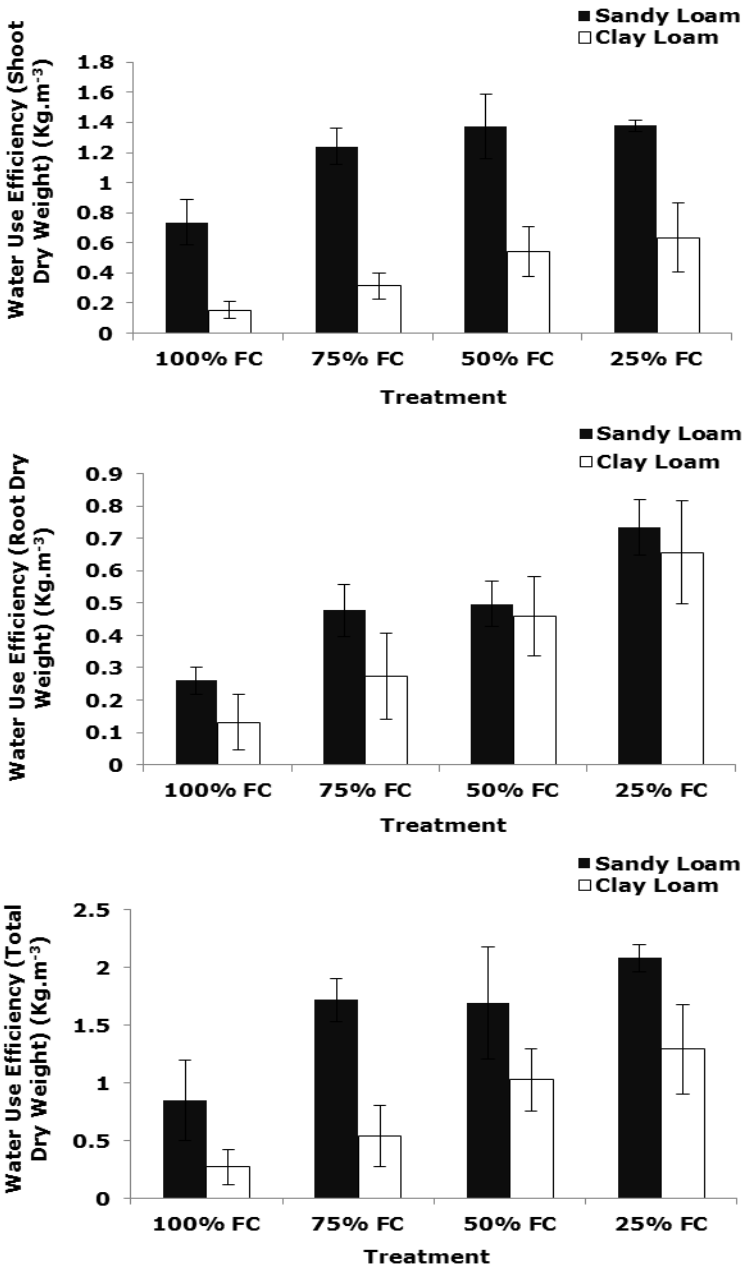


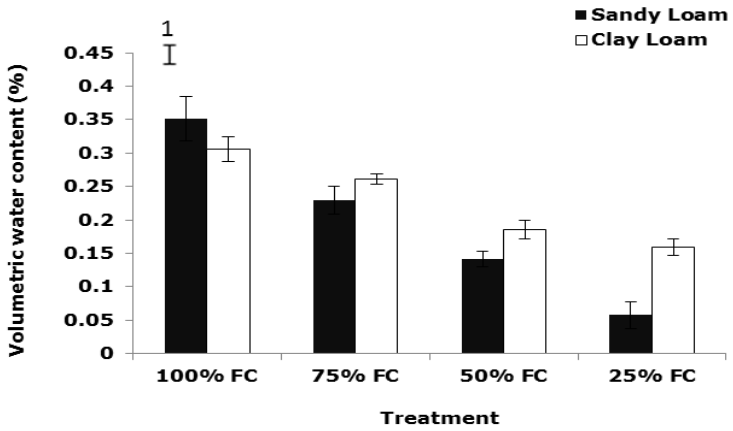
Figure 3.9: Water use efficiency (based on shoot, root and total dry weight) of wheat variety Zebedee grown under different moisture content (100%, 75%, 50% and 25% FC) 21 days after germination in the sandy loam and the clay loam soils. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on water use efficiency.

3.3.5 Soil pore characteristics

3.3.5.1 Volumetric water content

Volumetric water content 14 days post germination was 0.35, 0.23, 0.14, and 0.06 $g.g^{-1}$ at 100, 75, 50 and 25% FC respectively in the sandy loam soil whereas, it was 0.31, 0.26, 0.19 and 0.16 $g.g^{-1}$ at 100, 75, 50 and 25% FC respectively in the clay loam soil (P_{water}< 0.001) (Figure 3.10A). Volumetric water content 21 days post germination was 0.34, 0.22, 0.14, and 0.06 $g.g^{-1}$ at 100, 75, 50 and 25% FC respectively in the sandy loam soil whereas, it was 0.30, 0.25, 0.17 and 0.13 $g.g^{-1}$ at 100, 75, 50 and 25% FC respectively in the clay loam soil (P_{water}< 0.001) (Figure 3.10B). In general, the volumetric water content in the clay loam was significantly greater than in the sandy loam soil at all levels of water stress in both soil types, except at 100% FC when it was lower at 14 days (P_{soil}< 0.001) and at 21 days (P_{soil}< 0.01) post germination. The soil*water interaction was significant for volumetric water content ($P_{soil*water}$< 0.001) (Figure 3.10).

A



B

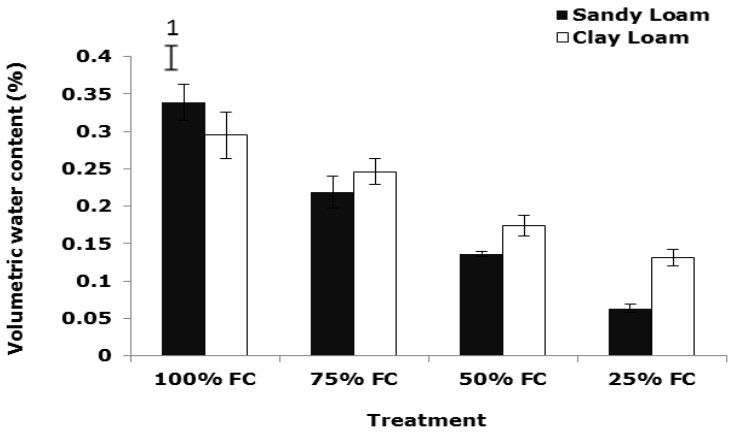


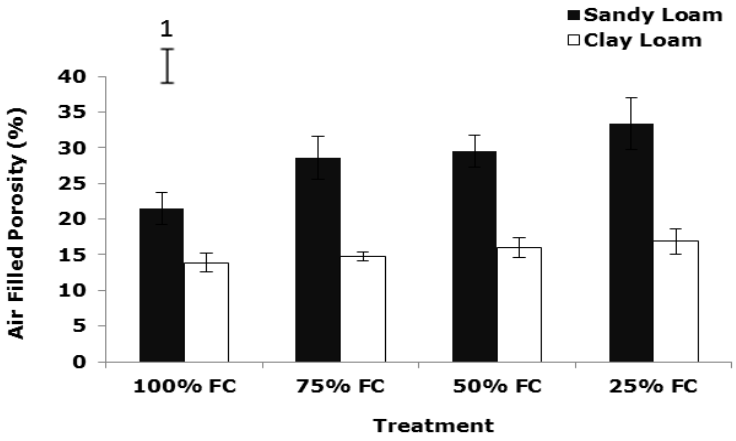
Figure 3.10: Volumetric water content 14 (A) and 21 (B) days post germination in the sandy loam and the clay loam soils. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on volumetric water content at both dates. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant at P< 0.001.

3.3.5.2 Air filled porosity

The X-ray CT images used to examine the root systems architecture could also be used to reveal the soil air filled pore space (for the given spatial resolution). The results found that air filled porosity 14 and 21 days post germination increased significantly with decreasing soil moisture content in both soil types (P_{water}< 0.001) (Figure 3.11). Air filled porosity in the sandy loam was significantly greater than in

the clay loam soil (P_{soil}< 0.001, $P_{soil*water}$< 0.05) at 14 and 21 days probably due to the pre-packing sieving used in this study (P_{soil}< 0.001, $P_{soil*water}$> 0.05) (Figure 3.11A & 3.11B).

A



B

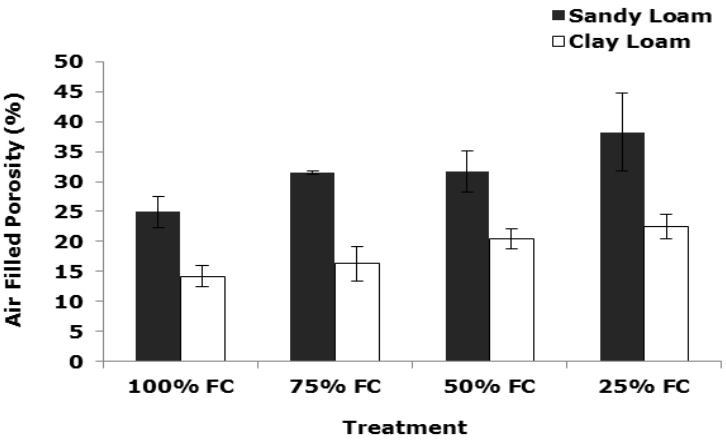


Figure 3.11: Air filled porosity of wheat variety Zebedee 14 (A) and 21 (B) days post germination in the sandy loam and the clay loam soils. Bars indicate means ± S.D (n=4). General analysis of variance (ANOVA) showed soil x water interaction on air filled porosity at P< 0.05 at 14 days (A). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

3.3.6 Effect of X-ray on plant measurements

It was important to investigate the potential effect of X-ray exposure on important root characteristics such as total root volume and mean root diameter of wheat plant, therefore WinRHIZO was used to analyse and compare the scanned and non-scanned data. The results found that no significant nor noticeable effect of X-ray exposure on wheat plant during the scanning process ($P > 0.05$). Scanned root systems had an average total root volume of 991 cm^3 compared to 985 cm^3 for non-scanned plants ($P > 0.05$). Root diameter was on average 0.36 mm for both scanned and non-scanned plants respectively ($P > 0.05$) as shown in Figure 3.12.

To explore the effect of X-ray exposure on photosynthesis parameters (photosynthesis, stomatal conductance and transpiration) of wheat plant during scanning process, measurements were taken before and after scanning by Licor. The results found that there was also no significant nor observable effect of X-ray exposure on photosynthesis parameters during the scanning time ($P > 0.05$). Photosynthesis was 11.95 and 12.12 $\mu mol.m^{-2}.s^{-1}$ before and after scanning, respectively of plants 21 days after germination ($P > 0.05$). Stomatal conductance averaged 157.2 and 162.1 $mmol.m^{-2}.s^{-1}$ before and after scanning plants, respectively ($P > 0.05$). Transpiration of plants before and after scanning also averaged 3.7 and 3.8 $\mu mol.m^{-2}.s^{-1}$, respectively as depicted in Figure 3.13.

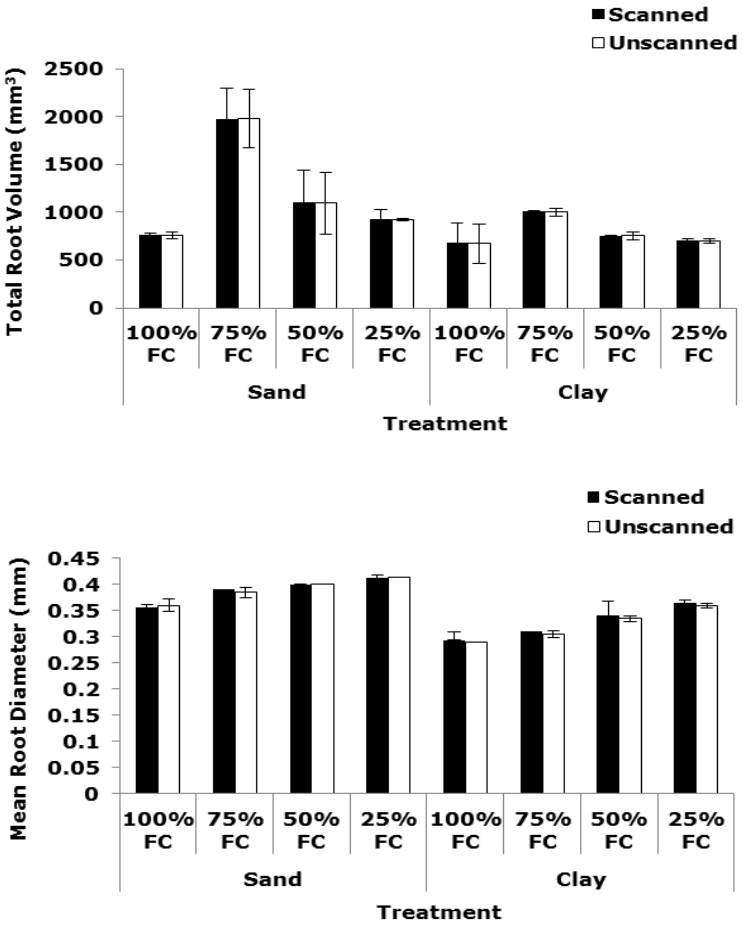


Figure 3.12: Impact of X-ray CT on root growth in wheat variety Zebedee. Zebedee at 21 days was grown under four levels of water stress (100%, 75%, 50% and 25% FC) in sandy loam and clay loam soil types. Bars indicate means ± S.D (n=4).

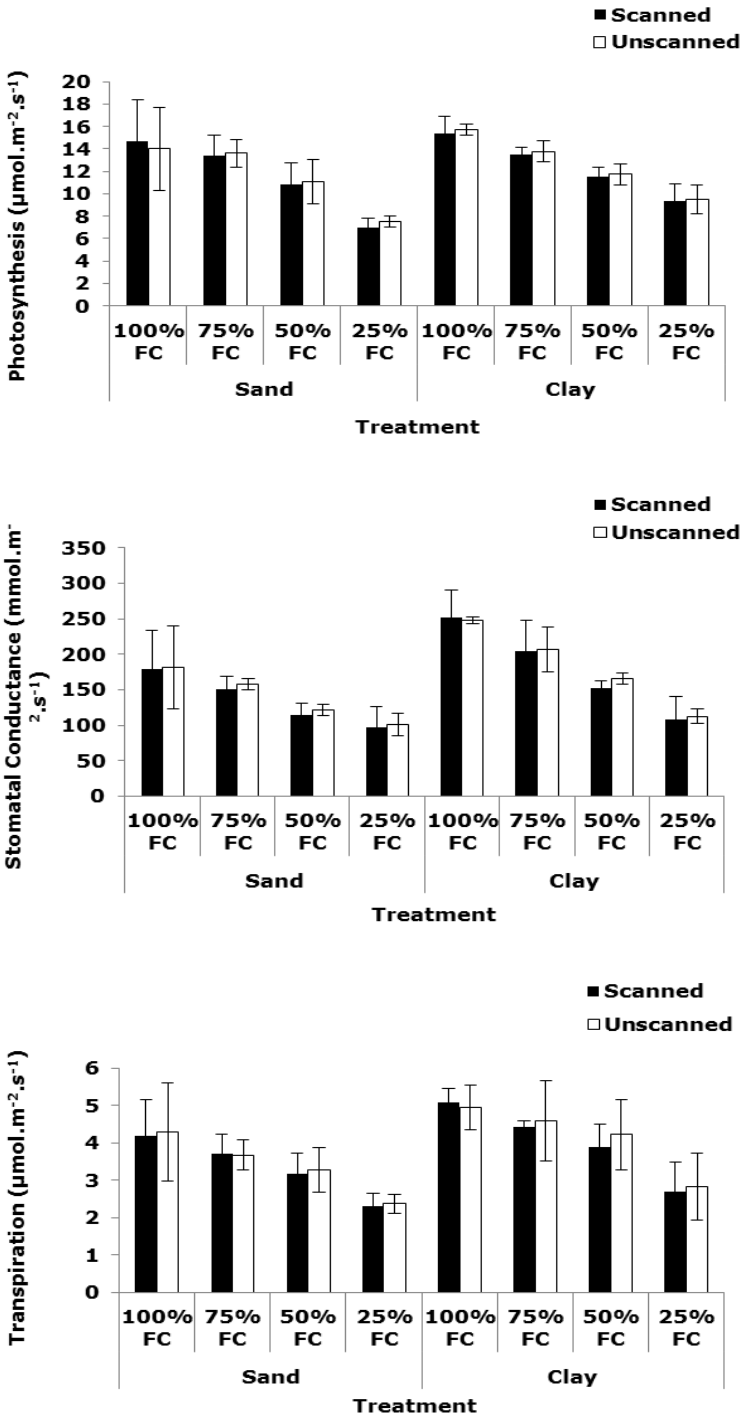


Figure 3.13: Impact of X-ray CT on wheat variety (Zebedee) shoot growth. Zebedee at 21 days was grown under four levels of water stress (100%, 75%, 50% and 25% FC) in sandy loam and clay loam soil types. Bars indicate means ±S.D (n=4).

3.3.7 Correlation matrix analysis

To identify potential relationships between measurements of shoot and root a correlation analysis was used. The results found that total dry weight, shoot dry weight, root dry weight and WinRHIZO total root volume were correlated at 21 days after germination of Zebedee under different soil water contents (100%, 75%, 50% and 25% FC) in the sandy loam and the clay loam soil types under high temperature 28°C. Photosynthesis at 21 days after germination also had a positive correlation with stomatal conductance, transpiration and volumetric water content. There was also positive correlation between X-ray CT mean root diameter at 21 days after germination and water use efficiency (based on shoot dry weight). X-ray CT mean root diameter at 14 days after germination positively correlated with air filled porosity at 14 days (Table 3. 2).

Table 3.2: Correlation Matrix between all variables (see list of abbreviations) of wheat variety Zebedee grown at different water content (100%, 75%, 50% and 25%FC) in the sandy loam and the clay loam soil types at P< 0.05.

Plantdwt	1	-																											
Psynth_14	2	0.33	-																										
Psynth_21	3	0.09	0.44	-																									
RSRatio	4	-0.55	0.19	0.08	-																								
Root_dwt	5	0.86	0.41	0.06	-0.13	-																							
STomC_14	6	-0.09	0.68	0.52	0.61	0.17	-																						
STomC_21	7	-0.12	0.35	0.90	0.31	-0.05	0.59	-																					
Shoot_dwt	8	0.98	0.28	0.10	-0.66	0.76	-0.18	-0.13	-																				
Transp_14	9	-0.10	0.65	0.39	0.67	0.21	0.92	0.45	-0.20	-																			
Transp_21	10	0.03	0.31	0.90	0.14	0.04	0.54	0.96	0.02	0.40	-																		
VWC_14	11	-0.04	0.25	0.83	0.05	-0.06	0.40	0.72	-0.03	0.28	0.70	-																	
VWC_21	12	-0.04	0.19	0.80	0.00	-0.08	0.37	0.70	-0.03	0.22	0.70	0.98	-																
WRDiam	13	0.51	-0.19	-0.40	-0.55	0.29	-0.55	-0.52	0.55	-0.50	-0.44	-0.46	-0.45	-															
WRRootvol	14	0.83	0.19	-0.03	-0.37	0.79	-0.07	-0.18	0.80	-0.12	-0.12	-0.11	-0.10	0.57	-														
WUE_R	15	0.24	-0.26	-0.71	-0.13	0.30	-0.48	-0.72	0.21	-0.28	-0.68	-0.80	-0.83	0.52	0.27	-													
WUE_SH	16	0.57	-0.22	-0.45	-0.70	0.31	-0.70	-0.59	0.62	-0.64	-0.45	-0.58	-0.56	0.72	0.46	0.68	-												
WUE_T	17	0.56	-0.23	-0.58	-0.54	0.40	-0.63	-0.67	0.58	-0.51	-0.57	-0.72	-0.72	0.73	0.52	0.86	0.91	-											
X_rayAvgDiam_14	18	0.58	-0.32	-0.31	-0.85	0.26	-0.73	-0.47	0.66	-0.77	-0.32	-0.30	-0.24	0.69	0.50	0.39	0.85	0.73	-										
X_rayAvgDiam_21	19	0.72	0.01	0.00	-0.70	0.41	-0.33	-0.24	0.78	-0.36	-0.09	-0.05	-0.03	0.68	0.71	0.20	0.65	0.56	0.74	-									
X_ray_RootVol_14	20	0.35	0.38	0.49	0.17	0.54	0.52	0.42	0.28	0.47	0.45	0.42	0.42	-0.14	0.46	-0.28	-0.26	-0.25	-0.13	0.15	-								
X_ray_RootVol_21	21	0.79	0.35	0.47	-0.44	0.63	0.15	0.27	0.80	-0.01	0.38	0.35	0.36	0.35	0.77	-0.21	0.19	0.14	0.37	0.68	0.57	-							
iwuepc_14	22	0.35	-0.17	-0.42	-0.64	0.09	-0.83	-0.54	0.42	-0.73	-0.51	-0.43	-0.42	0.56	0.22	0.49	0.79	0.69	0.72	0.36	-0.41	-0.01	-						
iwuepc_21	23	0.27	0.01	-0.28	-0.31	0.14	-0.31	-0.64	0.30	-0.24	-0.60	-0.15	-0.17	0.35	0.27	0.32	0.37	0.36	0.33	0.37	-0.03	0.11	0.35	-					
iwuept_14	24	0.39	-0.07	-0.22	-0.71	0.07	-0.67	-0.35	0.48	-0.79	-0.30	-0.25	-0.21	0.49	0.30	0.18	0.69	0.50	0.76	0.44	-0.33	0.23	0.85	0.29	-				
iwuept_21	25	0.05	0.23	0.02	0.02	0.04	-0.02	-0.30	0.06	0.05	-0.40	0.15	0.09	0.05	0.13	0.04	-0.05	-0.03	-0.07	0.08	0.08	0.05	0.12	0.83	0.07	-			
porosity_sp_14	26	0.48	-0.34	-0.47	-0.68	0.21	-0.72	-0.53	0.54	-0.71	-0.39	-0.54	-0.50	0.60	0.41	0.55	0.91	0.82	0.87	0.58	-0.32	0.17	0.73	0.20	0.70	-0.21	-		
porosity_sp_21	27	0.37	-0.30	-0.52	-0.62	0.10	-0.67	-0.62	0.45	-0.64	-0.52	-0.57	-0.55	0.67	0.31	0.60	0.88	0.80	0.80	0.61	-0.41	0.07	0.66	0.32	0.62	-0.05	0.87	-	
reg_p_ht_14	28	0.74	0.21	0.14	-0.37	0.65	0.01	-0.02	0.72	-0.02	0.11	0.01	0.04	0.33	0.74	0.07	0.46	0.35	0.46	0.71	0.47	0.65	0.13	0.16	0.21	-0.03	0.43	0.31	-
reg_p_ht_21	29	0.71	-0.05	-0.03	-0.74	0.44	-0.38	-0.21	0.77	-0.45	-0.04	-0.15	-0.10	0.55	0.64	0.18	0.75	0.57	0.78	0.76	0.14	0.58	0.46	0.24	0.57	-0.12	0.75	0.64	0.80	-
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Positive significant correlation between variables	Critical significant value	0.349
Negative significant correlation between variables	Number of observations:	32
No significant correlation between variables		

Key relationship

Table 3.3: P values by general analysis of variance (ANOVA) for all variables of wheat variety Zebedee grown at different water content (100%, 75%, 50% and 25%FC) in the sandy loam and the clay loam soil types 14 (A) and 21 (B) days after germination.

A

Measurements/Treatment	Soil	Water	Soil*Water
Total root volume (X-ray)	P> 0.05	P< 0.01	P> 0.05
Mean root diameter (X-ray)	P< 0.001	P> 0.05	P< 0.05
Photosynthesis	P< 0.05	P> 0.05	P> 0.05
Stomatal conductance	P< 0.001	P< 0.001	P> 0.05
Transpiration	P< 0.001	P< 0.001	P< 0.05
inWUE	P< 0.001	P< 0.05	P> 0.05
iWUE	P< 0.001	P= 0.001	P< 0.001
Shoot Length	P< 0.001	P< 0.001	P> 0.05
Air filled porosity	P< 0.001	P< 0.001	P< 0.05
Volumetric water content	P< 0.001	P< 0.001	P< 0.001

B

Measurements/Treatment	Soil	Water	Soil*Water
Total root volume (X-ray)	P< 0.01	P< 0.001	P< 0.01
Mean root diameter (X-ray)	P< 0.001	P< 0.05	P< 0.05
Total root volume (WinRHIZO)	P< 0.01	P< 0.05	P< 0.05
Mean root diameter (WinRHIZO)	P< 0.001	P< 0.05	P< 0.05
Photosynthesis	P> 0.05	P< 0.001	P> 0.05
Stomatal conductance	P< 0.01	P< 0.001	P> 0.05
Transpiration	P> 0.05	P< 0.001	P> 0.05
inWUE	P< 0.05	P> 0.05	P< 0.05
iWUE	P> 0.05	P> 0.05	P< 0.01
WUE (Shoot Dry Weight/ Total Water applied (T.W.app.))	P< 0.001	P< 0.001	P> 0.05
WUE (Root D.Wt/ T.W.app.)	P< 0.01	P< 0.001	P> 0.05
WUE (Total D.Wt/ T.W.app.)	P< 0.001	P< 0.001	P> 0.05
Shoot dry weight	P< 0.001	P< 0.05	P< 0.05
Root dry weight	P> 0.05	P> 0.05	P> 0.05
Shoot Length	P< 0.001	P< 0.001	P<0.001
Total dry weight	P< 0.001	P< 0.05	P> 0.05
Root to shoot ratio	P< 0.001	P> 0.05	P> 0.05
Air filled porosity	P< 0.001	P< 0.001	P> 0.05
Volumetric water content	P< 0.01	P< 0.001	P< 0.001

	Significant p values
	Non-significant p values

3.4 Discussion

Drought is an important environmental factor limiting crop growth and yield. However, many previous studies have not comprehensively considered the effect of water stress on early growth stages of wheat. In addition previous work has not investigated the effect of water stress on both shoot and root growth in undisturbed soil. In this study, the data showed that severe water stress (at 25% FC) had a significant negative effect on total root volume in both soil types (the sandy loam and the clay loam) as was hypothesized. There was a significant correlation between shoot dry weight and X-ray total root volume (r=0.80) (Table 3.2). There was also significant soil*water interaction for total root volume at day 21. This is probably due to the different responses to water stress shown by roots growing in the different soil types. Soil composition is highly influential for root architecture, impacting the mechanical impedance (physical stress) on root elongation through the soil, as well as affecting the availabilities of water, oxygen, and nutrients (Gregory, 2006). It was expected that Zebedee would grow better in the clay than the sandy soil due to higher water and nutrient availability in the clay soil. However, the soil moisture content negatively affected the plant growth in the clay loam soil more than in the sandy loam which was the converse of what was hypothesized (The root and shoot growth under water deficiency would be better in the clay than in the sandy soil). This could be due to growing plants under drought conditions in an environment with high temperature which led to the formation of large and observable

deep cracks in the clay soil type (Whitmore and Whalley, 2009). These cracks most likely allow even more water to evaporate. Another influencing factor might be the increased soil hardness and strength (Whalley et al., 2006, Whalley et al., 2008) in the clay soil. As soil strength increases, root elongation rate decreases due to increasing resistance of the soil particles to displacement. Strong soil can be a serious problem in agriculture as it can restrict access of root system to water and nutrients (Clark et al., 2003) and decrease plants growth. It is also worth noting that the soils in the study were not field structured, but sieved and packed into columns. As a result, the clay soil sieved to <2mm contained a larger portion of macropores than might exist in the field which may have contributed to enhanced drainage than observed in the sand soil.

This study found that the number of main roots decreased significantly with decreasing soil moisture content as shown in Figure 3.3. Water deficiency has a negative effect on plant growth and development and sharply decreases plant productivity (Pan et al., 2002). These findings are in line with Gao et al. (2007), who concluded water deficit hinders total root and shoot function, decreases root-shoot interactions (such as water and nutrients transport), and as a result, plant growth is reduced. A more serious water deficit leads to a longer the duration of unstable growth of shoot and root. Rewatering enhances root-shoot interactions, increases dry matter accumulation rate, and prolongs duration of instable growth (Gao et al., 2007).

The results have also shown root to shoot ratio increased with worsening water deficit. This ratio increased with increasing water deficit though not significantly in the clay loam. Root to shoot ratio in the clay loam was significantly greater than in the sandy loam soil. This meant that dry climate genotypes (where sandy soils are more prevalent) might have a well-developed root system with the stronger drought resistance (Wu and Bao, 2011). Ali et al. (2009) also reported water stress increases the root to shoot ratio. Lengthy roots have been shown to be very important for crop yield under water stress conditions (Ali et al., 2009). The results in this study contrast with Khan et al. (2010) who found that shoot growth is more affected than root length by water stress. Root to shoot ratio generally increases during water stress, although the effect of root growth by water stress is minimal (Khan et al., 2010). This might be due to more suppression of shoot than root growth under water stress environment. The reason for increased root to shoot ratio could be due to limited supply of water and nutrients hence root growth occurs at the expense of shoot (Khan et al., 2010).

Mean root diameter of plants in the sandy loam soil was thicker than in the clay loam. This could be due to the bulk density of the soil. However, plants were grown under the same bulk density (1.24 $g.cm^{-3}$) in both soil types. The thinner root diameter in clay soil could be due to the bulk density which restricted root growth. Thicker root diameters in the sandy soils over clay soils under bulk density (1.2-1.3 $g.cm^{-3}$) has been reported before (Tracy et al.,

2013). Mean root diameter at 100% FC increased sharply from 100% to 75% FC while it was slightly increased from 75 to 25% FC which is more likely due to water stress.

Aggarwal et al. (2006) found greater root diameters in drier soil conditions as in this experiment. Muñoz-Romero et al. (2010) found the greatest diameters were observed during tillering and/or stem elongation. However, they concluded that root diameter decreases during dry conditions and from the stem elongation stage onward. Smaller root diameters in dry years can be due to the greater resistance to penetration (Muñoz-Romero et al., 2010). However, Clark et al. (2008) concluded that good root penetration was consistently associated with greater root diameter and there was evidence that genetic control of root diameter led to genetic control of root penetration ability. Clark et al. (2008) also suggested that this is related to the cell wall relaxation needed for roots to develop growth pressure. Further studies are needed to extend the duration of plant growth until the harvesting time to quantify root diameters in all growth stages.

Thick roots might have greater capability for water absorption from deeper soil layers (Yambao et al., 1992). Thick roots are also hypothesized to confer greater water deficiency tolerance because root branching is associated with root thickness (Fitter, 1991). Thick roots persevere for a longer period and produce more and larger roots branches, therefore increasing water absorption capacity (Ingram et al., 1994). Correlation analysis showed a significant

negative relationship between mean root diameter and root to shoot ratio (r= -0.85) (Table 3.2). This is probably due to the fact that mean root diameter in sandy soil which was significantly greater than clay as mentioned above while the root to shoot ratio was significantly greater in clay soil type.

Interestingly, though similar trends were apparent, WinRHIZO® analysis was unable to detect significant treatment effects on root volume, which questions the sensitivity of this well-established technique. This could be due to the algorithms used to determine values for these variables as WinRHIZO® assumes that roots are perfectly cylindrical (Arsenault et al., 1995) and, in the case of root volume, the software determines 3-D values from 2-D images (dissimilar to X-ray CT where the actual 3-D root volume in soil is measured). As some soil particles attached to the root surface could not be removed during root washing, pixels corresponding to these would have been included in the WinRHIZO® images during image analysis. This might have led to overestimation of root volume and root diameter, and may explain why WinRHIZO® values were much greater than equivalent volumes derived from X-ray CT scans (Tracy et al., 2012).

Field capacity in a soil column such as in this study, based on two days drainage after saturation (100% FC), almost certainly had a negative impact due to excessive soil moisture. This decreased total root volume and shoot length of wheat. The high water content (volumetric water content) at this stage leads plants to hypoxia as

oxygen is needed in the soil for roots to respire (Yavas et al., 2012). The higher shoot and root characteristics values were observed at 75% FC in comparison to other water stress levels (100%, 50% and 25%). Therefore, it suggested that the optimal water content (%FC) for shoot and root growth of Zebedee was 75% FC.

Photosynthesis, stomatal conductance and transpiration were significantly and negatively affected by water stress conditions, as expected. Photosynthesis 21 days after germination had a significant positive relationship with volumetric water content (r= 0.83) (Table 3.2). These measurements in the clay loam were significantly greater than in the sandy loam soil. However, most previous studies on the effect of water stress on wheat growth have been performed on a single soil type, and no previous study, as yet, has been done on different soil types. Therefore, the differences reported in this study with regards to photosynthesis parameters in different soil types is unknown but it was expected to be greater in clay than sand due to the higher nutrient and water availability in the clay soil. Higher values of photosynthesis, stomatal conductance and transpiration were also shown in the clay loam soil under water deficit conditions, which were greater than in the sandy soil. This indicated that more light per unit leaf area could be utilized for carbohydrate production by this variety of wheat under these conditions of water stress (Wu and Bao, 2011). Drought is a significant environmental restriction that reduces crop photosynthesis, output and then yield (Shao et al., 2005, Huang et

al., 2009, Zheng et al., 2010). Photosynthetic efficiency can be decreased by an increase (waterlogging) or decrease in water availability (drought) (Wang et al., 2008), however, this is commonly associated with an increase in iWUE before any severe retardation of photosynthesis occurs.

Although other non-stomata factors effects on photosynthesis exist, based on the degree of water stress (Yu et al., 2009, Santos et al., 2009), stomatal closure is generally seen as the first stage when slight or mild water deficiency occurs (Yu et al., 2009, Santos et al., 2009, Souza et al., 2010). It is also regarded as a principal physiological characteristic for crops to manage with stress conditions and is a significant trait in stomatal resistance. The results of this study are in agreement with Ahmed et al. (2012) who concluded that a reduction in soil moisture content affected the photosynthesis rate, stomatal conductance and then transpiration rate. The results are also in agreement with the findings of Li et al. (2003), who conclude that lowered stomatal conductance reduces transpiration. A severe stress would be expected to result in complete stomatal closure.

In this study, the data showed that water use efficiency for Zebedee increased significantly due to decreasing soil water content. This could be due to stomatal conductance decreased with increasing soil water stress lead to decrease transpiration. It has been concluded by Abbate et al. (2004) that under limited supply, water use efficiency of wheat was greater than in well-watered conditions.

They correlated the higher water-use efficiency with stomatal closure to decrease the transpiration. In addition, it has been reported in a study on clover (*Trifolium alexandrinum*) that water use efficiency was increased due to lowered water loss under drought stress, mainly by decreased transpiration rate (Lazaridou and Koutroubas, 2004). Further discussions on water use efficiency of different wheat varieties can be seen with in the next chapter, chapter four.

The optimal matric potential (-kPa) at volumetric water content ($g.g^{-1}$) for both roots and shoots growth was approximately -10 kPa (0.32 $g.g^{-1}$) in the sandy loam and -150 kPa (0.34 $g.g^{-1}$) the clay loam soil at 75% FC. However, the optimal matric potential for photosynthesis and stomatal conductance and transpiration was -5 kPa (0.42 $g.g^{-1}$) in the sandy loam and -30 kPa (0.45 $g.g^{-1}$) the clay loam soil at 100% FC (Table 3.1). This means that roots and shoots growth were more affected by soil moisture content than photosynthesis characteristics. This could be due to other physiological processes such as low rate of respiration which might have hindered the plant growth. However, Batool et al. (2015) reported in *Abelmoschus esculentus L. Moench* plant that a higher rate of photosynthesis was observed at mild water stress level (60% F.C), but the highest biomass was recorded at low water stress level (100% F.C). This was suggested to be due to the high respiration rate which hindered the biomass accumulation of plant. The differences between these two studies could be due to the responses

of the two different plants were used in these studies. In contrast, Akhkha et al. (2011) who used different varieties of wheat and found out water stress affected the photosynthesis rates of wheat cultivars, but not at the same level. Their study indicated that the moderate water stress level (50% FC) did not affect the drought-tolerant wheat cultivars, while the impact was clear on the photosynthesis efficiency in most drought sensitive cultivars. However, the varieties of the wheat used in these two studies were different and experimental conditions, as well. In their study, the plants were kept at 80% FC until 21 days after sowing (DAS) then the water stress treatments were initiated for two more weeks.

3.5 Conclusions

Total root volume decreased significantly due to water stress. However, mean root diameter increased significantly. Whereas, the plants function assessed by photosynthesis, stomatal conductance, and transpiration decreased significantly with decreasing soil water content. Photosynthesis measurements in the clay loam soil were significantly greater than in the sandy loam soil. The results suggest an optimal matric potential (based on an approximated measure for matric potential) for both roots and shoots growth was -10 kPa and -150 kPa at 75% FC in the sandy loam and the clay loam soil respectively. However, the optimal matric potential for photosynthesis and stomatal conductance and transpiration was -5 kPa and -30 kPa at 100% FC in the sandy loam and the clay loam soil respectively. Interestingly the matric values for sand soil are similar to the accepted values for field capacity whereas for clay soil they are much higher. The notional 100% FC in this experiment was most likely too wet for optimal growth conditions. However, the impact of water stress on the precise root system architecture is not well known, in this study the root architecture in sandy soil was much better than in clay which was surprising and converse to our hypothesis. Therefore, further experiments are required to explore this further including examining the response of drought resistant varieties to the same moisture stress conditions.

4.0 Assessing the responses of different varieties of wheat to water deficit

4.1 Introduction

To confirm the influence of water stress on the precise root system architecture and to further detect the response of different drought resistant varieties to the water stress conditions, three different contrasting but drought tolerant varieties of winter wheat (Hereward, Xi 19 and Istabraq) were used. These varieties have been chosen because Hereward has been the "gold standard" breadmaking wheat for over 20 years and shows high stability but with low yield and low transpiration, Xi19 is a breadmaking wheat with high yield, high transpiration and water use efficiency and Istabraq is a feed wheat with high yield, average transpiration and water use efficiency (John Foulkes, 2013 unpublished data). Ober et al. (2013) studied water use efficiency under drought conditions in UK winter wheat varieties in the field during crop development growth stages and reported that Istabraq was a drought tolerant genotype.

Water stress negatively affects plant growth and development and severely reduces crop yield (Pan et al., 2002). Severe water stress can also have a detrimental effect on the local economy, even if only for a short period (Khakwani et al., 2011).

An annual average increase of 0.3 °C in world temperature may change water availability and usage, hence, can affect cereal

production to a greater extent (McCarthy, 2001). Differences in water availability and instability in environmental temperature during plant growth can affect the whole growth and development through altering the main physiological processes such as photosynthesis, nutrient and water uptake (Ahmed et al., 2012).

Many studies have examined the effect of water stress on shoot and root growth and photosynthesis; however, no previous study has visualized and measured the 3D root systems of different wheat varieties under drought conditions in soil. This study aimed to examine the impact of water stress on roots and shoots growth of three different varieties of wheat (Hereward, Xi19 and Istabraq). It was hypothesized water shortage has a consistent negative effect on roots and shoots growth and development, and photosynthesis, stomatal conductance and transpiration. It was also hypothesized water deficiency has a significant influence on the growth of contrasting wheat cultivars.

4.2 Materials and methods

4.2.1 Sample preparation

To prepare samples the same procedure used as in the first experiment was used except for the following: Two soil textures were used (clay soil type was used instead of clay loam). We intended to collect the same soil type as in chapter three, however, when the soil was collected it was discovered it had different soil physical properties (e.g., soil texture). In addition, three different drought tolerant wheat varieties (Hereward, Xi 19 and Istabraq) were used. Three moisture treatments were also chosen to represent a range of saturated, field and dry conditions namely 75%, 50% and 25% of a notional field capacity (2 day drainage after saturation) in both soil types. Details of the exact volumetric water content, matric potentials and other relevant soil properties are given in Table 4.1. Three replicates were prepared for each soil type and treatment combination to give a total of 54 columns.

4.2.2 Plant Measurements

Photosynthesis, stomatal conductance and transpiration were measured one day after scanning by infrared gas analysis (LI-6400XT Portable Photosynthesis System, Licor, Illinois). After CT scanning and measuring photosynthesis, stomatal conductance and transpiration, the roots were washed from the soil and analysed using WinRHIZO®2002c scanning equipment and software as mentioned before in chapter 2 (2.4).

4.2.3 Statistical analysis

The results from this experiment were also analysed by ANOVA.

Table 4.1: The values of volumetric water content (VWC), matric potential (ψ)(-kPa) at different water content (100%, 75%, 50% and 25% FC), the percentage of the sand, silt, the clay, Organic Matter by Loss on Ignition, pH and nutrient content for the loamy sand and the clay soils in Newport and Worcester.

Soil Properties	Sandy Loam	Clay
VWC ($g.g^{-1}$) at 100%FC	0.39	0.50
VWC ($g.g^{-1}$) at 75%FC	0.29	0.37
VWC ($g.g^{-1}$) at 50%FC	0.2	0.25
VWC ($g.g^{-1}$) at 25%FC	0.1	0.13
Ψ (-kPa) at 100%FC	5	20
Ψ (-kPa) at 75%FC	20	100
Ψ (-kPa) at 50%FC	75	900
Ψ (-kPa) at 25%FC	1100	1500
% sand	76	10
% silt	10	29
% clay	14	61
Organic Matter by Loss on Ignition (%)	3.9	8.9
pH	6.9	6.5
Carbon (mg/kg soil)	17355	30862
Nitrogen (mg/kg soil)	1486	3176
Phosphorus (mg/kg soil)	778	927
Potassium (mg/kg soil)	10384	25233
Magnesium (mg/kg soil)	2352	12281
Sulphur (mg/kg soil)	2258	2857
Sodium (mg/kg soil)	1479	2431
Calcium (mg/kg soil)	2071	4437

4.3 Results

4.3.1 Root characteristics

4.3.1.1Total root volume

To examine the effect of water stress on important root characteristics such as total root volume and mean root diameter of Hereward, Xi19 and Istabraq wheat varieties 21 days after germination, X-ray CT and WinRHIZO were used for measuring these root characteristics. The analysed data revealed that total root volume for Hereward, Xi19 and Istabraq measured by X-ray CT decreased significantly with decreasing soil moisture content in the sandy loam and the clay soils 21 days post germination (P_{water}< 0.001) (Figure 4.1). There were significant interactions between all treatments, except the soil*water*wheat varieties interactions ($P_{soil*water}$< 0.001, $P_{soil*wheat\ varieties}$< 0.05, $P_{water*wheat\ varieties}$< 0.05, $P_{soil*water*wheat\ varieties}$> 0.05). There was no significant difference between all three wheat varieties in the sandy loam soil. Total root volume in the clay soil type for Hereward (36.1 mm^3) was significantly smaller than Istabraq (107.5 mm^3) and Xi 19 (84.4 mm^3) which was significantly smaller than Istabraq ($P_{wheat\ varieties}$< 0.01). The p-values for all variables at 21 days after germination of all wheat varieties are shown in Table 4.4.

Similar trends were found by WinRHIZO® analysis as total root volume decreased slightly with decreasing soil water content in the sandy loam although not significantly. Similarly, total root volume reduced significantly with reducing soil water content in the clay

(P_{water}= 0.001) (Figure 4.2). The soil*water interaction was significant while no significant interactions were found between the other interactions ($P_{soil*water}$< 0.01, $P_{soil*wheat\ varieties}$> 0.05, $P_{water*wheat\ varieties}$> 0.05, $P_{soil*water*wheat\ varieties}$> 0.05). In the sandy loam soil, total root volume for Hereward (3214 mm^3) was significantly less than Istabraq (3720 mm^3). In the clay soil, total root volume was significantly less for Hereward (1553 mm^3) and Xi 19 (1912 mm^3) than to Istabraq (3130 mm^3) ($P_{wheat\ varieties}$< 0.001). Generally, total root volume in the sandy loam was significantly greater than the clay soil at all levels of water stress, except at 75% FC for Istabraq (P_{soil}< 0.001) (Figure 4.1 and 4.2).

4.3.1.2 Mean root diameter

Mean root diameter for all varieties measured by X-ray CT increased with declining soil moisture content in the sandy loam and the clay soils, though not significantly 21 days post germination (P_{water}> 0.05) (Figure 4.1). There were no significant differences between all wheat varieties in both soil types ($P_{wheat\ vatieties}$> 0.05, P_{soil}> 0.05).

Similar trends were in the WinRHIZO® analysis as mean root diameter in the clay (0.56 mm) was significantly greater than the sandy loam (0.46 mm) at all levels of water stress (P_{soil}< 0.001) (Figure 4.2). Mean root diameter increased with decreasing soil water content in the sandy loam and the clay soil types though not significantly (P_{water}> 0.05) (Figure 4.2). There were no significant differences between all water treatment in both soil types, except 25% and 50% FC for Hereward and 25% and 75% FC for Istabraq

in the clay soil. There was no significant difference between all wheat varieties in both soil types ($P_{wheat\ varieties} > 0.05$), except Xi 19 (0.53 mm) in the clay soil decreased significantly in comparison to Istabraq (0.59 mm).

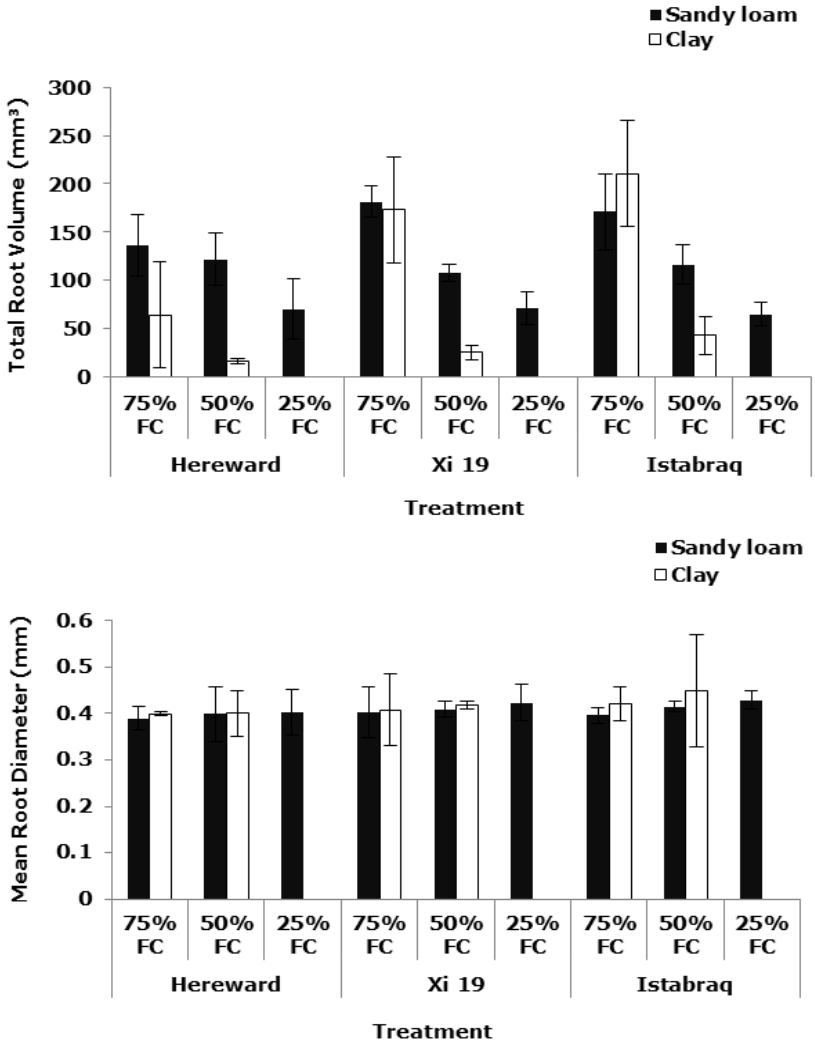


Figure 4.1: The effect of water stress on total volume and mean diameter of root by X-ray CT of three varieties of wheat (Hereward, Xi 19 and Istabraq) grown under water stress (75%, 50% and 25% field capacity) for the sandy loam and the clay soils after 21 days after germination. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water x wheat varieties interaction on total root volume and mean root diameter.

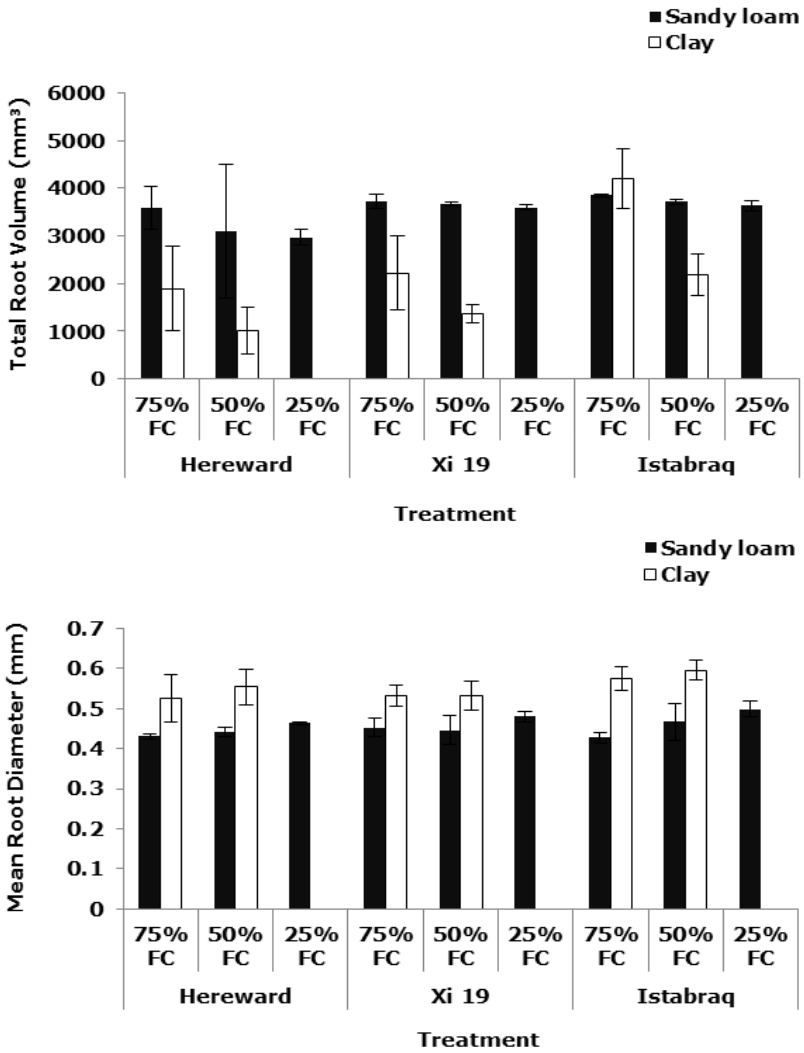


Figure 4.2: The effect of water stress on total volume and average diameter of root by WinRHIZO of three varieties of wheat (Hereward, Xi 19 and Istabraq) grown under water stress (75%, 50% and 25% field capacity) for the sandy loam and the clay soils after 21 days after germination. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water x wheat varieties interaction on total root volume and mean root diameter.

4.3.1.3 Comparison of root imaging methods

Examples of images of root architecture for both methods are shown in Figure 4.3. It can be seen that main roots decreased significantly in size due to water stress for all wheat varieties.

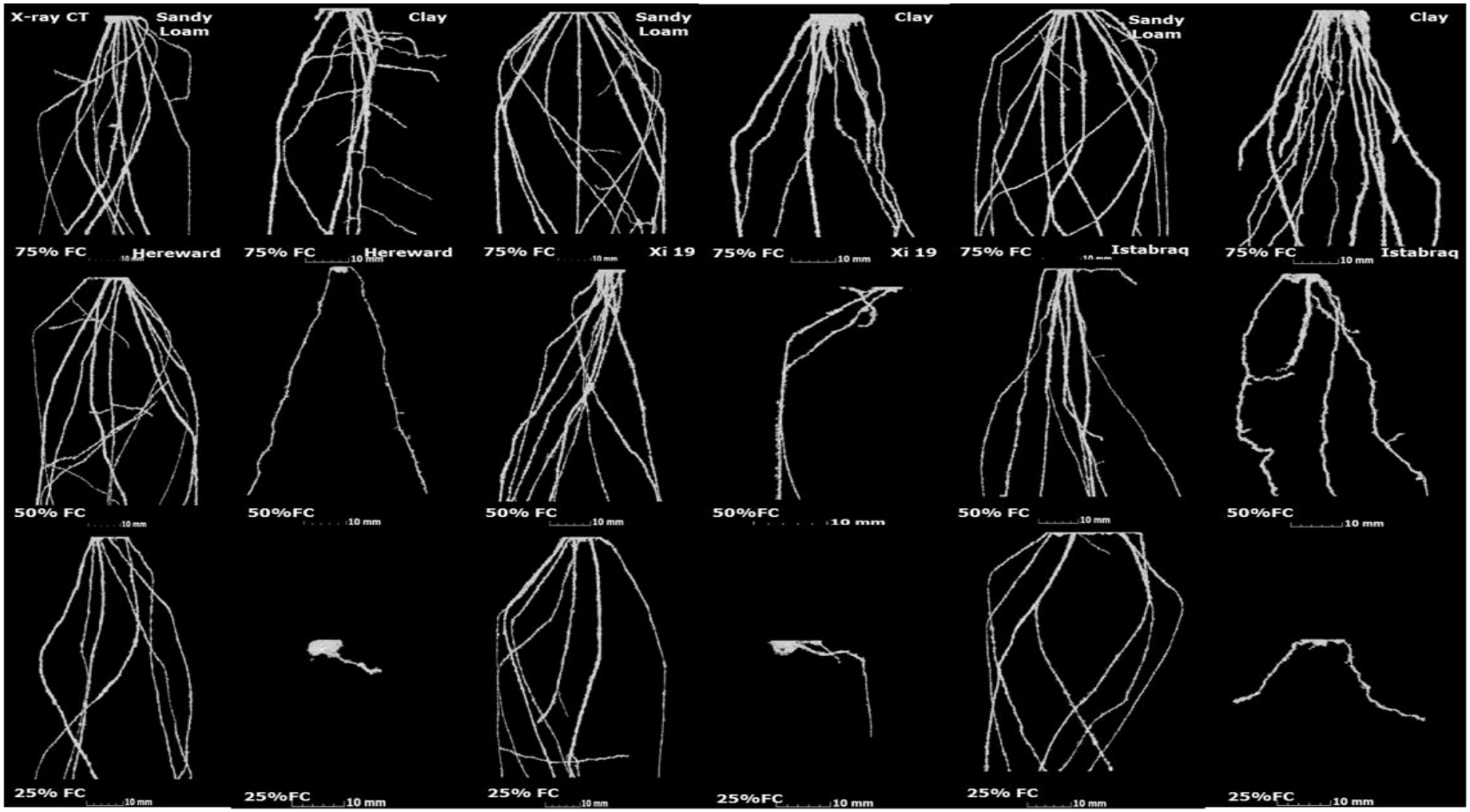


Figure 4.3A. CT images of the roots of three varieties of wheat (Hereward, Xi 19 and Istabraq) 21 days after germination grown under water stress (75%, 50% and 25% field capacity) for the sandy loam and the clay soils types. Scale bars represent 10mm.

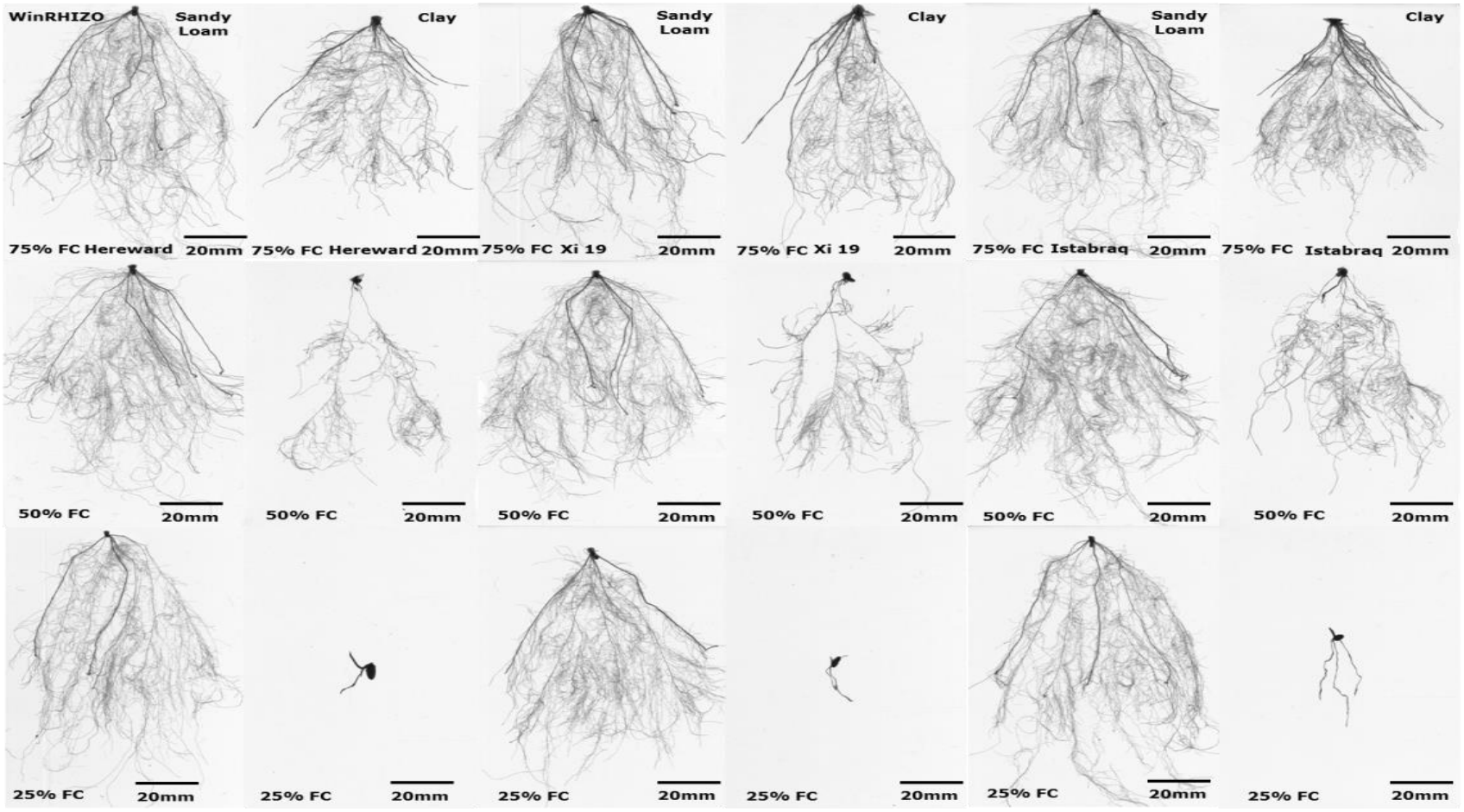


Figure 4.3B: WinRHIZO images of root systems of three varieties of wheat (Hereward, Xi 19 and Istabraq) after extraction and analysis using WinRHIZO® equipment 21 days after germination after scanning grown under conditions 28 °C under three different moisture content levels (75%, 50%, and 25% field capacity) in the sandy loam and the clay soil types Scale bar represents 20mm.

4.3.2 Photosynthesis, stomatal conductance and transpiration

4.3.2.1 Photosynthesis

To study the effect of water stress on photosynthesis, stomatal conductance and transpiration and the interaction between photosynthesis and root of Hereward, Xi 19 and Istabraq, infrared gas analysis (Licor6400XT, Licor, Nebraska) was used. The analysed data showed the soil*wheat varieties and soil*water*wheat varieties interactions were significant for photosynthesis ($P_{soil*wheat\ varieties} < 0.001$, $P_{soil*water*wheat\ varieties} < 0.05$, $P_{soil*water} > 0.05$, $P_{water*wheat\ varieties} > 0.05$). In the clay soil, photosynthesis for Hereward (15 $\mu mol.m^{-2}.s^{-1}$) and Xi 19 (13.4 $\mu mol.m^{-2}.s^{-1}$) were significantly lower than Istabraq (16.4 $\mu mol.m^{-2}.s^{-1}$) ($P_{wheat\ varieties} < 0.001$) Photosynthesis for Hereward, Xi 19 and Istabraq 21 days after germination increased with decreasing soil moisture content in the sandy loam and the clay soil types from 75% FC to 50% FC, and then decreased to 25% FC while it reduced with declining soil moisture content in the clay soil type for Hereward ($P_{water} > 0.05$, $P_{soil} > 0.05$). The biggest value for photosynthesis was 21.8 $\mu mol.m^{-2}.s^{-1}$ at 50% FC for Istabraq in the clay while the lowest value was 8.6 $\mu mol.m^{-2}.s^{-1}$ at 75% FC for Xi 19 in the clay soil type (Figure 4.4).

4.3.2.2 Stomatal conductance

The soil*water and soil*water*wheat varieties interactions were significant for stomatal conductance ($P_{soil*water} < 0.01$, $P_{soil*water*wheat\ varieties} < 0.05$, $P_{soil*wheat\ varieties} > 0.05$, $P_{water*wheat\ varieties} > 0.05$).

Stomatal conductance for Hereward, Xi 19 and Istabraq 21 days after germination increased with decreasing soil moisture content in the sandy loam and the clay soil type from 75% FC to 50% FC and then reduced to 25% FC while it decreased significantly with declining soil moisture content in the clay soil type for Hereward (P_{water}> 0.05, $P_{wheat\ varieties}$> 0.05, P_{soil}> 0.05). The highest value for stomatal conductance was 173.9 $mmol.m^{-2}.s^{-1}$ at 50% FC for Hereward in the sandy loam while the lowest value was 73 $mmol.m^{-2}.s^{-1}$ at 50% FC for Hereward in the clay soil type (Figure 4.4).

4.3.2.3 Transpiration

Transpiration in the sandy loam was significantly greater than in the clay in all three wheat varieties at almost all levels of water stress, except at 75% FC for Hereward where it was lower (P_{soil}< 0.001) (Figure 4.4). The soil*water and soil*water*wheat varieties interactions were significant for transpiration ($P_{soil*water}$< 0.05, $P_{soil*water*wheat\ varieties}$< 0.05, $P_{soil*wheat\ varieties}$> 0.05, $P_{water*wheat\ varieties}$> 0.05). The highest value for transpiration was 5.1 $\mu mol.m^{-2}.s^{-1}$ at 50% FC for Hereward in the sandy loam while the lowest value was 2 $\mu mol.m^{-2}.s^{-1}$ at 50% FC for Hereward in the clay soil type (Figure 4.4).

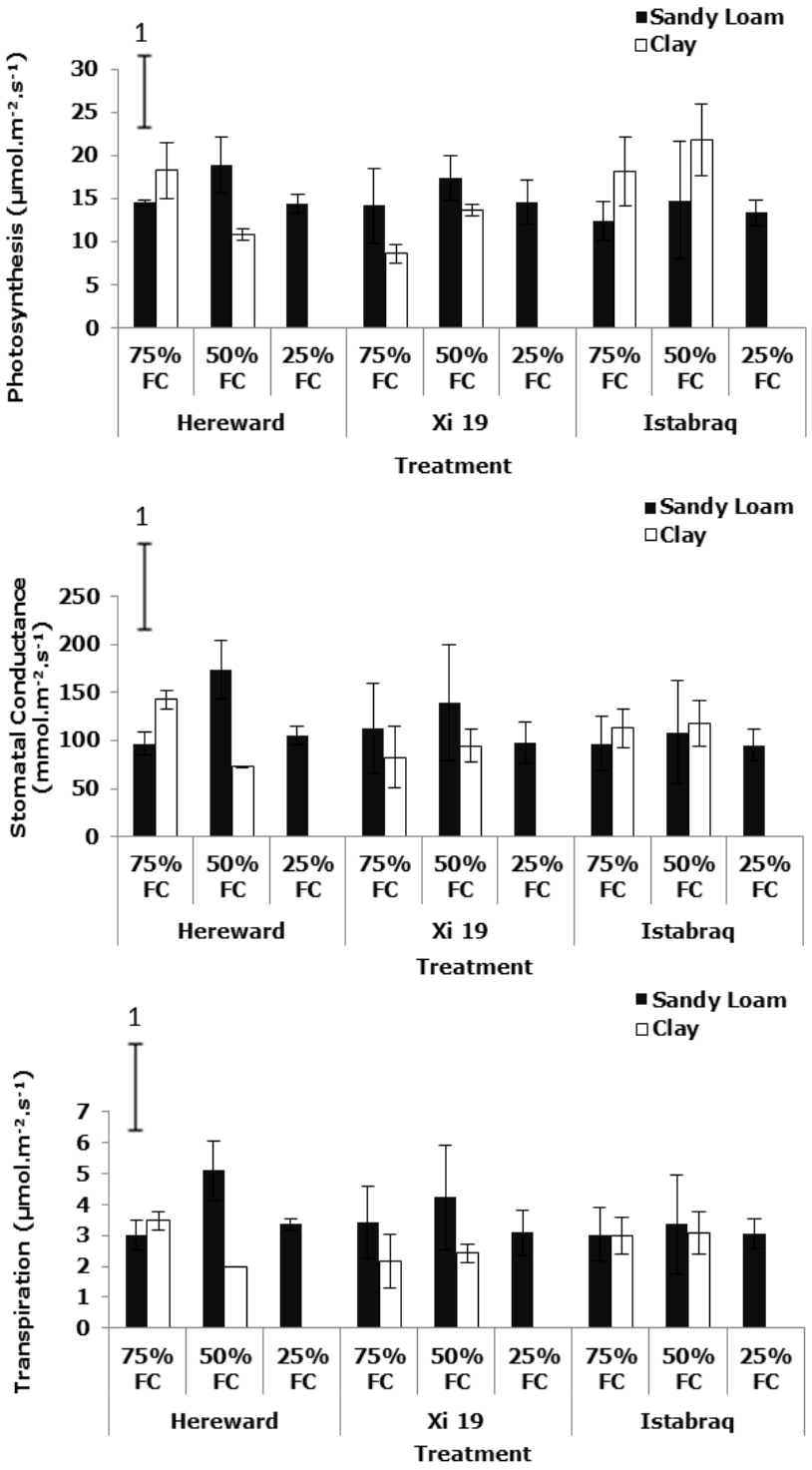


Figure 4.4: The effect of soil water content on photosynthesis, stomatal conductance and transpiration one day after scanning , 21 days after germination of three varieties of wheat (Hereward, Xi 19 and Istabraq) grown under water stress (75%, 50% and 25% FC) in the sandy loam and the clay soils. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on photosynthesis, stomatal conductance and transpiration at $P< 0.05$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

4.3.2.4 Intrinsic water use efficiency (photosynthesis/ stomatal conductance)

The soil*water and soil*wheat varieties interactions were significant for intrinsic water use efficiency (inWUE) ($P_{soil*water}$< 0.01, $P_{soil*wheat\ varieties}$< 0.05, $P_{water*wheat\ varieties}$> 0.05, $P_{soil*water*wheat\ varieties}$> 0.05). inWUE for Hereward, Xi 19 and Istabraq 21 days after germination increased with decreasing soil moisture content in the sandy loam and the clay soil type whereas, it reduced with declining soil moisture content from 75% FC to 50% FC and then increased to 25% in the sandy loam soil type for Hereward (P_{water}> 0.05). In the clay soil, inWUE for Istabraq (0.17) was significantly greater than Hereward (0.14) and Xi 19 (0.13) ($P_{wheat\ varieties}$< 0.05) while there were no significant differences between all these wheat varieties in the sandy loam soil. inWUE in the clay was significantly greater than in the sandy loam at almost levels of water stress, except at 75% FC for Hereward and Xi 19 and also for Istabraq at 75% and 50% FC was lower (P_{soil}< 0.05) (Figure 4.5).

4.3.2.5 Instantaneous water use efficiency (photosynthesis/ transpiration)

The soil*water and soil*wheat varieties interactions were significant for instantaneous water use efficiency (iWUE) ($P_{soil*water}$< 0.05, $P_{soil*wheat\ varieties}$= 0.01, $P_{water*wheat\ varieties}$> 0.05, $P_{soil*water*wheat\ varieties}$> 0.05). In the clay soil, iWUE for Istabraq (6.5) was significantly greater than Hereward (5.3) and Xi 19 (5.1) ($P_{wheat\ varieties}$< 0.05). iWUE in the clay (5.6) was significantly greater than in the sandy loam (4.4) at all levels of water stress (P_{soil}< 0.001) (Figure 4.5).

iWUE for Hereward, Xi 19 and Istabraq 21 days after germination increased with decreasing soil moisture content in the sandy loam and the clay soil type while it reduced from 75% FC to 50% FC and then increased to 25% in the sandy loam soil type for Hereward (P_{water}> 0.05).

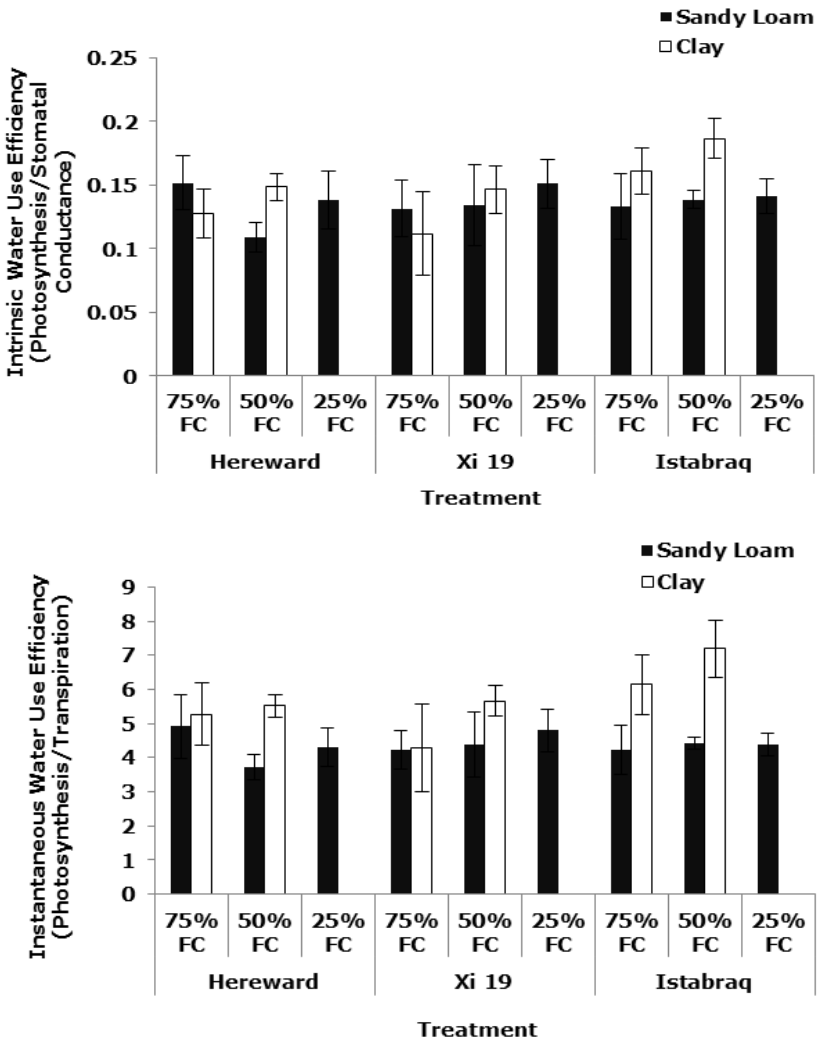


Figure 4.5: inWUE and iWUE of wheat (Hereward, Xi 19 and Istabraq) under different moisture content (75%, 50% and 25% FC) for the sandy loam and the clay soils one day after scanning, 21 days after germination. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on inWUE and iWUE.

4.3.3 Combined shoot and root characteristics

4.3.3.1 Shoot dry weight

To estimate the effect of different levels of water stress (75%, 50 and 25% FC) on shoot growth of three different varieties (Hereward, Xi19 and Istabraq), shoot dry weight was measured. The data found that shoot dry weight for all varieties decreased significantly with decreasing soil moisture content in the sandy loam and the clay soils (P_{water}< 0.001). The soil*water and water*wheat varieties interactions were significant ($P_{soil*water}$< 0.01, $P_{water*wheat\ varieties}$< 0.05). Shoot dry weight in the sandy loam soil for Hereward (0.18 g) reduced significantly in comparison to Istabraq (0.26 g) and Xi 19 (0.23 g). In the clay soil, shoot dry weight declined significantly for Hereward (0.12 g) and Xi 19 (0.15 g) in comparison to Istabraq (0.23 g) ($P_{wheat\ vatieties}$< 0.001). Shoot dry weight in the sandy loam (0.22 g) was significantly higher than in the clay soil (0.17 g) at all levels of water stress, except at 75% FC for Istabraq was lower (P_{soil}< 0.001) (Figure 4.7). Examples of images of leaves of these three wheat varieties grown under different moisture content (75%, 50% and 25% FC) in the sandy loam and the clay soils 21 days after germination are shown in Figure 4.6.

4.3.3.2 Root dry weight

Root dry weight was measured to examine the influence of water stress in Hereward, Xi19 and Istabraq. The data show that root dry weight for all wheat varieties decreased dramatically with lowering soil moisture content in the sandy loam and the clay soils (P_{water}<

0.001). The soil*water interaction was significant ($P_{soil*water}$= 0.001). Root dry weight in the sandy loam soil for Hereward (0.20 g) reduced significantly in comparison to Istabraq (0.24 g). In the clay soil, root dry weight for Hereward (0.07 g) and Xi 19 (0.10 g) declined significantly in comparison to Istabraq (0.14 g) ($P_{wheat\ vatieties}$< 0.001). Root dry weight in the sandy loam (0.22 g) was considerably greater than in the clay soil (0.10 g) (P_{soil}< 0.001) (Figure 4.7).

4.3.3.3 Shoot length

The impact of different levels of water stress on the length of shoot of Hereward, Xi19 and Istabraq was measured. The data show that shoot length for all varieties reduced greatly with declining soil moisture content in both soil types (P_{water}< 0.001). The soil*water interaction was significant ($P_{soil*water}$< 0.01). Shoot length in the sandy loam soil for Hereward (20.78 cm) decreased significantly in comparison to Istabraq (24.9 cm) and Xi 19 (24.7 cm). In the clay soil, shoot length for Hereward (16.9 cm) reduced significantly in comparison to Xi 19 (22.2 cm) and Istabraq (20.5 cm) ($P_{wheat\ vatieties}$< 0.001). Shoot length in the sandy loam was significantly larger than in the clay (P_{soil}< 0.001) (Figure 4.7).

4.3.3.4 Total dry weight

To estimate the influence of water stress on growth of Hereward, Xi19 and Istabraq, total dry weight of plants of these three varieties was measured. The data revealed that total dry weight of these three varieties also decreased significantly with reducing soil

moisture content in the sandy loam and the clay soils (P_{water}< 0.001). The soil*water and water*wheat varieties interactions were significant ($P_{soil*water}$< 0.001, $P_{water*wheat\ varieties}$< 0.05). Total dry weight in the sandy loam soil for Hereward (0.38 g) also was significantly lower than that of Istabraq (0.50 g) and Xi 19 (0.46 g). In the clay soil, total dry weight for Hereward (0.20 g) and Xi 19 (0.25 g) dramatically reduced in comparison to Istabraq (0.37 g) ($P_{wheat\ vatieties}$< 0.001). Total dry weight in the sandy loam (0.44 g) soil was significantly greater than in the clay (0.27 g) at all levels of water stress (P_{soil}< 0.001) (Figure 4.6 and 4.7).

4.3.3.5 Root to shoot ratio

To assess the shoot and root responses to water stress, root to shoot ratio of Hereward, Xi19 and Istabraq was calculated. The data found that root to shoot ratio for all varieties increased dramatically with declining soil moisture content in both soil types (P_{water}< 0.001). Root to shoot ratio in the sandy loam (1.04) was significantly higher than in the clay soil (0.65) (P_{soil}< 0.001). Root to shoot ratio (0.98) for Istabraq and Xi 19 also reduced greatly in comparison to Hereward (1.15) in the sandy loam soil, although there was no significant difference between all wheat varieties in both soil types ($P_{wheat\ vatieties}$> 0.05) (Figure 4.7).

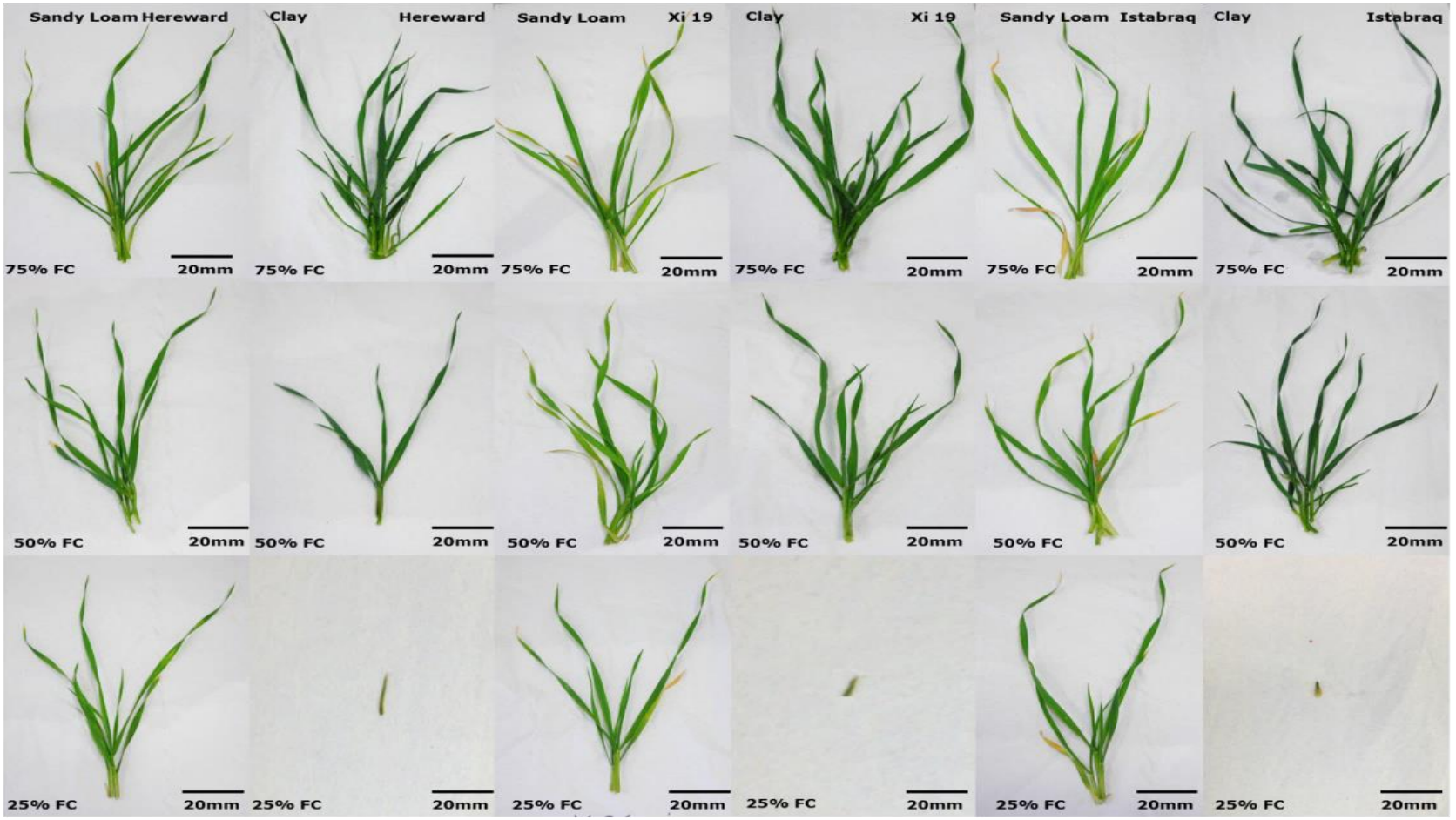


Figure 4.6: Leaves of three varieties of wheat (Hereward, Xi 19 and Istabraq) under different moisture content (75%, 50%, and 25% FC) in the sandy loam and the clay soils types after 21 days after germination after scanning. Scale bar represents 20mm.

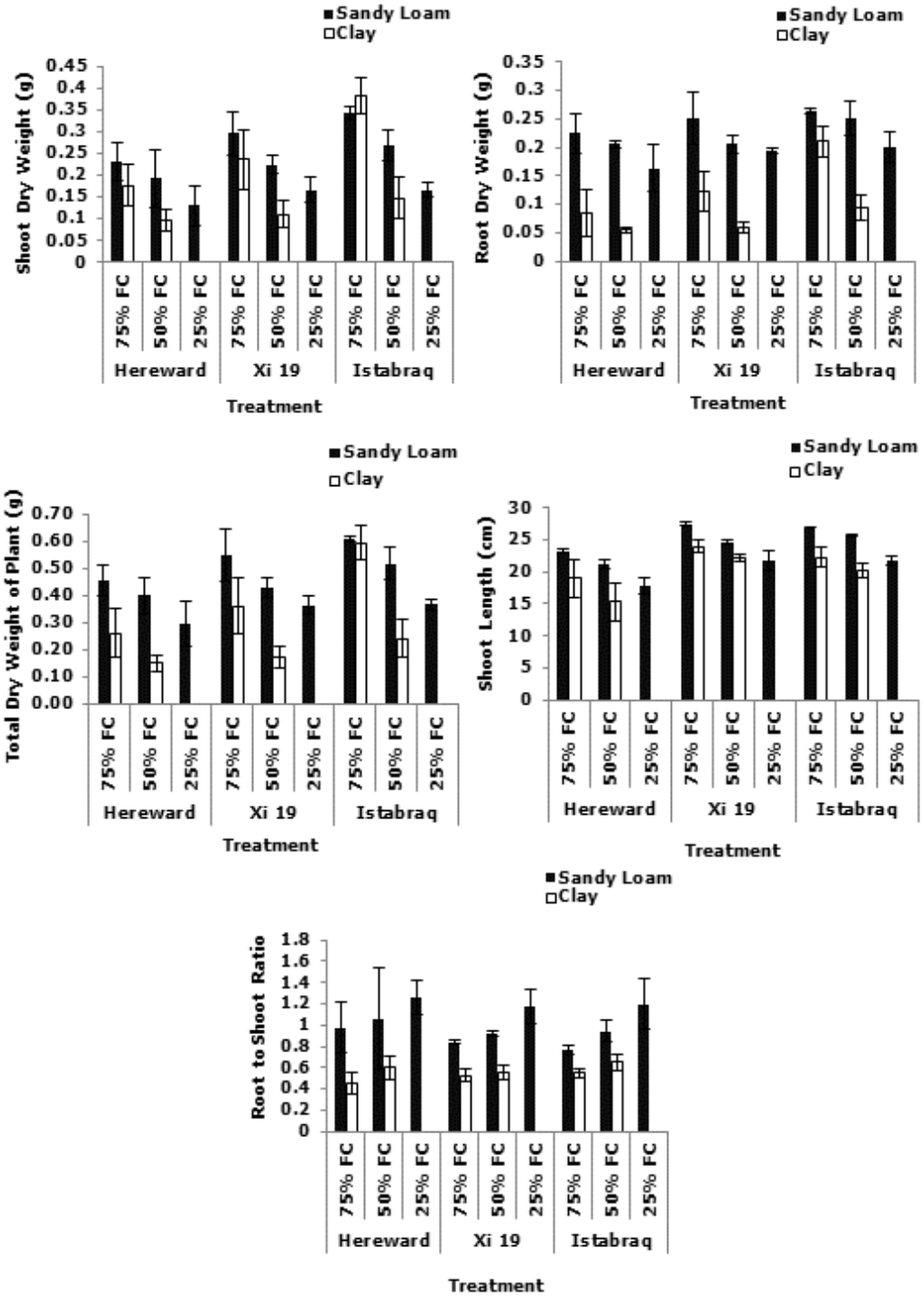


Figure 4.7: The effect of water stress on shoot, root and total dry weight of plant, shoot length and root to shoot ratio of three varieties of wheat (Hereward, Xi 19 and Istabraq) for the sandy loam and the clay soils. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on shoot, root and total dry weight of plant, shoot length and root to shoot ratio.

4.3.4 Water use efficiency

4.3.4.1 Water use efficiency (shoot dry weight)

Water use efficiency (shoot dry weight) for Hereward, Xi 19 and Istabraq 21 days after germination significantly increased with lowering soil water content in both soil types (P_{water}< 0.01). Water use efficiency in the clay soil for Hereward (1.25 kg.m^{-3}) greatly reduced in comparison to Istabraq (1.71 kg.m^{-3}) and Xi 19 (1.52 kg.m^{-3}) ($P_{wheat\ varieties}$< 0.01). Water use efficiency in the clay (1.49 kg.m^{-3}) was greater than in the sandy loam soil (1.05 kg.m^{-3}) (P_{soil}< 0.001) (Figure 4.8).

4.3.4.2 Water use efficiency (root dry weight) (WUE)

Water use efficiency (root dry weight) for all varieties 21 days after germination enhanced considerably with declining soil water content in the sandy loam and the clay soils (P_{water}< 0.001). In the clay soil, Hereward (0.75 kg.m^{-3}) had lower WUE than Istabraq (1.17 kg.m^{-3}) and Xi 19 (0.98 kg.m^{-3}) ($P_{wheat\ varieties}$< 0.01). The soil*wheat varieties interaction was significant ($P_{soil*wheat\ varieties}$< 0.05). Water use efficiency in the sandy loam was significantly greater than in the clay soil, except at 75% FC for Xi 19 and at 75% and 50% FC for Istabraq was lower (P_{soil}< 0.05) (Figure 4.8).

4.3.4.3 Water use efficiency (total dry weight)

Water use efficiency (total dry weight) for all varieties 21 days after germination increased significantly with declining soil water content in both soil types, except in the clay soil for Hereward and Xi 19

reduced dramatically (P_{water}< 0.001). Water use efficiency in the clay soil for Hereward (1.91 $kg.m^{-3}$) and Xi 19 (2.38 $kg.m^{-3}$) considerably reduced in comparison to Istabraq (2.90 $kg.m^{-3}$), and there was also a considerable difference between Xi 19 and Hereward. In the sandy loam soil, water use efficiency for Istabraq (2.28 $kg.m^{-3}$) was bigger than Xi 19 (2.09 $kg.m^{-3}$) and Hereward (2.04 $kg.m^{-3}$) though not significantly ($P_{wheat\ varieties}$< 0.01). Water use efficiency in the clay was significantly greater than in the sandy loam soil, except at 50% FC for Hereward which was lower (P_{soil}< 0.05) (Figure 4.8).

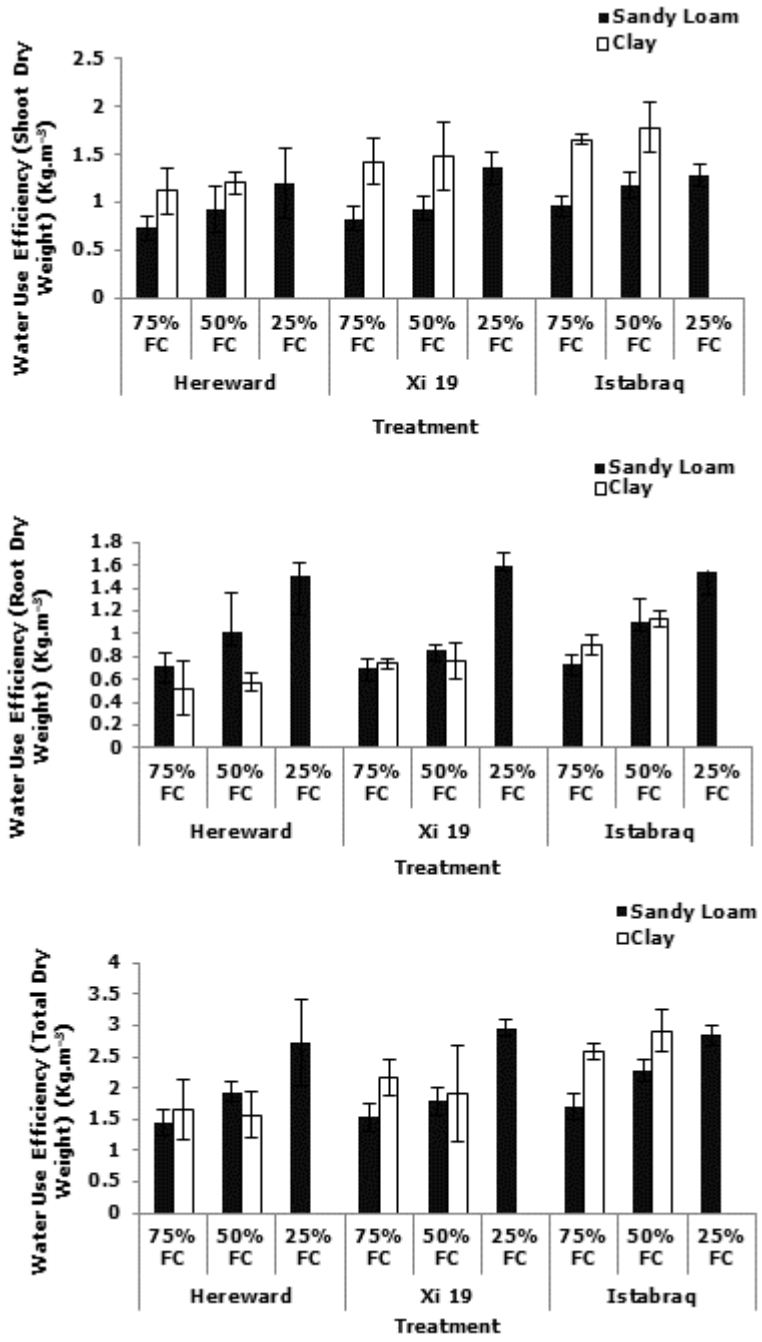


Figure 4.8: Water use efficiency (based on shoot, root and total dry weight) of wheat (Hereward, Xi 19 and Istabraq) under different moisture content (100%, 75%, 50% and 25% field capacity) for the sandy loam and the clay soils after 21 days after germination. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water x wheat varieties interaction on water use efficiency.

4.3.5 Soil characteristics

4.3.5.1 Volumetric water content

Volumetric water content in the clay soil (0.23 $g.g^{-1}$) was higher than in the sandy loam (0.17 $g.g^{-1}$) at all levels of water stress for all varieties 21 days after germination (P_{soil}< 0.001) (Figure 4.9). There was a significant difference between all water treatments (P_{water}< 0.001). Although volumetric water content for Hereward was greater than Xi 19 and Istabraq in both soil types, no significant difference between these varieties ($P_{wheat\ varieties}$> 0.05) was found. The highest values for volumetric water content were 0.30 $g.g^{-1}$, 0.27 $g.g^{-1}$, and 0.25 $g.g^{-1}$ for Hereward, Xi 19 and Istabraq, respectively at 75% FC in the clay soil type. Whereas, the lowest values volumetric water content were 0.20 $g.g^{-1}$, 0.18 $g.g^{-1}$, and 0.17 $g.g^{-1}$ for Hereward, Xi 19 and Istabraq, respectively at 25% FC in the sandy loam soil type.

4.3.5.2 Air filled porosity

The X-ray CT images were used to reveal the soil air filled porosity (for the given spatial resolution) and to examine the root systems architecture of Hereward, Xi 19 and Istabraq 21 days after germination in sandy loam and clay soil types. The results found that air filled porosity increased significantly with decreasing soil moisture content in the sandy loam and the clay soils (P_{water}< 0.001). Air filled porosity in the sandy loam soil for Hereward (32.9 %) was greater than Istabraq (30.8 %) and Xi 19 (30.8 %). In the clay soil, air filled porosity for Xi 19 (17.7 %) declined dramatically

in comparison to Hereward (23.3 %) ($P_{wheat\ varieties} < 0.05$) (Figure 4.9). Air filled porosity in the sandy loam (31.5 %) was markedly higher than in the clay soil (20.8 %) for all varieties ($P_{soil} < 0.001$).

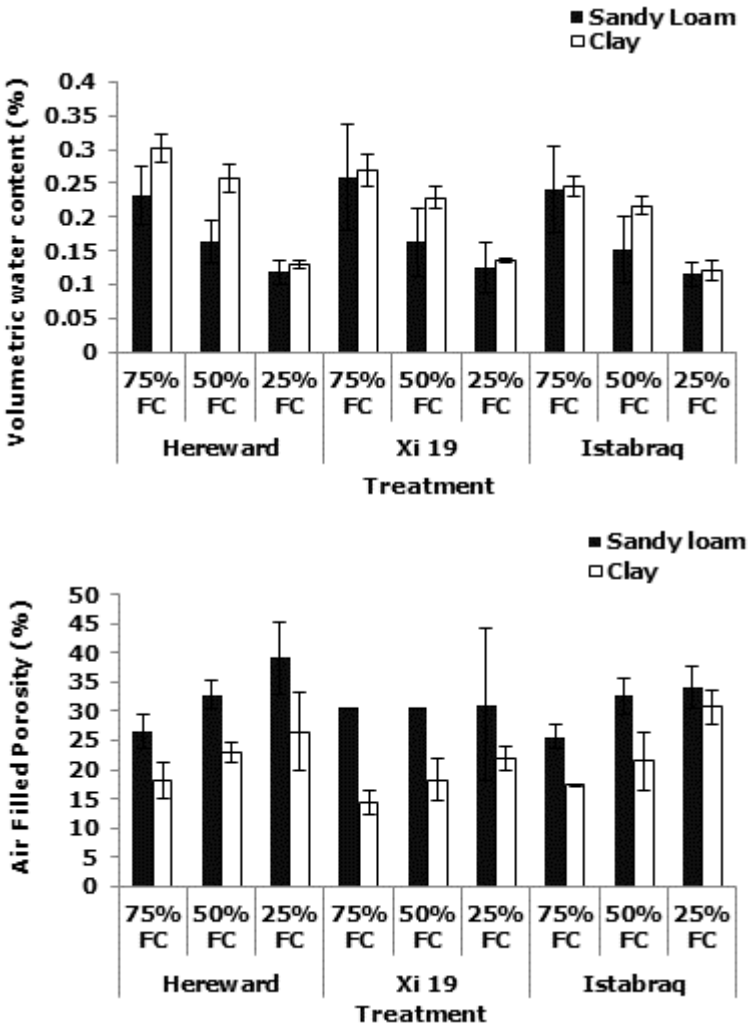
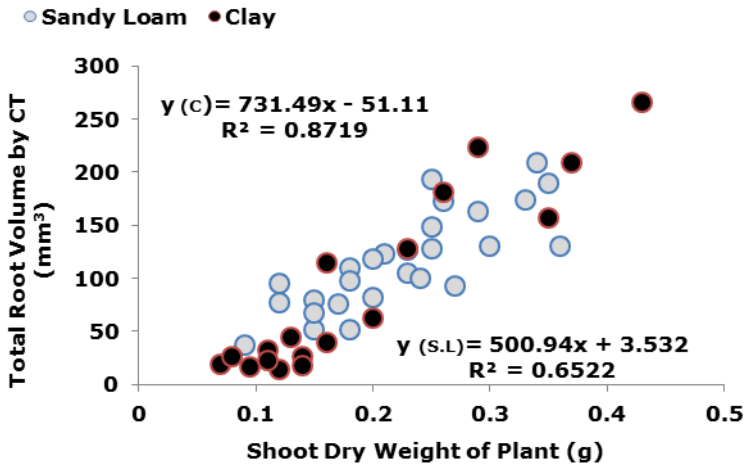


Figure 4.9: Volumetric water content and air filled porosity of three wheat varieties (Hereward, Xi 19 and Istabraq) in the sandy loam and the clay soils after 21 days after germination grown under different moisture content (100%, 75%, 50% and 25% field capacity). Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water x wheat varieties interaction on volumetric water content and air filled porosity.

4.3.6 Correlation matrix analysis

Here the relationship between multiple variables is investigated and it is shown that total dry weight, shoot dry weight, X-ray CT root volume and root dry weight had positive correlations with each other under (75%, 50% and 25% FC) in the sandy loam and the clay soil types under high temperature 28°C (Table 4. 2). The data also found important correlations between shoot and total dry weight with total root volume by X-ray CT in clay soil type with typical R^2 values of 0.9, higher than with typical R^2 values of 0.6-0.7 in sandy loam (Figure 4.10).

A



B

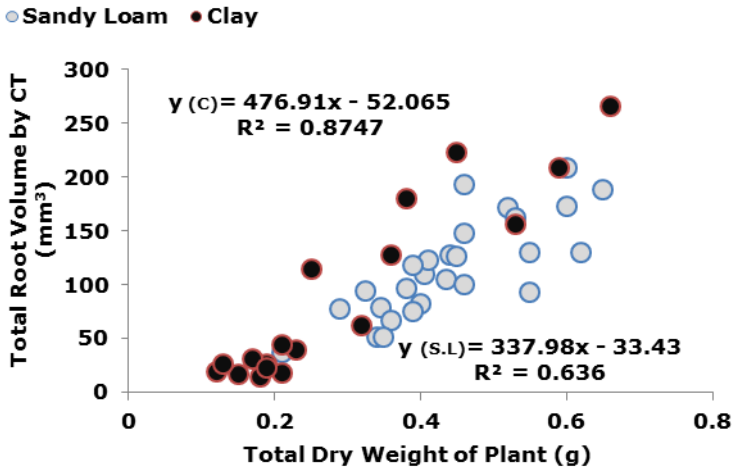


Figure 4.10: Scatter diagram between each of the shoot (A) and total dry weight of plant (B) with the total root volume by CT of three wheat varieties (Hereward, Xi 19 and Istabraq) grown in the sandy loam (S.L) and the clay (C) soil types, respectively 30 days after germination after scanning under conditions 28 °C and three different moisture content (75%, 50% and 25% FC).

Table 4.2: Correlation matrix between all variables (see list of abbreviations) of three wheat varieties (Hereward, Xi 19 and Istabraq) in the sandy loam and the clay soil types 21 days after germination grown under different moisture content (75%, 50% and 25% FC) at P< 0.05.

Plantdwt	**1**	-																	
Psynth	**2**	0.03	-																
RSRatio	**3**	0.10	-0.01	-															
Root_dwt	**4**	0.91	0.02	0.47	-														
STomC	**5**	0.08	0.76	0.04	0.10	-													
Shoot_dwt	**6**	0.94	0.03	-0.22	0.72	0.05	-												
Transp	**7**	0.20	0.69	0.24	0.30	0.95	0.10	-											
VWC	**8**	-0.05	-0.11	-0.68	-0.32	-0.16	0.18	-0.35	-										
WRDiam	**9**	-0.42	0.03	-0.46	-0.58	-0.26	-0.23	-0.43	0.26	-									
WRRootvol	**10**	0.86	0.21	0.35	0.89	0.17	0.72	0.34	-0.36	-0.49	-								
WUE_R	**11**	0.07	0.13	0.66	0.30	-0.01	-0.13	0.12	-0.72	-0.14	0.36	-							
WUE_SH	**12**	-0.11	0.21	-0.37	-0.29	-0.07	0.04	-0.19	0.02	0.50	-0.09	0.39	-						
WUE_T	**13**	0.07	0.23	0.20	0.10	0.00	0.03	0.01	-0.42	0.11	0.25	0.84	0.78	-					
X_rayAvgDiam	**14**	-0.08	-0.08	0.07	-0.08	-0.17	-0.08	-0.18	-0.17	0.13	-0.07	0.10	0.05	0.10	-				
X_ray_RootVol	**15**	0.86	-0.02	-0.08	0.68	0.09	0.89	0.14	0.16	-0.27	0.68	-0.13	-0.09	-0.06	-0.08	-			
iwuepc	**16**	-0.09	0.18	-0.02	-0.10	-0.47	-0.06	-0.49	0.06	0.41	0.04	0.15	0.29	0.23	0.16	-0.20	-		
iwuept	**17**	-0.28	0.28	-0.34	-0.41	-0.32	-0.14	-0.48	0.30	0.63	-0.24	-0.07	0.44	0.16	0.20	-0.24	0.88	-	
porosity_sp	**18**	0.14	0.07	0.74	0.45	0.17	-0.13	0.39	-0.65	-0.48	0.38	0.49	-0.39	0.10	0.04	-0.11	-0.14	-0.45	-
reg_p_ht	**19**	0.73	-0.04	0.02	0.68	0.04	0.67	0.14	0.02	-0.54	0.61	-0.06	-0.17	-0.08	-0.07	0.64	-0.14	-0.27	0.06	-
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**

Positive significant correlation between variables	**Critical significant value**	**0.294**
Negative significant correlation between variables	**Number of observations:**	**24**
No significant correlation between variables		

Key relationship

Table 4.3: P values for all variables analysed by ANOVA for three wheat varieties (Hereward, Xi 19 and Istabraq) in the sandy loam and the clay soils after 21 days after germination grown under different moisture content (75%, 50% and 25% FC).

Measurements/Treatment	Soil	Water	Wheat Varieties	Soil*Water* Wheat Varieties
Total root volume (X-ray)	P< 0.001	P< 0.001	P< 0.01	P> 0.05
Mean root diameter (X-ray)	P> 0.05	P> 0.05	P> 0.05	P> 0.05
Total root volume (WinRHIZO)	P< 0.001	P= 0.001	P< 0.001	P> 0.05
Mean root diameter (WinRHIZO)	P< 0.001	P> 0.05	P> 0.05	P> 0.05
Photosynthesis	P> 0.05	P> 0.05	P< 0.05	P< 0.05
Stomatal conductance	P> 0.05	P> 0.05	P> 0.05	P< 0.05
Transpiration	P< 0.001	P> 0.05	P> 0.05	P< 0.05
inWUE	P< 0.05	P> 0.05	P< 0.05	P> 0.05
iWUE	P< 0.001	P> 0.05	P< 0.05	P> 0.05
WUE (based on shoot dry weight)	P< 0.001	P< 0.01	P< 0.01	P> 0.05
WUE (based on root dry weight)	P< 0.05	P< 0.001	P< 0.01	P> 0.05
WUE (based on total dry weight)	P< 0.05	P< 0.001	P< 0.01	P> 0.05
Shoot dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Root dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Shoot length	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Total dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Root to shoot ratio	P< 0.001	P< 0.001	P> 0.05	P> 0.05
Air filled porosity	P< 0.001	P< 0.001	P< 0.05	P> 0.05
Volumetric water content	P< 0.001	P< 0.001	P> 0.05	P> 0.05

	Significant p values
	Non-significant p values

4.4 Discussion

Three different wheat varieties (Hereward, Xi 19 and Istabraq) were chosen to further test the impact of water stress on the precise root system architecture. This novel study aimed to assess the responses of root and shoot growth of three wheat varieties to water stress in undisturbed soil. The data showed that severe water stress level (25% FC) has a significant negative effect on total volume of roots in both soil types (sandy and clay). The soil moisture content negatively affected the plants growth in the clay more than in the sandy soil. This could be due to the soil moisture content (VWC at 25% FC was 0.13 $g.g^{-1}$) in clay soil type which was lower than wilting point (-1500 kPa) as shown in water release curve (Figure 2.1 and 2.2). Therefore, plants growth was more affected especially under severe water stress in this soil type. Alternatively, this could be due to increased soil hardness or soil strength under water deficit as discussed in chapter three (Whalley et al., 2006, Whalley et al., 2008). The data also revealed Istabraq had a higher total volume of roots than Xi 19 and Hereward. This implied that Istabraq was more drought-resistant than Xi 19 and Hereward, although all of these varieties did not grow very well at very high water stress level (25% FC) in the clay soil type. Researchers considered that the increase in root growth as an indicator of the ability of plants to withstand water stress, as well as to screen plant cultivars for drought tolerance (Boutraa et al., 2010). Roots are the key plant organ for adaptation to drought. If tolerance is defined as the ability to maintain leaf area and growth under prolonged vegetative stage

stress, the main basis of variation appears to be constitutive root system architecture that allows the maintenance of more favorable plant water status (Nguyen et al., 1997). The possession of a deep and greater root system allowed access to water deep in the soil, which was considered important in determining drought resistance in the plant area (Kavar et al., 2008). It has been suggested that it is quality, i.e. the distribution and structure, and not quantity of roots that determines the most efficient strategy for extracting water during the crop-growing season (Farooq et al., 2009).

Root to shoot ratio increased with worsening water stress. Root to shoot ratio in the sandy soil was significantly greater than in the clay soil. This could be due to dry climate genotypes having a well-developed root system with the stronger drought resistance as mentioned before (Wu and Bao, 2011). The continuous increase of root growth in water stressed soil is mainly important to manage the effects of water stress (Khan et al., 2010). It has been reported that plants respond to water shortage through declining leaf and stem growth with a simultaneous increase in root growth to explore the soil volume of water (Spollen et al., 1993). The roots are considered a sensitive part of the plant, being the first organ to sense water shortage via their tips (Shimazaki et al., 2005).

Mean root diameters of plants grown at the early growth stage (vegetative stage) in the clay soil were greater in the sandy soil for all varieties. This could be due to gap formation between the roots and soil which led to decrease the mean root diameter by shrinkage

in the sandy loam soil (Carminati et al., 2013). In general, Istabraq had a larger root system such as total root volume in both soil types than others under water stress conditions. This could be due to Istabraq having a larger root diameter than Hereward and Xi 19 (Figure 4.1 and 4.2) as discussed before in chapter three (the relationship between root thickness and root branching). Thick roots lead to increased branching which increases water absorption capacity (Ingram et al., 1994), and increased root system architecture. This would be consistent with Istabraq being more drought-resistant than the other varieties tested and would lead to a recommendation to use Istabraq for wheat production under drought condition at early growth stages, backed up by water use efficiency data (below). However this is based on a seedling study only, our study confirm Ober et al. (2013) work different varieties of winter wheat in the field were evaluated under irrigated and drought conditions. They found that istabraq was a drought tolerant wheat variety as mentioned before in this chapter and provide more justification by using istabraq under different levels of water stress in the small soil columns in the growth room.

Photosynthesis and stomatal conductance were higher in the sandy soil than in the clay for Hereward and Xi 19, except at 75% FC all these parameters were lower for Hereward. In addition, photosynthesis and stomatal conductance were also lower at 75% and 50 % FC for Istabraq although overall a consistent pattern for drought- responses of photosynthesis was not apparent in this

experiment. This could be due to a high respiration rate which could hinder the biomass accumulation of plant, as it has been seen that higher rate of respiration interfere with biomass accumulation in *Abelmoschus esculentus L. Moench* (Batool et al. (2015). However, the data presented here contrasted with those found earlier in chapter three where the highest values of photosynthesis were observed at 75% FC.

The different varieties showed a different photosynthetic trend at 50% FC. The highest value of photosynthesis for all wheat varieties was at 50% FC, except of that in the clay soil type where it was at 75% FC for Hereward. As previously discussed, a decline of photosynthesis associated with water deficit often affects plant growth and yield. This study indicated that a larger proportion of photosynthates were allocated to the root, while higher root to shoot ratio has been considered as one of the mechanisms involved in the adaptation of plants to drought as discussed above (Turner, 1997). The root to shoot ratio showed significant variation with water supply and with the three genotypes that had different sensitivities to water deficit.

The results showed that the water use efficiency (based on shoot, root and total dry weight of plant) for Istabraq was significantly greater than Xi 19 and Hereward investigated in the current study. This could be due to shoot and root dry weight and the amount of water consumed during the plant growth, Istabraq had greater shoot and root dry weights and water loss. Or this could be due to

decreased stomatal conductance and transpiration as mentioned before in chapter three and/or increased photosynthesis to increase water use efficiency. Plant intrinsic water use efficiency (inWUE) based on stomatal conductance does not provide direct information about actual water use efficiency of the whole plant although it indicates a physiological response of plants to environmental variables. The values of instantaneous water use efficiency (iWUE), and, especially, transpiration efficiency, are empirical and can often be more readily associated to the growth rate of plant than the inWUE (Feng, 1999). However neither iWUE or inWUE showed a consistent responses to treatment whilst whole plant WUE increased with declining soil water availability. This could be due to the use of smaller plants where effects of drought on shoots may be more extreme than whole plants or that root responses were more important in determining water use efficiency responses than leaf physiology. The higher root to shoot ratio (dry weight) of Istabraq under drought coincided with higher water use efficiency in comparison to the other wheat varieties. Our results are in contrast to Boutraa et al. (2010) who used different wheat varieties at seedling growth stages under contrasting levels of water stress (80%, 50% and 30% FC) and found out that the WUE (based on the total dry weight for whole plant) was not affected by the moderate water stress in any of the wheat cultivars investigated in their study. Whereas, under severe water stress (30% FC) WUE decreased in Hab-Ahmar and Sindy-2 (Hab-Ahmar was the most drought sensitive wheat variety followed by Sindy-2), but Sindy-1 and Al-

gaimi (Al-gaimi was the most drought tolerant wheat variety followed by Sindy-1) were not affected. The decrease in WUE as a result of water stress was also shown by a number of researchers (Shangguan et al., 2000, El Hafid et al., 1998b) using cultivars of wheat plant. These data are in line with studies which showed that drought-tolerant wheat cultivars showed an increase in WUE, while the drought-sensitive cultivars showed a decrease in WUE (Michihiro et al., 1994, Varga et al., 2015). Whereas, Balouchi (2010) did not observe any effect of water stress on WUE in any of the Australian wheat cultivars but a significant variation between the used wheat cultivars in terms of WUE was observed. Water use efficiency (WUE) is a physiological parameter of key importance, expressing the ability of the crop to preserve the water reserves of the soil, thus combining drought tolerance and high yield potential (Fang et al., 2010). Many researchers have demonstrated substantial differences between the WUE values of individual winter wheat cultivars (Miranzadeh et al., 2011, Zhang et al., 2013), but have also emphasised the fact that WUE values change if the water supplies to the crop are limited (Varga et al., 2013).

Under both rain-fed and irrigated farming conditions, there is an increasing demand for an improvement in WUE (Condon et al., 2004). For farmers, WUE can best be interpreted as the correlation between the harvested yield and the water supplies (rainfall + irrigation water) available to the crop (Condon et al., 2004). However, the yield depends on both the water reserves stored in the

soil and on the temporal distribution of rainfall and irrigation (Francia et al., 2011). Zhang et al. (1999) found wheat to be the most sensitive to water deficit during the shooting to heading and heading to milky ripeness stages, so balanced water supplies are most critical during these periods.

4.5 Conclusions

Total root volume decreased significantly due to water stress. However, mean root diameter increased. It has also been found that photosynthesis, stomatal conductance, and transpiration for all wheat varieties increased with decreasing soil water content in the sandy loam and the clay soil type from the low water stress level (75% FC) to the medium (50% FC) and then decreased to high water stress level (25% FC). While it reduced with declining soil moisture content in the clay soil type for Hereward. In the sandy loam, Hereward had higher photosynthesis than Xi 19 and Istabraq while in the clay Istabraq was greater than the others. Photosynthesis measurements in the sandy soil were significantly greater than in the clay soil for (Hereward and Xi 19), except at 75% FC all these parameters were lower for Hereward. Photosynthesis and stomatal conductance were also lower at 75% and 50 % FC for Istabraq. Istabraq had larger root system than Xi 19 and Hereward and even than Zebedee in both soil types. Istabraq was more drought-resistant than the other three varieties. Therefore it is recommended using these wheat varieties under the same levels of water stress over longer periods to confirm the effect of water stress on these varieties.

5.0 Effect of elevated CO_2 and temperature on root and shoot growth of wheat grown under water deficit conditions

5.1 Introduction

Determining the effects of elevated CO_2 and temperature under water stress conditions during early periods of vegetative growth on root architecture systems and photosynthetic traits would be helpful to understand their combined effect. Previous studies have focussed on the individual impact of temperature climatic conditions across the wheat-growing regions of the world which are predicted to change with potentially severe impacts on wheat (*Triticum aestivum* L.) production over the next 50 years (Tubiello et al., 2007, Forster et al., 2007). The roots and shoots of a plant have an inter-dependence and could be thought of as either in competition with each other or cooperating in terms of extracting water, minerals and nutrients (Shangguan et al., 2004). Water availability affects photosynthesis and hence the growth of leaves and roots (Blum, 1996). Plants respond to water stress mainly by decreasing leaf elongation rate and closing stomata to reduce water loss through transpiration which in turn reduces photosynthesis.

On a global level, atmospheric CO_2 concentrations and temperatures will increase, but rainfall should decrease (Ragab and Prudhomme, 2002). Many studies on the impact of climate change on wheat have focused on the independent effects of elevated CO_2 (Gutiérrez et al., 2009, Zhu et al., 2009), high temperatures (Wall et al., 2011,

Ottman et al., 2012) or water shortage (Inoue et al., 2004). Few studies have examined the combined effects of elevated CO_2 and high temperature (Wheeler et al., 1996) or elevated CO_2 and water deficit (Manderscheid and Weigel, 2007), but none have studied the effects of all three variables combined on shoot and root growth in wheat. Elevated CO_2 generally increases biomass production in wheat and other plants as a result of greater rates of net leaf photosynthesis (Högy et al., 2009). This chapter has investigated the impact of elevated CO_2 and temperature on root and shoot growth in wheat under water deficit conditions. Photosynthesis, stomatal conductance and transpiration were measured after CT scanning to examine the shoot behaviour under water stress and temperature at different CO_2 concentrations. The null hypothesis was that elevated CO_2 and temperature has a significant positive effect on shoot and root growth and could reduce the negative impact of drought on their growth. It was also hypothesised that elevated CO_2 enhances photosynthesis and root architecture systems.

5.2 Materials and methods

5.2.1 Sample preparation and X-ray µCT

A Newport series sandy loam soil (FAO Class brown soil) was prepared and packed to a bulk density of 1.24 $g.cm^{-3}$. Two soil moisture content levels were chosen to represent a range of saturated, field and dry conditions namely 75% and 25% FC. Details of the exact volumetric water content, matric potentials and other relevant soil properties are given in Table 5.1 and 3.1 in chapter 3. Three replicates were prepared for each treatment combination to give a total of 24 columns. The columns were then water-saturated and drained to 100% FC then left to drain to reach these two levels (75% and 25% FC). Seeds of Xi 19 were germinated for 48 hrs before being planted 5 mm below the soil surface. Thereafter, they were placed in a growth room under conditions of 22/17 and 28/17 °C day/night, at 400 and 800 ppm CO_2 levels with 12 hrs photoperiod, at 70% relative humidity. All columns were placed in a transpired propagator to maintain high relative humidity levels during germination and seedling growth. They were weighed daily and sufficient water was added to maintain soil moisture content close to the two pre-determined moisture contents (75% and 25% FC). At day 14 post germination, the columns were scanned using a Phoenix Nanotom® (GE Measurement & Control Solutions, Wunstorf, Germany) X-ray µCT scanner set at 110 kV and 130 µA, with a 0.15 mm copper filter and an image averaging of 3. Pixel/voxel resolution was set at 55 µm and each scan took 32.5 min to complete. For each column, 1300 image projections were captured on all sampling

dates; each sample volume had a size of approx. 5GB. Thereafter, these images were reconstructed using VG StudioMAX (Volume graphics), an image processing software.

A further 24 columns were prepared and grown in the same conditions. These columns were scanned at day 30 after germination using a phoenix v|tome|x m Computed Tomography X-ray µCT scanner set at 150 kV and 160 µA, and an image averaging of 3. Pixel/voxel resolution was set at 56 µm and each scan took 76 min to complete. For each column, 2160 image projections were captured on all sampling dates; each sample volume had a size of approx. 46GB. Thereafter, these images were reconstructed using VG StudioMAX (Volume graphics).

5.2.2 Image processing and analysis

Root systems were non-destructively segmented from the grey-scale µCT images using the Region Growing selection tool in VG StudioMAXV2.0 and 2.2 software as described by Tracy et al. (2012). Region Growing classifies voxels in a certain grey-value range from a starting seed point. Tolerance values were adjusted to ensure that only root material was included in the growing region of interest (ROI) from the original seed points. The root system data segmented from the µCT image data were used for quantitative determination of total root volume and mean root diameter. The mean root diameter was measured by the distance measurement tool. As the study was concerned with the impact and the interaction between roots and soil, the soil pore characteristics were also

measured in addition to root measurements by using the calibration wizard and volume analyser in VGStudioMAX®.

5.2.3 Plant measurements

Light-saturated photosynthesis (A_{max}), stomatal conductance (G_s) and transpiration (T) were measured the day after scanning by infrared gas analysis (LI-6400XT Portable Photosynthesis System, Licor, Illinois). The settings were as per growth room conditions for temperature and CO_2 i.e. cuvette temperature 22 and 28 °C, at 400 and 800 ppm CO_2 levels, and pressure 100 kPa, flow rate 500 $\mu mol.s^{-1}$, and 1500 $\mu mol.m^{-2}.s^{-1}$ PAR (saturating light).

To determine the optimal temperature for photosynthesis, plants grown under 400 ppm CO_2 at 28 °C, a soil moisture content (75% FC) in sandy loam soil were measured using the Licor 6400XT under a range of different cuvette temperatures (22, 24, 28, 30, 32, 35 °C) under after 30 days after germination after scanning. Leaves were left in the chamber at each temperature for 3 minutes before measuring and proceeding to the next temperature, moving from low to high. The columns were scanned during the light part of the photoperiod in randomized order to ensure that all six treatment combinations were equally exposed to any diurnal variation in root growth that may have occurred. After CT scanning, the roots were washed from the soil and analysed using WinRHIZO®2002c scanning equipment and software to calculate total root volume and mean root diameter as described above in chapter 2 (2.4). The images obtained were collected to compare with the X-ray µCT images. Root

and shoot dry weight was also measured by oven drying at 75°C for 24 hrs.

5.2.4 Statistical analysis

The results from this experiment were also analysed the same as the first and second experiment described in chapter 3 and chapter 4.

Table 5.1. The values of matric potential (ψ)(-kPa) at different water content (saturation, 100%, 75% and 25% FC) for the sandy loam soil type in Newport under conditions 400 and 800 ppm CO_2 at 22 and 28 °C during 14 and 30 days after germination.

Treatment	Sandy Loam VwC (cm^3/cm^3)	M.P Sandy Loam (-kPa)
Saturation,(22,28°C),(400,800)	0.49	0
100% FC,(22,28°C),(400,800)	0.39	5
75% FC,(22,28°C),(400,800)	0.30	20
25% FC,(22,28°C),(400,800)	0.10	1080

5.3 Results

5.3.1 Root characteristics

5.3.1.1 Total root volume

To examine the effect of elevated CO_2 and temperature on total root volume and mean root diameter of Xi19 at 14 and 30 days after germination under water stress, X-ray CT and WinRHIZO were used. The data revealed that total root volume of Xi 19 obtained by X-ray CT after 14 days post germination reduced greatly at 28˚C (42%) in comparison to 22˚C ($P_{temperature}$< 0.001). The highest value for total root volume was 115.4 mm at 22˚C at 400 CO_2 at 25% FC while the lowest value was 54.3 mm at 28˚C at 400 CO_2 at 75% FC. Similar trends were found by WinRHIZO® as total root volume increased significantly with decreasing soil water content in the sandy loam (P_{water}< 0.001) (Figure 5.2A). Total root volume increased significantly for 400 ppm CO_2 by 17% in comparison to 800 ppm (P_{CO2}< 0.05). There was a significant interaction between all treatments ($P_{water*CO2}$= 0.001, $P_{water*temperature}$< 0.001, $P_{CO2*temperature}$< 0.001, $P_{water*CO2*temperature}$< 0.01). Total root volume decreased significantly at 28˚C (66%) in comparison to 22˚C ($P_{temperature}$< 0.001). The highest value for total root volume was 1871.7 mm^3 at 22˚C at 400 ppm CO_2 at 25% FC while the lowest value was 293 mm^3 at 28˚C at 400 ppm CO_2 at 75% FC.

Total root volume for Xi 19 obtained by X-ray CT 30 days after germination reduced significantly with declining soil moisture content in the sandy loam (P_{water}< 0.001) (Figure 5.1B). Total root

volume for 400 ppm decreased significantly by 31% in comparison to 800 ppm (P_{CO2}< 0.001). The water*CO_2 and water*temperature interactions were significant ($P_{water*CO2}$< 0.05, $P_{water*temperature}$< 0.05). The highest value for total root volume was 2020.6 mm^3 at 22°C at 800 ppm CO_2 at 75% FC while the lowest value was 348.3 mm^3 at 28°C at 400 ppm CO_2 at 25% FC. Similar trends were found by WinRHIZO® as total root volume decreased significantly with decreasing soil water content in the sandy loam (P_{water}< 0.001) (Figure 5.2B). Total root volume decreased significantly for 400 ppm CO_2 by 23% in comparison to 800 ppm (P_{CO2}< 0.001). Total root volume also decreased significantly at 28°C (44%) in comparison to 22°C ($P_{temperature}$< 0.001). There was significant interaction between all treatment ($P_{water*CO2}$< 0.001, $P_{water*temperature}$< 0.001, $P_{CO2*temperature}$< 0.001, $P_{water*CO2*temperature}$< 0.05). The highest value for total root volume was 5908 mm^3 at 22°C at 800 ppm CO_2 at 75% FC while the lowest value was 1509.5 mm^3 at 28°C at 400 ppm CO_2 at 25% FC. The p-values for all variables at 14 and 30 days after germination of Xi 19 are shown in Table 5.4.

5.3.1.2 Mean root diameter

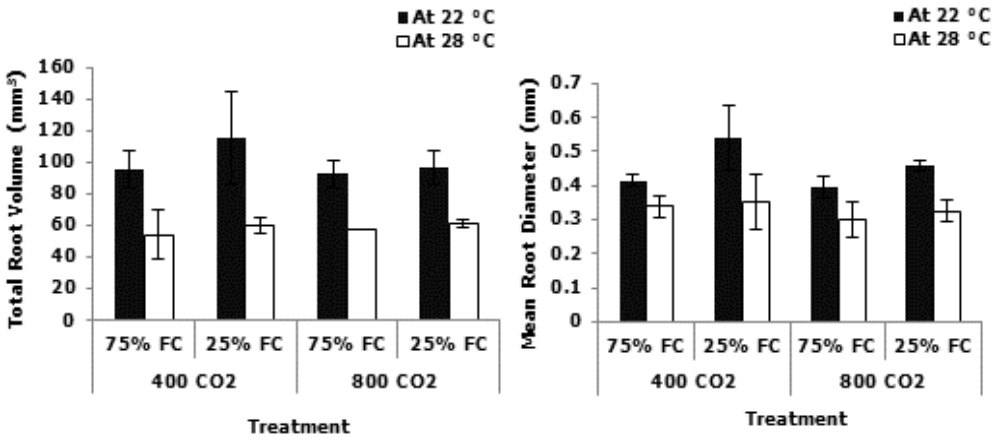
The data show that mean root diameter of Xi 19 by X-ray CT 14 days after germination increased significantly with decreasing soil moisture content in the sandy loam for the same levels of CO_2 (P_{water}< 0.05) (Figure 5.1A). Mean root diameter decreased significantly at 28°C (27%) in comparison to 22°C ($P_{temperature}$< 0.001). The highest value for mean root diameter was 0.54 mm at

22°C at 400 ppm CO_2 at 25% FC while the lowest value was 0.30 mm at 28°C at 800 ppm CO_2 at 75% FC. Similar trends were found by WinRHIZO® as mean root diameter increased significantly with decreasing soil water content in the sandy loam (P_{water}< 0.001) (Figure 5.2A). Mean root diameter increased significantly for 400 ppm CO_2 by 16 % in comparison to 800 ppm (P_{CO2}< 0.001). Mean root diameter decreased significantly at 28°C (37%) in comparison to 22°C ($P_{temperature}$< 0.001). There was a significant interaction between all treatments ($P_{water*CO2}$, $P_{water*temperature}$, $P_{CO2*temperature}$, $P_{water*CO2*temperature}$ < 0.001). The highest value for mean root diameter was 0.74 mm at 22°C, 400 ppm CO_2 and 25% FC while the lowest value was 0.29 mm at 28°C, 800 ppm CO_2 and 75% FC.

Mean root diameter for Xi 19 by X-ray CT 30 days after germination increased with decreasing soil moisture content in the sandy loam for the same levels of CO_2 (P_{water}< 0.001) (Figure 5.1B). Mean root diameter for 400 ppm decreased significantly by 14 % in comparison to 800 ppm (P_{CO2}< 0.001). Mean root diameter decreased significantly at 28°C (9%) in comparison to 22°C ($P_{temperature}$< 0.05). The highest value for mean root diameter was 0.70 mm at 22°C at 800 ppm CO_2 at 25% FC while the lowest value was 0.42 mm at 28°C at 400 ppm CO_2 at 75% FC. Similar trends were found by WinRHIZO® as mean root diameter increased significantly with decreasing soil water content in the sandy loam (P_{water}< 0.001) (Figure 5.2B). Mean root diameter decreased significantly for 400 ppm CO_2 by 7 % in comparison to 800 ppm

(P_{CO2}< 0.001). Mean root diameter decreased significantly at 28˚C (25%) in comparison to 22˚C ($P_{temperature}$< 0.001). There were significant interactions between the water*temperature and water*CO_2*temperature ($P_{water*CO2}$> 0.05, $P_{water*temperature}$< 0.001, $P_{CO2*temperature}$> 0.05, $P_{water*CO2*temperature}$= 0.001). The highest value for mean root diameter was 0.65 mm at 22˚C, 800 ppm CO_2 and 25% FC while the lowest value was 0.37 mm at 28˚C, 400 ppm CO_2 and 75% FC.

A



B

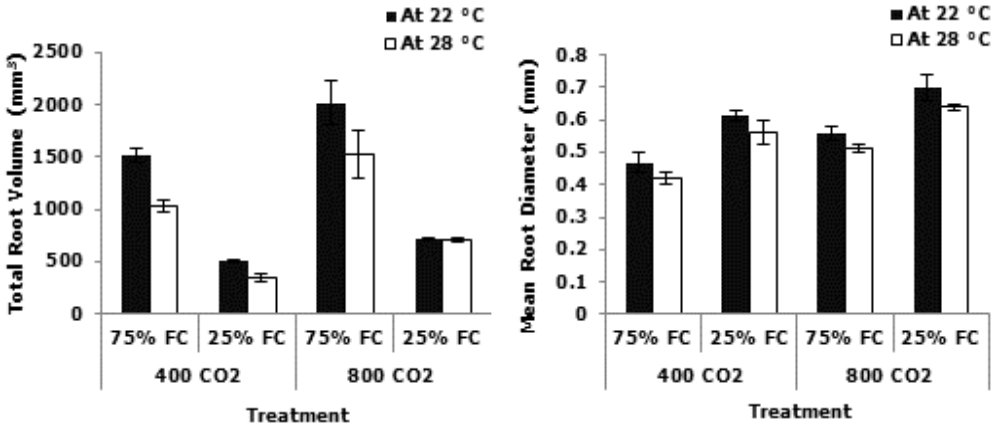
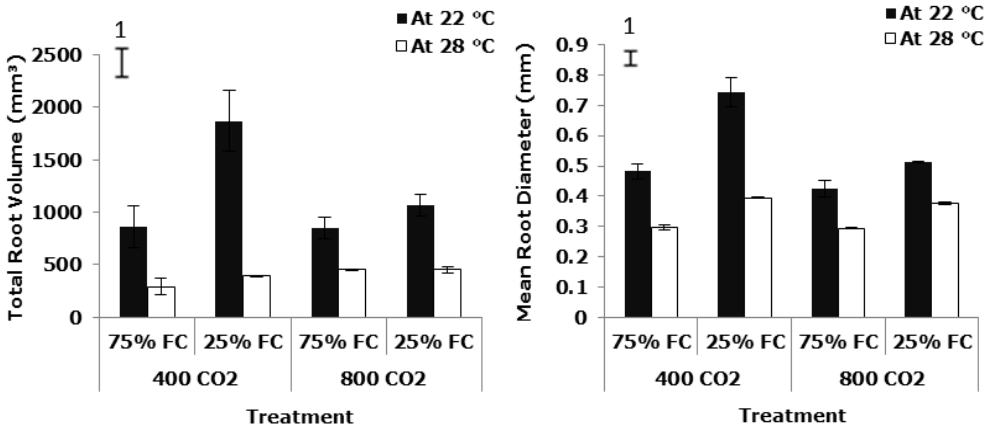


Figure 5.1: Total root volume and mean root diameter of root by using X-ray CT of Xi 19 wheat variety grown under 400 and 800 CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on total root volume and mean root diameter.

A



B

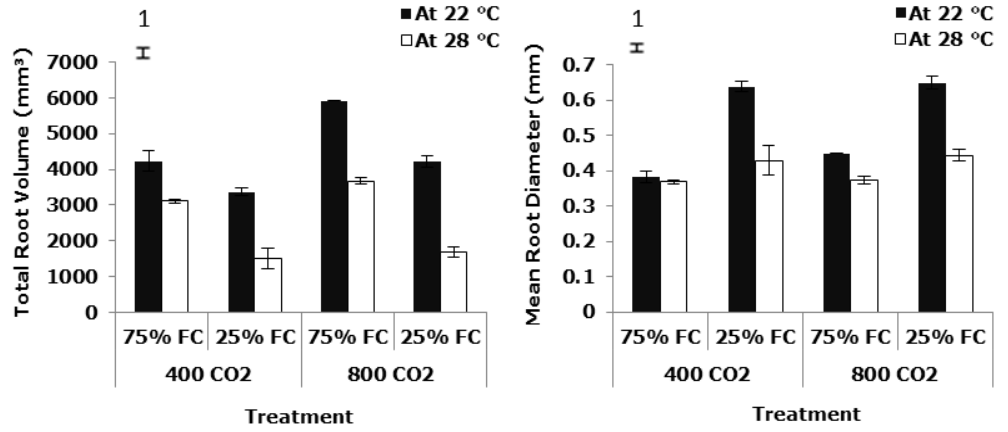


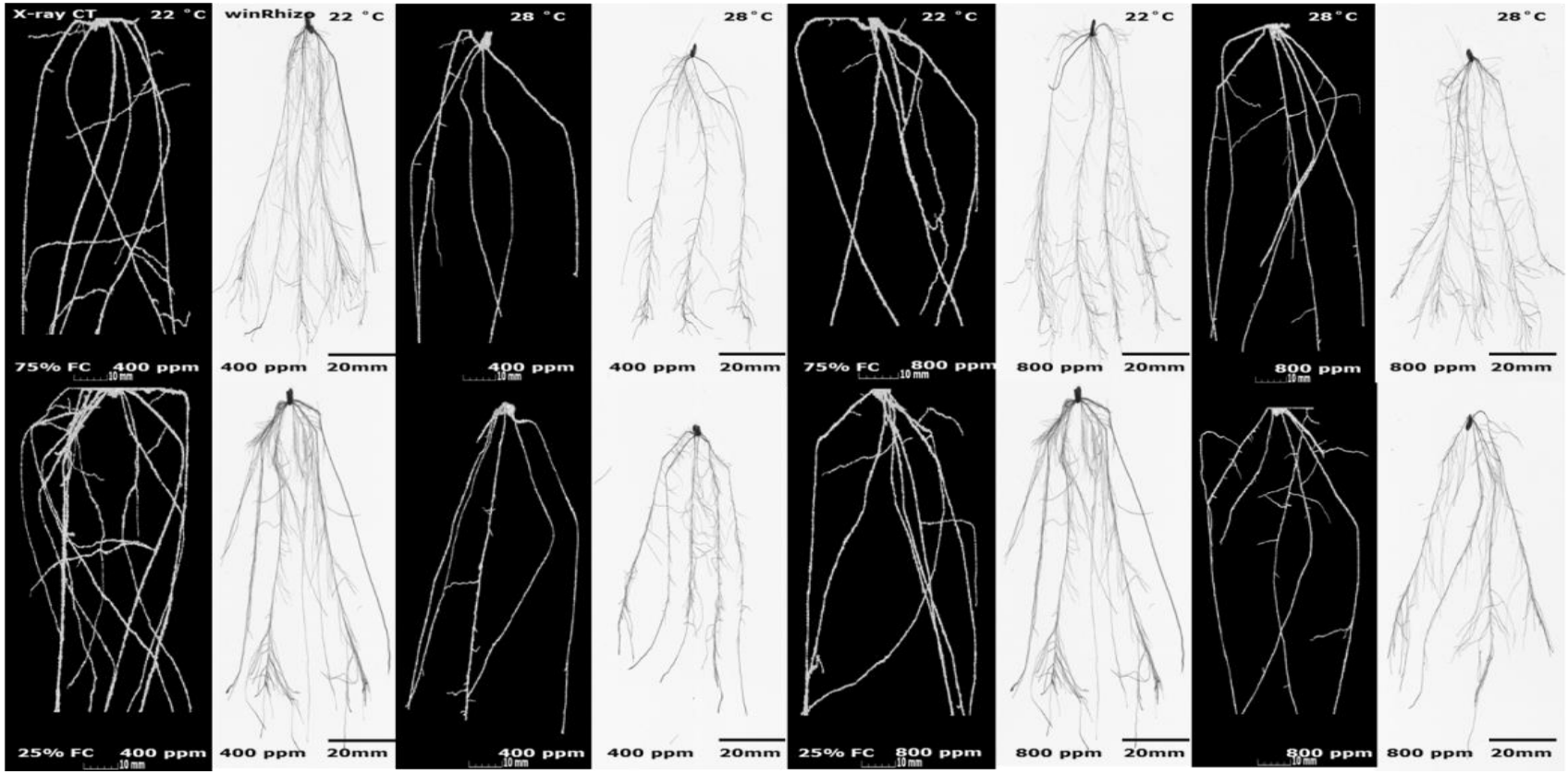
Figure 5.2: Total root volume and mean root diameter of root by using WinRHIZO of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on total root volume and mean root diameter. Vertical bar (1) represents standard errors of difference (SED) between means of total root volume in (A) at $P< 0.01$ and (B) at $P< 0.05$ and mean root diameter in (A) at $P< 0.001$ and (B) at $P= 0.001$.

5.3.2 Comparison of root imaging methods

An example of images of root architecture for both methods is shown in Figure 5.3. As it can be seen there was no significant difference between main roots for both levels of CO_2 under water stress conditions for Xi 19 by both methods. The main roots for

plant grown during 14 days at 25% FC were greater than at 75% FC in Figure 5.3A. While for those grown during 30 days at 75% FC were greater than at 25% FC in Figure 5.3B. The total root length and lateral roots increased significantly at day 30 compared to day 14 (Figure 5.3).

A


X-ray CT 22 °C
winRhizo 22 °C
28 °C
28°C
22 °C
22°C
28°C
28°C
75% FC 400 ppm
400 ppm 20mm
400 ppm
400 ppm 20mm
75% FC 800 ppm
800 ppm 20mm
800 ppm
800 ppm 20mm
25% FC 400 ppm
400 ppm 20mm
400 ppm
400 ppm 20mm
25% FC 800 ppm
800 ppm 20mm
800 ppm
800 ppm 20mm

B

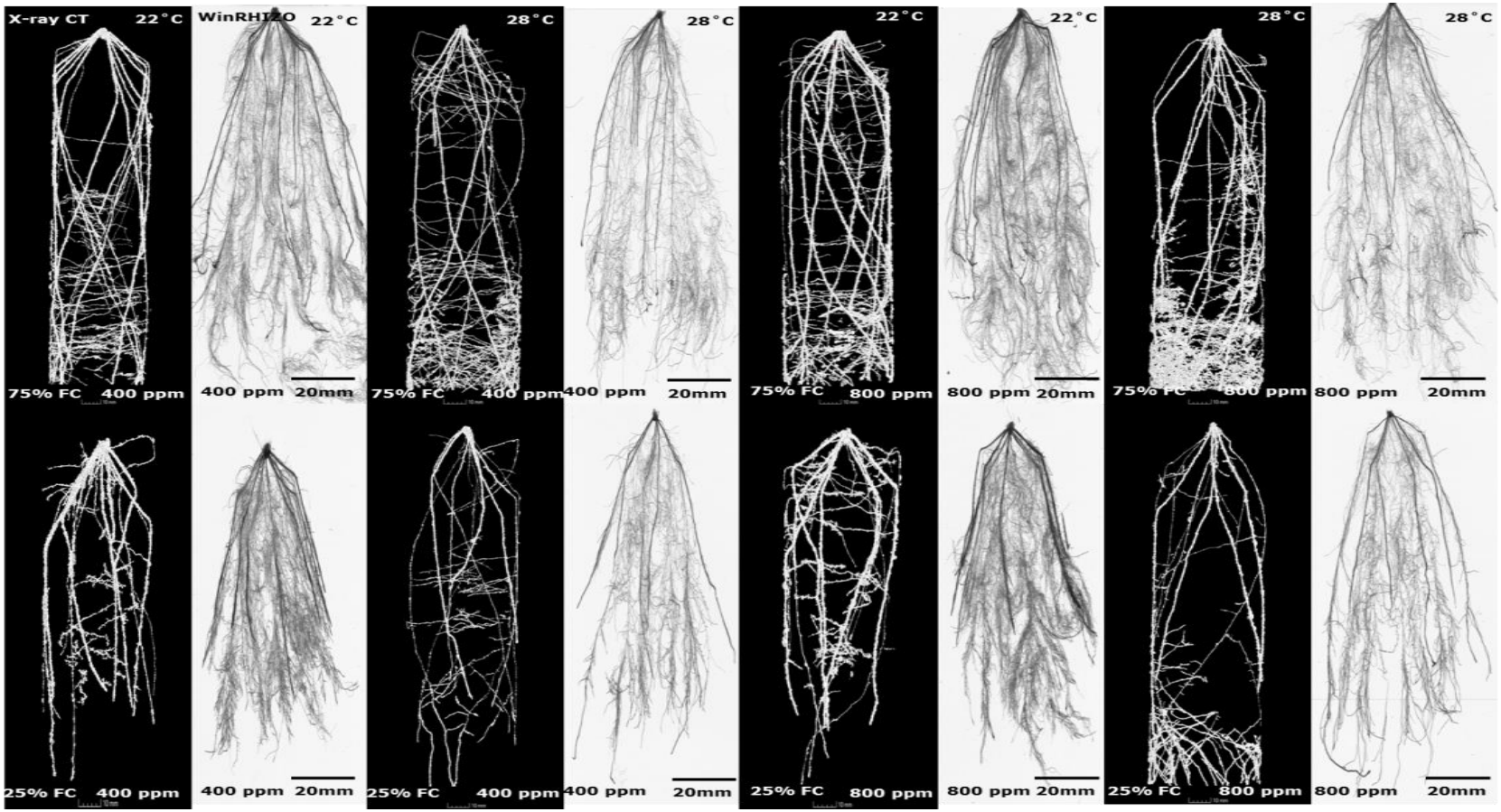


Figure 5.3: CT images of the roots of Xi 19 wheat variety after 14 (A) and 30 (B) days after germination which were grown under 400 and 800 ppm CO_2 levels at 22°C and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil and root systems of plants after extraction and analysis using WinRHIZO equipment. Scale bars represent 10mm and 20mm.

5.3.3. Photosynthesis, stomatal conductance and transpiration

5.3.3.1 Photosynthesis (A_{max})

To investigate the optimal temperature for light – saturated photosynthesis, stomatal conductance and transpiration, these were measured under six different temperatures (22, 24, 28, 30, 32 and 35°C) in plants grown at 28°C at 75% FC at 400 ppm CO_2. The data revealed that photosynthesis increased sharply with increasing temperature from 22°C to 24°C then increased slightly to 30°C, thereafter decreased suddenly to 35°C (Figure 5.4). Therefore, the optimal temperature is between 24 and 30°C, as expected for a C3 plant under these conditions. Stomatal conductance and transpiration increased greatly with increasing temperature from 22°C to 30°C and then decreased to 35°C showing a higher but narrower optimal temperature of around 30 °C in comparison with photosynthesis. These data show that there should be little difference between using 28 or 30 °C for measurement or growth in order to expose temperature-dependent differences

To study the effect of elevated CO_2 and temperature on photosynthesis, stomatal conductance and transpiration of Xi19 and the interaction between photosynthesis and root growth under water stress 14 and 30 days after germination gas exchange was used. Plants were measured using the same [CO_2] they were grown under. The data revealed that photosynthesis for Xi19 at 14 days after germination at 25% FC decreased significantly by 16% in

comparison to 75% FC at both CO_2 levels at 28 °C (P_{water}< 0.01). Photosynthesis for 400 ppm CO_2 was 30% lower than 800 ppm (P_{CO2}< 0.001) (Figure 5.5A). Photosynthesis at 28°C (26.6 μmol.m^{-2}.s^{-1}) was significantly greater than 22°C (23.9 μmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.01). The water*temperature interaction was significant ($P_{water*temperature}$< 0.05). The highest value for photosynthesis was 33.5 μmol.m^{-2}.s^{-1} at 28°C, 800 ppm CO_2 and 75% FC while the lowest value was 18.9 μmol.m^{-2}.s^{-1} at 22°C, 400 ppm CO_2 and 25% FC.

Photosynthesis for Xi19 at 30 days after germination at 25% FC was 22% less than 75% FC at both CO_2 levels (P_{water}< 0.001). Photosynthesis for 400 ppm was 18% lower than 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5.5B). Photosynthesis at 28°C (25.3 μmol.m^{-2}.s^{-1}) was significantly greater than 22°C (22.3 μmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.05). The highest value for photosynthesis was 31.1 μmol.m^{-2}.s^{-1} at 28°C, 800 ppm CO_2 and 75% FC while the lowest value was 17 μmol.m^{-2}.s^{-1} at 22°C, 400 ppm CO_2 and 25% FC.

5.3.3.2 Stomatal conductance (G_s)

The data show that stomatal conductance of Xi19 at 14 days after germination at 25% FC decreased significantly by 19% in comparison to 75% FC at both CO_2 levels (P_{water}< 0.05). Stomatal conductance for 400 ppm CO_2 increased significantly by 28% in comparison to 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5.5A). Stomatal conductance at 28 °C (268.6 mmol.m^{-2}.s^{-1}) was significantly greater than 22 °C (147.1 mmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.001). The

CO_2*temperature interaction was significant ($P_{CO2*temperature}$< 0.05). The highest value for stomatal conductance was 384.2 mmol.m^{-2}.s^{-1} at 28˚C, 400 ppm CO_2 and 75% FC while the lowest value was 131.4 mmol.m^{-2}.s^{-1} at 22˚C, 800 ppm CO_2 and 25% FC.

Stomatal conductance 30 days after germination at 25% FC was 27% lower than at 75% FC at both CO_2 levels (P_{water}< 0.001). Stomatal conductance for 800 ppm CO_2 decreased significantly by 41% in comparison to 400 ppm CO_2 (P_{CO2}< 0.001) (Figure 5.5B). Stomatal conductance at 28 ˚C (302.5 mmol.m^{-2}.s^{-1}) was significantly greater than 22 ˚C (199.6 mmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.001). The water*temperature, CO_2*temperature and water*CO_2*temperature interactions were significant ($P_{water*temperature}$< 0.05, $P_{CO2*temperature}$< 0.01, $P_{water*CO2*temperature}$< 0.05). The highest value for stomatal conductance was 452.2 mmol.m^{-2}.s^{-1} at 28˚C, 400 ppm CO_2 and 75% FC while the lowest value was 118.7 mmol.m^{-2}.s^{-1} at 22˚C, 800 ppm CO_2 and 25% FC.

5.3.3.3 Transpiration (T)

The data show that transpiration of Xi19 at 14 days after germination at 25% FC was 19% less than at 75% FC at both CO_2 levels (P_{water}< 0.001). Transpiration was greater at 400 ppm CO_2 than 800 ppm CO_2 (P_{CO2}< 0.001). T at 28˚C (7.7 µmol.m^{-2}.s^{-1}) was significantly greater than 22˚C (3.3 µmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.001). The water*temperature and CO_2*temperature interactions were significant ($P_{water*temperature}$< 0.01, $P_{CO2*temperature}$= 0.001). The highest value for transpiration was 9.9 µmol.m^{-2}.s^{-1} at 28˚C, 400

ppm CO_2 and 75% FC while the lowest value was 2.3 μmol.m^{-2}.s^{-1} at 22°C, 800 ppm CO_2 and 25% FC (Figure 5.5A).

Transpiration at 30 days after germination at 25% FC also decreased significantly by 19% in comparison to 75% FC at both CO_2 levels (P_{water}< 0.001). Transpiration for 400 ppm CO_2 increased significantly by 30% in comparison to 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5.5B). T at 28°C (6.4 μmol.m^{-2}.s^{-1}) was significantly greater than 22°C (2.8 μmol.m^{-2}.s^{-1}) ($P_{temperature}$< 0.001). The CO_2*temperature interaction was significant ($P_{CO2*temperature}$= 0.001). The highest value for transpiration was 8.4 μmol.m^{-2}.s^{-1} at 28°C, 400 ppm CO_2 and 75% FC while the lowest value was 2 μmol.m^{-2}.s^{-1} at 22°C, 800 ppm CO_2 and 25% FC.

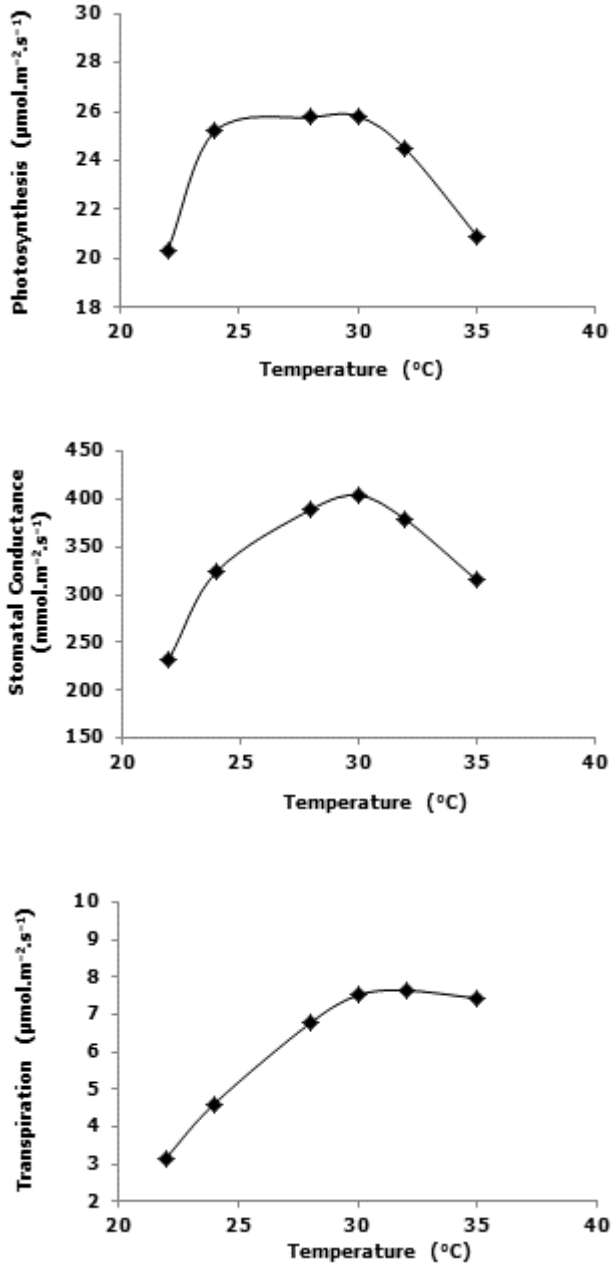


Figure 5.4. Photosynthesis, stomatal conductance and transpiration of Xi 19 wheat variety grown under 400 ppm CO_2 level at 28 °C under soil moisture content (75% FC), means of three replicates but it measured under different temperatures (22, 24, 28, 30, 32, 35 °C) in the sandy loam soil after 30 days after germination after scanning.

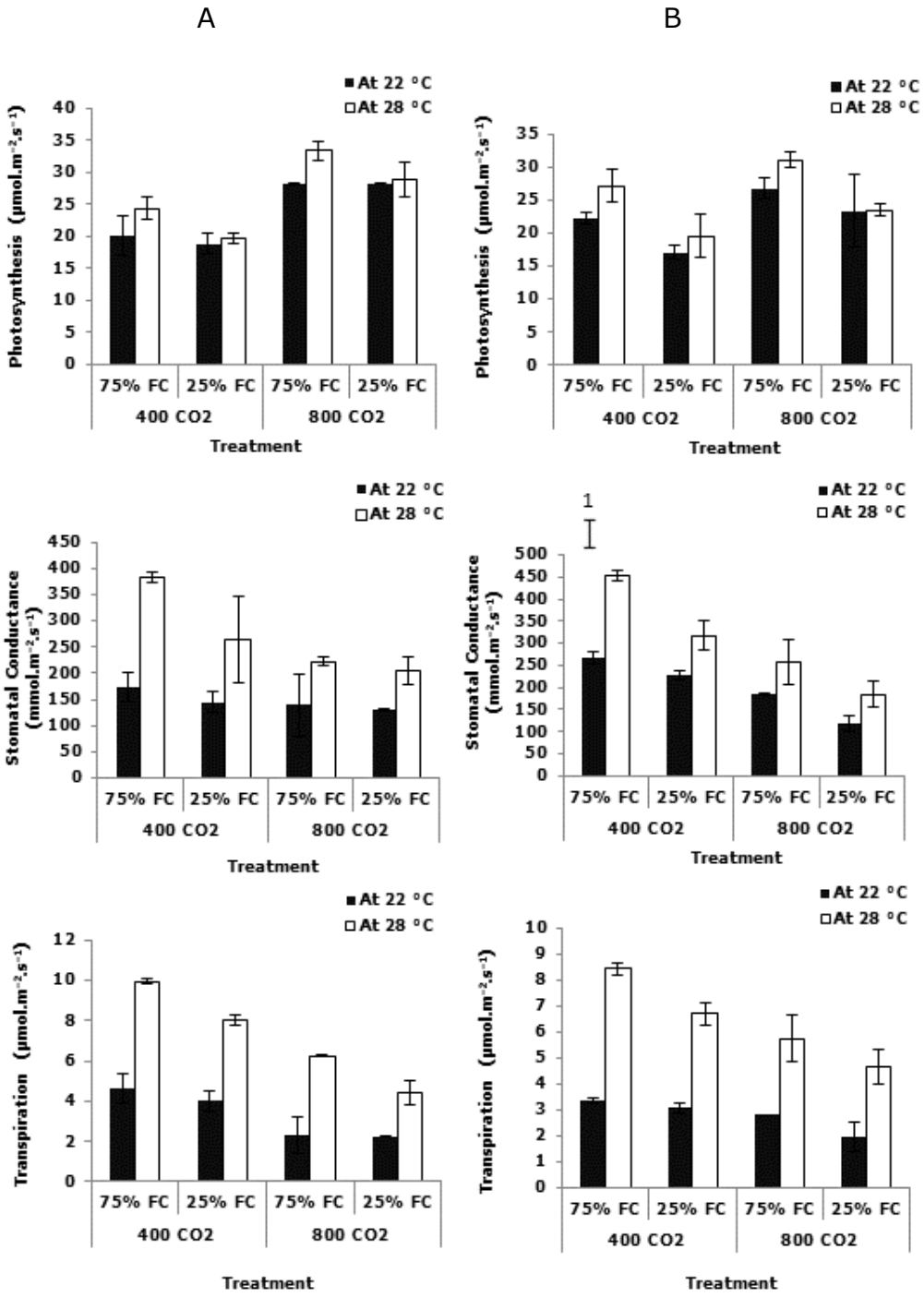


Figure 5.5. Photosynthesis, stomatal conductance and transpiration of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (column A) and 30 (column B) days after germination after scanning. Plants were measured using the same [CO_2] they were grown under. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on photosynthesis, stomatal conductance and transpiration. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant, at $P< 0.05$ of stomatal conductance in column B.

5.3.3.4 Intrinsic water use efficiency (photosynthesis / stomatal conductance) (inWUE)

To study the combined effect of elevated CO_2 and temperature on water use efficiency (photosynthesis / stomatal conductance) of Xi19 under water stress 14 and 30 days after germination, intrinsic water use efficiency was calculated. The data found that inWUE 14 days after germination at 400 ppm CO_2 was 48% less than 800 ppm (P_{CO2}< 0.001) (Figure 5.6A). inWUE at 22˚C (0.2) was significantly greater than 28˚C (0.1) ($P_{temperature}$= 0.001). The highest value for inWUE was 0.24 at 22˚C, 800 ppm CO_2 and 75% FC while the lowest value was 0.06 at 28˚C, 400 ppm CO_2 and 75% FC.

inWUE 30 days after germination increased with decreasing soil water content, except at 22˚C at 400 ppm CO_2 where it slightly decreased (P_{water}> 0.05). inWUE for 400 ppm CO_2 decreased significantly by 53% in comparison to 800 ppm (P_{CO2}< 0.001) (Figure 5. 6B). inWUE at 22˚C (0.13) was significantly greater than 28˚C (0.09) ($P_{temperature}$< 0.001). The water*CO_2 interaction was significant ($P_{water*CO2}$ < 0.05). The highest value for inWUE was 0.2 at 22˚C, 800 ppm CO_2 and 25% FC while the lowest value was 0.06 at 28˚C, 400 ppm CO_2 and 75% FC.

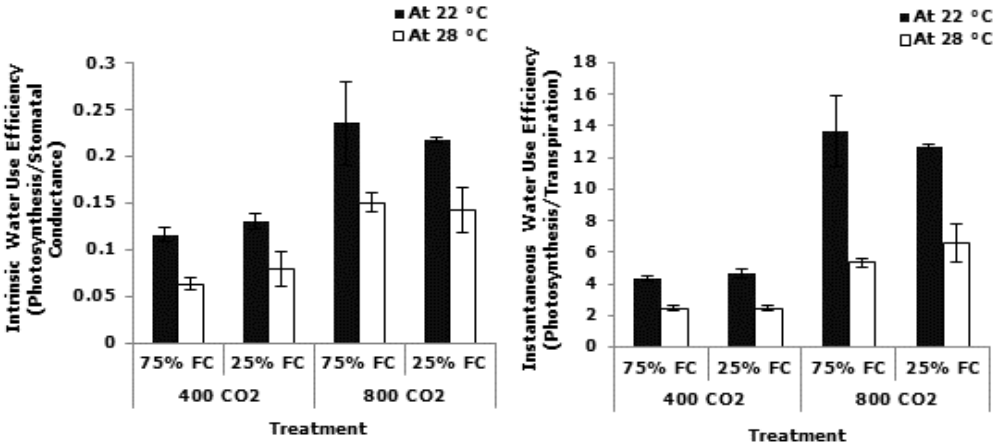
5.3.3.5 Instantaneous water use efficiency (photosynthesis / transpiration) (iWUE)

To examine the combined effect of elevated CO_2 and temperature on water use efficiency (photosynthesis / transpiration) of Xi19 under water stress 14 and 30 days after germination, instantaneous water use efficiency was calculated. The data revealed that iWUE of Xi19

at 14 days after germination for 400 ppm CO_2 decreased significantly by 64% in comparison to 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5. 6A). iWUE at 22°C (8.8) was significantly greater than 28 °C (4.2) ($P_{temperature}$< 0.001). The CO_2*temperature interaction was significant ($P_{CO2*temperature}$< 0.05). iWUE increased with decreasing soil water content at 400 ppm at 22°C and at 800 ppm CO_2 at 28°C while at 400 CO_2 at 28°C and at 800 CO_2 at 22°C it decreased (P_{water}> 0.05). The highest value for iWUE was 13.7 at 22°C at 800 ppm CO_2 at 75% FC while the lowest value was 2.5 at 28°C at 400 ppm CO_2 at 75% and 25% FC.

iWUE for Xi19 at 30 days after germination decreased with reducing soil water content at both CO_2 levels and at both temperature degrees, except at 800 ppm CO_2 at 22°C increased (P_{water}< 0.05). iWUE for 400 ppm CO_2 decreased significantly by 43% in comparison to 800 ppm (P_{CO2}< 0.001) (Figure 5. 6B). iWUE at 22°C (8.4) was significantly greater than 28°C (4.2) ($P_{temperature}$< 0.001). The water*CO_2, CO_2*temperature and water*CO_2*temperature interactions were all significant ($P_{water*CO2}$< 0.01, $P_{CO2*temperature}$< 0.001, $P_{water*CO2*temperature}$< 0.01). The highest value for iWUE was 12 at 22°C at 800 ppm CO_2 at 25% FC while the lowest value was 2.9 at 28°C at 400 ppm CO_2 at 25% FC.

A



B

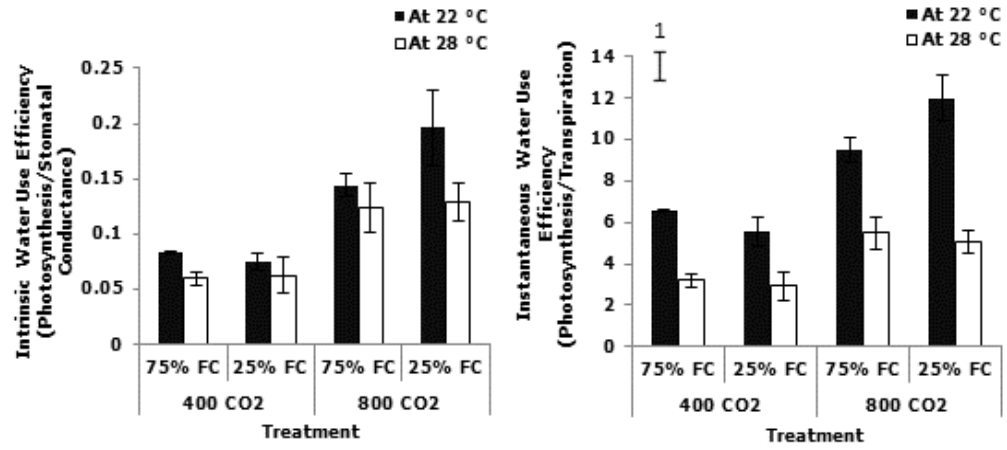


Figure 5.6: inWUE and iWUE of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Plants were measured using the same [CO_2] they were grown under. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on inWUE and iWUE at $P< 0.01$ in (B). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

5.3.4 Combined shoot and root characteristics

5.3.4.1 Shoot dry weight

To estimate the combined effect of elevated CO_2 and temperature on shoot growth of Xi19 under water stress 14 and 30 days after germination, shoot dry weight was measured. The data showed that shoot dry weight 14 days after germination declined significantly with reducing soil water content (P_{water}< 0.001). Shoot dry weight for 400 ppm CO_2 decreased significantly by 16% in comparison to 800 ppm (P_{CO2}< 0.05). Shoot dry weight at 22˚C was greater than that at 28˚C at both CO_2 levels, except at 800 ppm where it was lower ($P_{temperature}$> 0.05). The CO_2*temperature and water*CO_2*temperature interactions were significant ($P_{CO2*temperature}$, $P_{water*CO2*temperature}$< 0.05) (Figure 5. 8A). The highest value for shoot dry weight was 0.10 g at 28˚C at 800 ppm CO_2 at 75% FC while the lowest value was 0.05 g at 28˚C at 400 ppm CO_2 at 25% FC. Examples of images of leaves of Xi 19 grew under different CO_2 levels (400 and 800 ppm CO_2); two different temperatures (22˚C and 28˚C) under different moisture content (75% and 25% FC) 14 days after germination are shown in Figure 5. 7A.

Shoot dry weight at 30 days was reduced significantly with declining soil water content at both CO_2 levels and at both temperatures (P_{water}< 0.001). Shoot dry weight for 400 ppm CO_2 decreased significantly by 27% in comparison to 800 ppm (P_{CO2}< 0.001). Shoot dry weight at 28˚C decreased significantly by 39% in comparison to 22˚C ($P_{temperature}$< 0.001). The water*CO_2*temperature interaction

was significant ($P_{water*CO2}$< 0.001, $P_{water*temperature}$< 0.01, $P_{CO2*temperature}$ < 0.05) (Figure 5. 8B). The highest value for shoot dry weight was 1.34 g at 22°C at 800 CO_2 at 75% FC, while the lowest value was 0.26 g at 28°C at 400 CO_2 at 25% FC. Examples of images of leaves of Xi 19 grew under different CO_2 levels (400 and 800 ppm CO_2); two temperatures (22°C and 28°C) under different water stress levels (75% and 25% FC) 30 days after germination are shown in Figure 5.7B.

5.3.4.2 Root dry weight

Root dry weight was measured to study the effect of elevated CO_2 and temperature on root growth under water stress of Xi19 at 14 and 30 days after germination. The data showed that root dry weight 14 days after germination at 28°C decreased significantly by (21%) in comparison to 22°C ($P_{temperature}$< 0.05). The CO_2*temperature interaction was significant ($P_{CO2*temperature}$< 0.01). Root dry weight increased with decreasing soil moisture content in the sandy soil at both CO_2 levels, except at 800 ppm CO_2 at 28°C where it decreased. At the same temperature (28°C) at 400 ppm CO_2 it was the same (P_{water}> 0.05). The highest value for root dry weight was 0.04 g at 28°C at 400 ppm CO_2 at 25% FC while the lowest value was 0.02 g at 28°C at 400 ppm CO_2 at 75% FC and 25% FC (Figure 5. 8A).

Root dry weight 30 days after germination at 25% FC decreased significantly by 40% in comparison to 75% FC (P_{water}< 0.001). Root dry weight at 400 ppm CO_2 level decreased significantly by 27% in

comparison to 800 ppm CO_2 (P_{CO2}< 0.001). Root dry weight at 28˚C (46%) decreased significantly in comparison to 22˚C ($P_{temperature}$< 0.001). The water*temperature and CO_2*temperature interactions were significant ($P_{water*temperature}$< 0.05, $P_{CO2*temperature}$= 0.001). The highest value for root dry weight was 0.65 g at 22˚C at 800 ppm CO_2 at 75% FC while the lowest value was 0.14 g at 28˚C at 400 ppm CO_2 at 25% FC (Figure 5. 8B).

5.3.4.4 Total dry weight

To estimate the effect of elevated CO_2 and temperature on plant growth under water stress of Xi19 at 14 and 30 days after germination, total dry weight was measured. The data show that total dry weight 14 days after germination at 25% FC decreased significantly by 23% in comparison to 75% FC (P_{water}< 0.01). The CO_2*temperature interaction was significant ($P_{CO2*temperature}$< 0.01) (Figure 5. 8A). Total dry weight for 800 ppm CO_2 was slightly greater than at 400 ppm CO_2 (P_{CO2}> 0.05). The highest value for total dry weight was 0.13 g at 22˚C under 400 CO_2 and at 28˚C under 800 ppm at low water stress level (75% FC) while the lowest value was 0.07 g at 28˚C at 400 ppm CO_2 at 25% FC.

Total dry weight 30 days after germination at 25% FC decreased significantly by 51% in comparison to 75% FC (P_{water}< 0.001). Total dry weight for 400 ppm CO_2 decreased significantly by 27% in comparison to 800 ppm CO_2 (P_{CO2}< 0.001). Total dry weight at 28˚C decreased significantly by 41% in comparison to 22˚C ($P_{temperature}$< 0.001). The water*CO_2 and CO_2*temperature were significant

interactions ($P_{water*CO2}$= 0.001, $P_{CO2*temperature}$< 0.01) (Figure 5. 8B). The highest value for total dry weight was 2 g at 22°C at 800 ppm CO_2 at 75% FC while the lowest value was 0.38 g at 28°C at 400 ppm CO_2 at 25% FC.

5.3.4.3 Shoot length

To further examine the effect of elevated CO_2 and temperature on shoot growth under water stress of Xi19 at 14 and 30 days after germination, shoot length was measured. The data revealed that shoot length 14 days after germination at 25% FC reduced by 15% in comparison to 75% FC (P_{water}< 0.001). Shoot length at 400 ppm CO_2 level declined by 14% in comparison to 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5. 8A). The highest value for shoot length was 24.3 cm at 28°C at 800 ppm CO_2 at 75% FC, while the lowest value was 19.7 cm at 28°C at 400 ppm CO_2 at 25% FC.

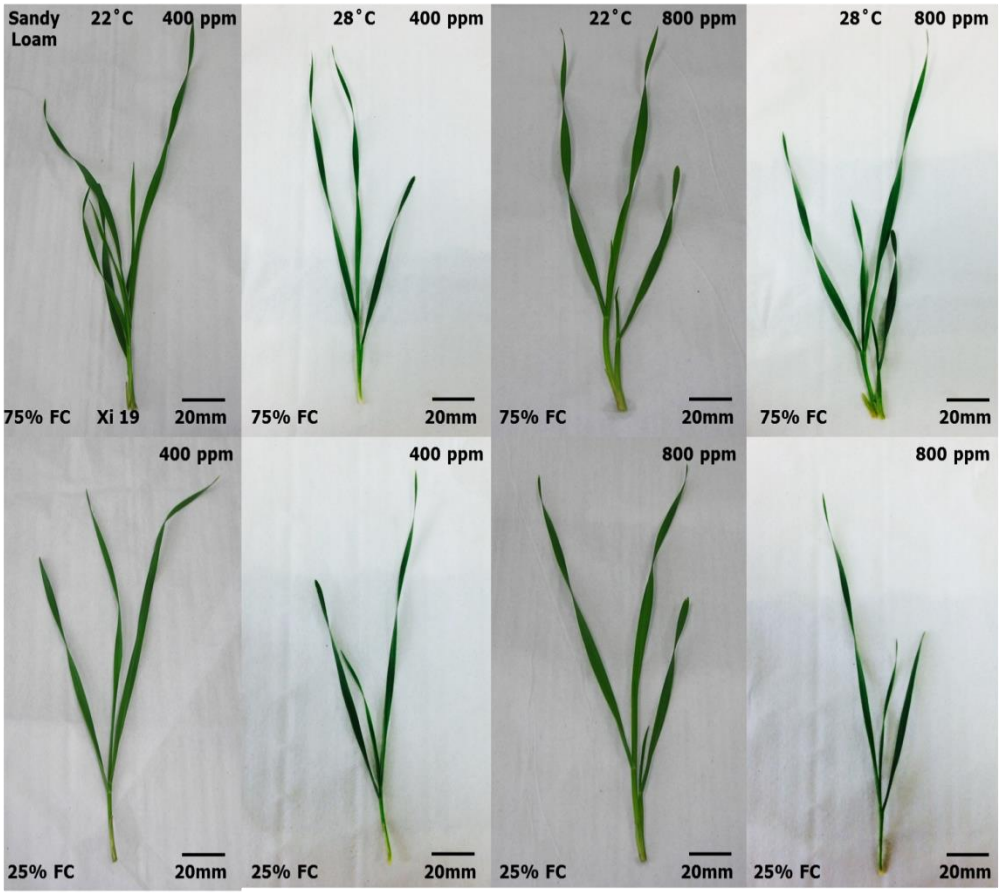
Shoot length 30 days after germination decreased significantly with decreasing soil moisture content in the sandy loam (P_{water}< 0.001). Shoot length at 400 ppm CO_2 level decreased significantly by 7% in comparison to 800 ppm CO_2 (P_{CO2}< 0.05). Shoot length at 22°C was significantly greater than those at 28°C ($P_{temperature}$< 0.001) (Figure 5. 8B). The highest value for shoot length was 39.7 cm at 22°C at 800 ppm CO_2 at 75% FC, while the lowest value was 27.8 cm at 28°C at 400 ppm CO_2 at 25% FC.

5.3.4.5 Root to shoot ratio

Root and shoot ratio responses to elevated CO_2 and temperature under water stress of Xi19 were tested at 14 and 30 days after germination. The results found root to shoot ratio 14 days after germination at 25% FC increased significantly by 41% in comparison to 75% FC (P_{water}< 0.001). Root to shoot ratio for 800 ppm CO_2 decreased significantly by 21% in comparison to 400 ppm CO_2 (P_{CO2}< 0.001). Root to shoot ratio at 22˚C was significantly greater than at 28˚C, except at 800 ppm CO_2 at 25% FC was lower ($P_{temperature}$< 0.01). The water*CO_2, CO_2*temperature and water*CO_2*temperature interactions were significant ($P_{water*CO2}$< 0.01, $P_{CO2*temperature}$< 0.001, $P_{water*CO2*temperature}$< 0.001) (Figure 5. 8A). The highest value for root to shoot ratio was 0.81 at 22˚C at 400 ppm CO_2 at 25% FC, while the lowest value was 0.26 at 28˚C at 800 ppm CO_2 at 75% FC.

Root to shoot ratio 30 days after germination increased significantly with decreasing soil moisture content (P_{water}< 0.001). Root to shoot ratio at 22˚C was significantly greater than at 28˚C, except at 400 ppm CO_2 at 75% FC which was lower ($P_{temperature}$< 0.001). The water*temperature interaction was significant ($P_{water*temperature}$< 0.001) (Figure 5. 8B). The highest value for root to shoot ratio was 0.75 at 22˚C at 800 ppm CO_2 at 25% FC, while the lowest value was 0.47 at 28˚C at 800 ppm CO_2 at 75% FC.

A


Sandy Loam
22˚C
400 ppm
75% FC
Xi 19
20mm
28˚C
400 ppm
75% FC
20mm
22˚C
800 ppm
75% FC
20mm
28˚C
800 ppm
75% FC
20mm
400 ppm
25% FC
20mm
400 ppm
25% FC
20mm
800 ppm
25% FC
20mm
800 ppm
25% FC
20mm

B

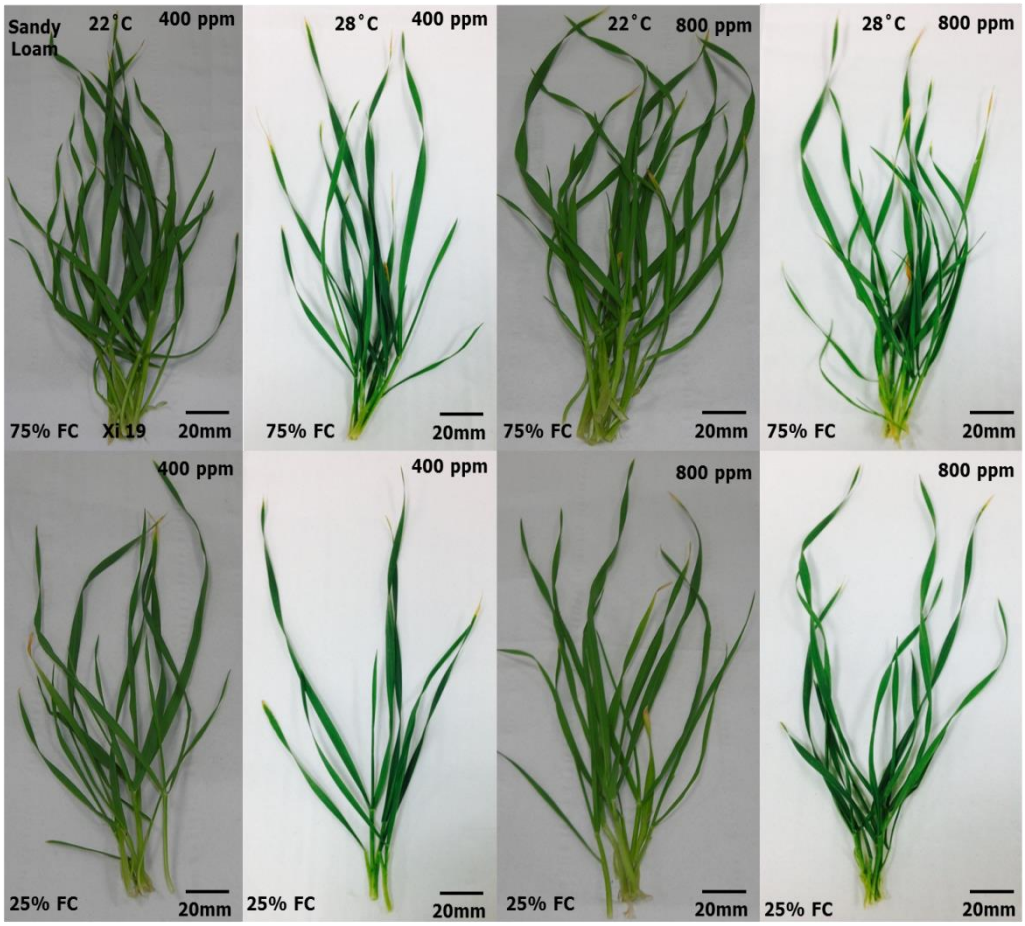
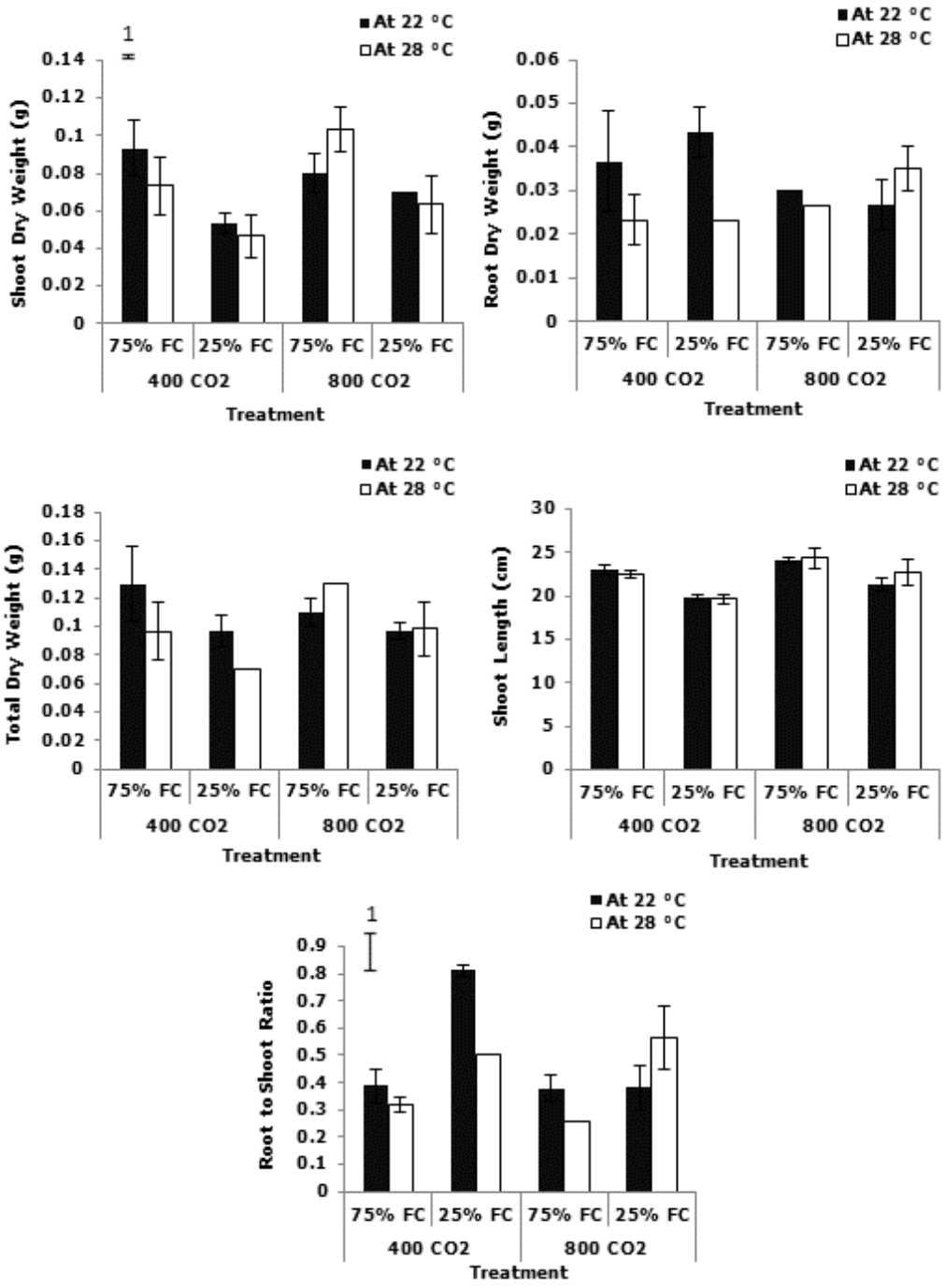


Figure 5.7: Leaves of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22°C and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Scale bar represents 20mm.

A


At 22 °C
At 28 °C
Shoot Dry Weight (g)
Root Dry Weight (g)
Total Dry Weight (g)
Shoot Length (cm)
Root to Shoot Ratio
75% FC
25% FC
400 CO2
800 CO2
Treatment

B

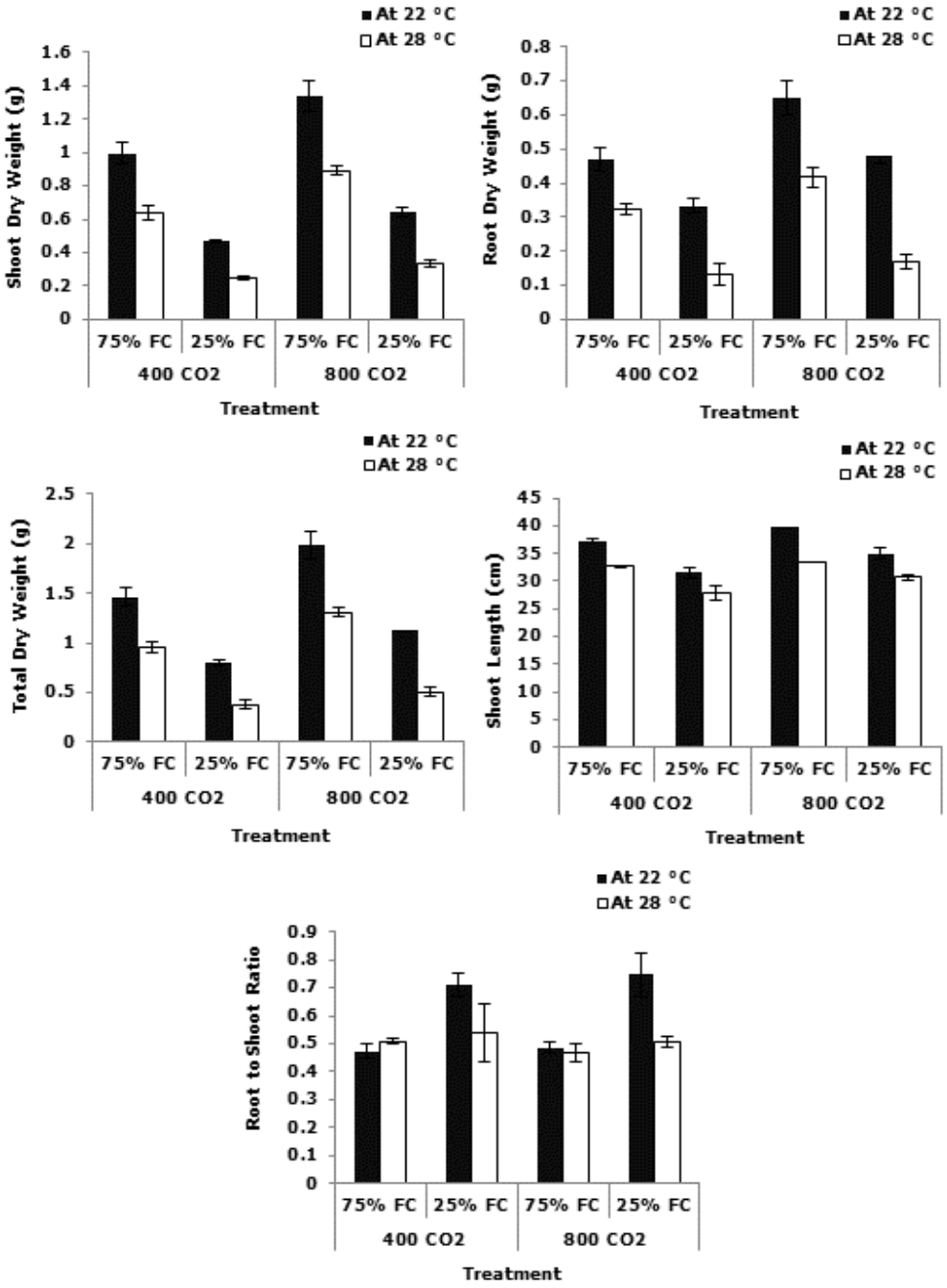


Figure 5.8: Shoot, root and total dry weight, shoot length and root to shoot ratio of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 days (B) after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on shoot (at $P< 0.05$ in column A), root and total dry weight, shoot length and root to shoot ratio (at $P< 0.001$ in column A). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

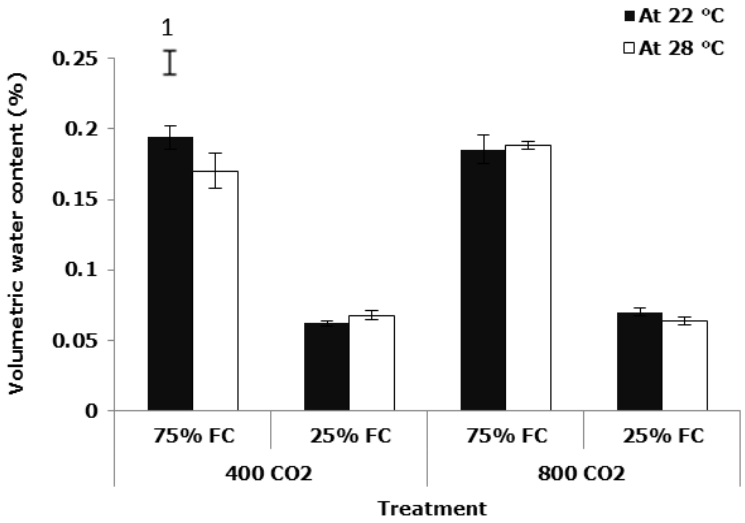
5.3.5 Soil characteristics

5.3.5.1 Volumetric water content

Volumetric water content 14 days after germination at 25% FC decreased significantly by 64% in comparison to 75% FC (P_{water}< 0.001). The water*CO_2*temperature interaction was significant ($P_{water*CO2*temperature}$< 0.01) (Figure 5. 9A). Volumetric water content at 22˚C was greater than that at 28˚C, except at 400 ppm CO_2 at 25% FC and at 800 ppm CO_2 at 75% FC which was lower ($P_{temperature}$> 0.05, P_{CO2}> 0.05).

Volumetric water content 30 days after germination at 25% FC decreased significantly by (67%) in comparison to 75% FC (P_{water}< 0.001). There was no significant difference between CO_2 treatments (P_{CO2}> 0.05). Volumetric water content at 22˚C was greater than that at 28˚C but not significantly ($P_{temperature}$> 0.05) (Figure 5. 9B).

A



B

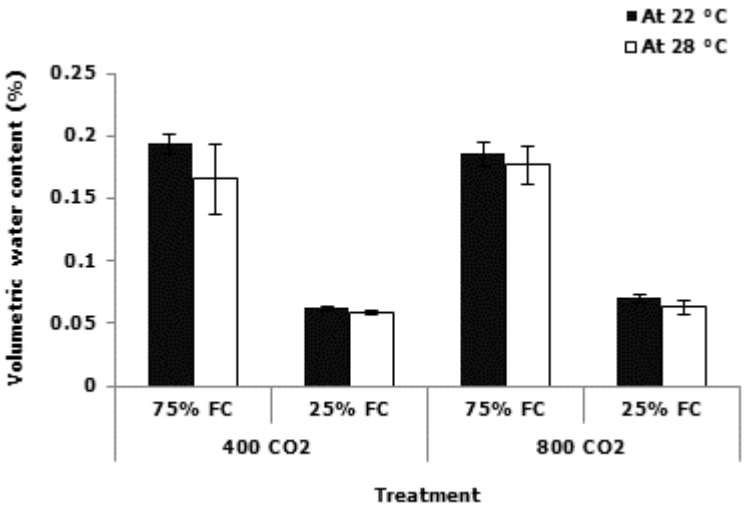


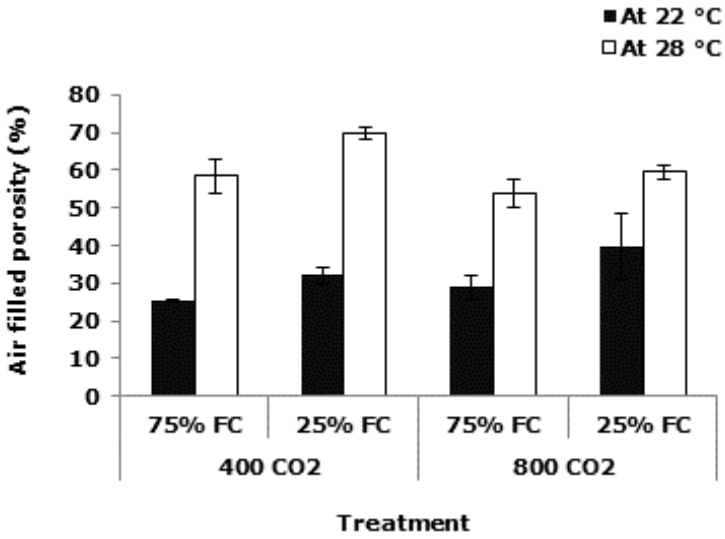
Figure 5.9: Volumetric water content of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on volumetric water content (at $P< 0.01$ in column A). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

5.3.5.2 Air filled porosity

X-ray CT was used to study the effect of elevated CO_2 and temperature under water stress condition on air filled porosity 14 and 30 days after germination. The data showed that air filled porosity 14 days after germination increased significantly with decreasing soil moisture content in the sandy loam (P_{water}< 0.001). Air filled porosity at 28°C was significantly greater than that at 22°C ($P_{temperature}$< 0.001). The CO_2*temperature interaction was significant ($P_{CO2*temperature}$= 0.001) (Figure 5. 10A). Air filled porosity at 800 ppm CO_2 was greater than at 400 ppm CO_2 at 22°C, while at 400 ppm CO_2 was greater than at 800 ppm CO_2 at 28°C (P_{CO2}> 0.05). The highest value for air filled porosity was 69.8% at 28°C at 400 ppm CO_2 at 25% FC, while the lowest value was 25.2 % at 22°C at 400 ppm CO_2 at 75% FC.

Air filled porosity 30 days after germination increased significantly with decreasing soil moisture content (P_{water}< 0.001). Air filled porosity at 800 ppm CO_2 was significantly greater than at 400 ppm CO_2 (P_{CO2}< 0.001). Air filled porosity at 28°C was significantly greater than that at 22°C ($P_{temperature}$< 0.001). The water*temperature and CO_2*temperature interactions were significant ($P_{water*temperature}$< 0.01, $P_{CO2*temperature}$< 0.05) (Figure 5.10B). The highest value for air filled porosity was 45.8% at 28°C at 800 ppm CO_2 at 25% FC, while the lowest value was 32.5% at 22°C at 400 ppm CO_2 at 75% FC.

A



B

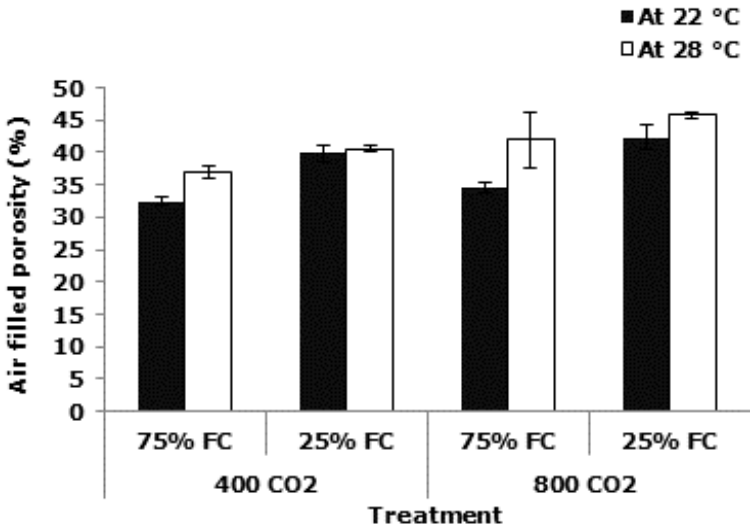


Figure 5.10: Air filled porosity of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 levels at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on air filled porosity.

5.3.6 Water use efficiency

5.3.6.1 Water use efficiency (shoot dry weight)

Water use efficiency (shoot dry weight) 14 days after germination decreased with decreasing soil water content at both CO_2 levels at both temperature degrees (P_{water}< 0.05). Water use efficiency for 800 ppm CO_2 (0.89 $kg.m^{-3}$) was significantly greater than 400 ppm CO_2 (0.66 $kg.m^{-3}$) (P_{CO2}< 0.001). Water use efficiency at 22˚C (0.92 $kg.m^{-3}$) was significantly greater than that at 28˚C (0.63 $kg.m^{-3}$) ($P_{temperature}$< 0.001) (Figure 5. 11A).

Water use efficiency (shoot dry weight) 30 days after germination decreased significantly with decreasing soil water content (P_{water}< 0.001). Water use efficiency for 800 ppm CO_2 (2.2 $kg.m^{-3}$) was significantly greater than 400 ppm CO_2 (1.3 $kg.m^{-3}$) (P_{CO2}< 0.001). Water use efficiency at 22˚C (2.24 $kg.m^{-3}$) was significantly greater than that at 28˚C (1.23 $kg.m^{-3}$) ($P_{temperature}$< 0.001). The water*CO_2, CO_2*temperature and water*CO_2*temperature interactions were significant ($P_{water*CO2}$< 0.001, $P_{CO2*temperature}$< 0.001, $P_{water*CO2*temperature}$< 0.05) (Figure 5. 11B).

5.3.6.2 Water use efficiency (root dry weight)

Water use efficiency (root dry weight) of Xi19 at 14 days after germination for 800 ppm CO_2 (0.32 $kg.m^{-3}$) was significantly greater than 400 ppm CO_2 (0.26 $kg.m^{-3}$) (P_{CO2}< 0.05). Water use efficiency at 22˚C (0.35 $kg.m^{-3}$) was significantly greater than that at 28˚C (0.23 $kg.m^{-3}$) ($P_{temperature}$< 0.001). The water*temperature interaction was significant ($P_{water*temperature}$< 0.05) (Figure 5. 11A).

Water use efficiency decreased with decreasing soil water content at 22°C while it increased at 28°C (P_{water}> 0.05).

Water use efficiency (root dry weight) for Xi19 at 30 days after germination increased significantly with decreasing soil water content (P_{water}< 0.001). Water use efficiency for 800 ppm CO_2 (1.29 $kg.m^{-3}$) was significantly greater than 400 ppm CO_2 (0.65 $kg.m^{-3}$) (P_{CO2}< 0.001). Water use efficiency at 22°C (1.32 $kg.m^{-3}$) was significantly greater than that at 28°C (0.62 $kg.m^{-3}$) ($P_{temperature}$< 0.001). The water*temperature and CO_2*temperature interactions were significant ($P_{water*temperature}$= 0.001, $P_{CO2*temperature}$< 0.001) (Figure 5.11B).

5.3.6.3 Water use efficiency (total dry weight)

Water use efficiency (total dry weight) of Xi 19 at 14 days after germination for 400 ppm CO_2 was 25% less than 800 ppm CO_2 (P_{CO2}< 0.001) (Figure 5.11A). Water use efficiency at 22°C (2.24 $kg.m^{-3}$) was significantly greater than that at 28°C (1.23 $kg.m^{-3}$) ($P_{temperature}$< 0.001). Water use efficiency decreased with decreasing soil water content at both CO_2 levels at both temperatures, except at 28°C at 800 ppm CO_2 where it increased slightly (P_{water}> 0.05).

Water use efficiency (total dry weight) for Xi 19 at 30 days after germination decreased significantly with decreasing soil water content, except at 400 ppm CO_2 at 22°C increased (P_{water}< 0.05). Water use efficiency for 800 ppm CO_2 (1.22 $kg.m^{-3}$) was significantly greater than at 400 ppm (0.92 $kg.m^{-3}$) (P_{CO2}< 0.001). Water use

efficiency at 22 °C (1.26 $kg.m^{-3}$) was significantly greater than at 28°C (0.88 $kg.m^{-3}$) ($P_{temperature} < 0.001$). The water*CO_2, water*temperature and CO_2*temperature interactions were significant ($P_{water*CO2} < 0.05$, $P_{water*temperature} < 0.01$, $P_{CO2*temperature} < 0.001$) (Figure 5.11B).

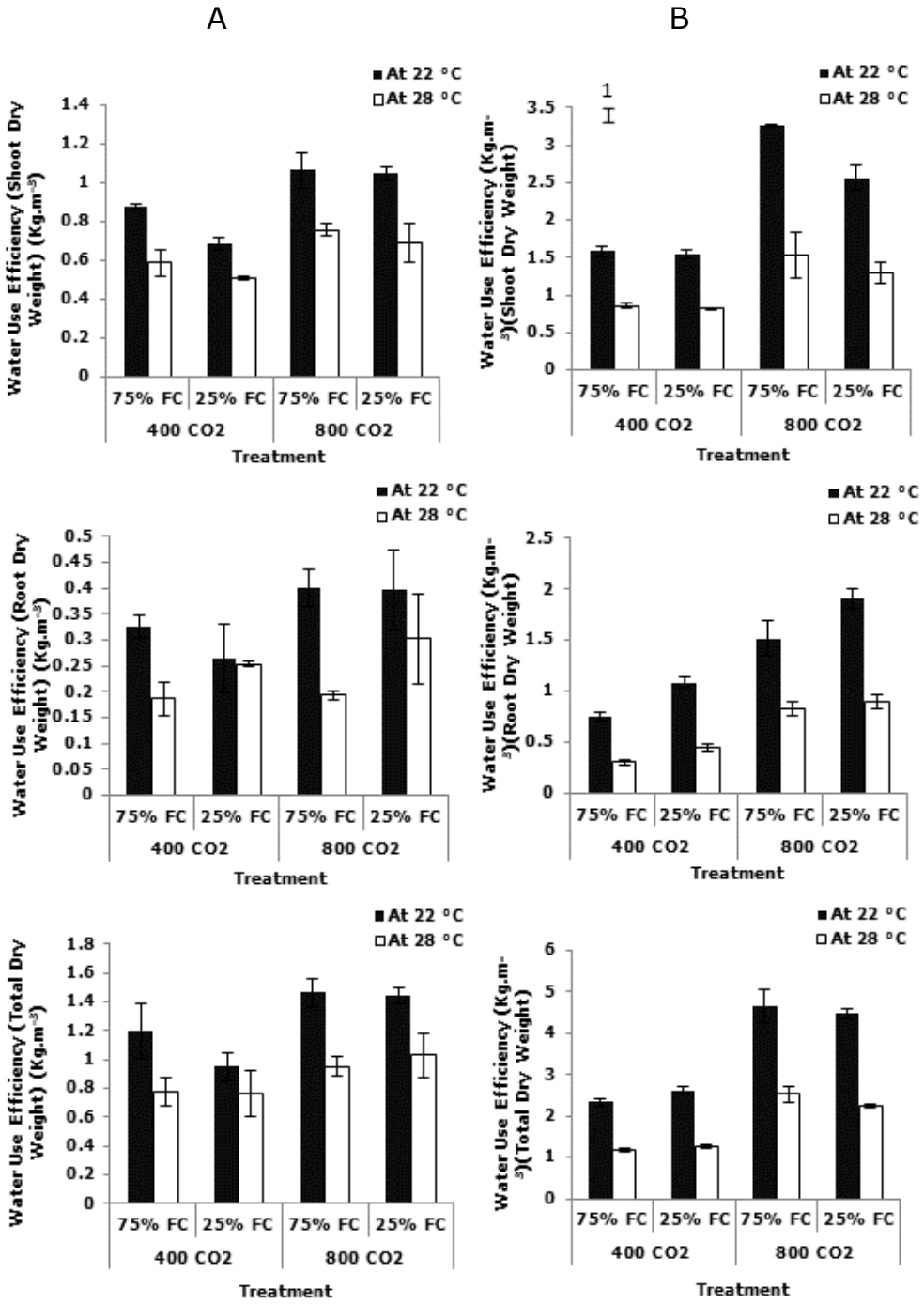


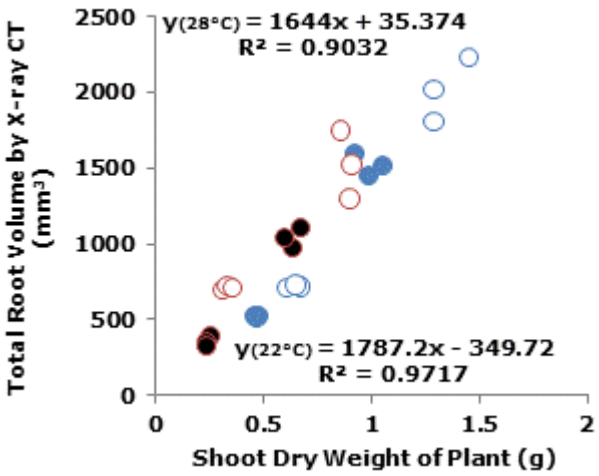
Figure 5.11: Water use efficiency (based on shoot, root and total dry weight) of Xi 19 wheat variety grown under 400 and 800 ppm CO_2 at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (column A) and 30 (column B) days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed water x CO_2 x temperature interaction on water use efficiency (based on shoot, root and total dry weight). Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant (at P< 0.01 of water use efficiency (based on shoot dry weight in column B)).

5.3.7 Correlation matrix analysis

Summary statistical analysis was performed to determine correlation between measurements of shoot and root of Xi 19 grew under elevated CO_2 and temperature under water stress at 14 and 30 days independently after germination. The results found that Plant (total) dry weight at 14 days after germination was strongly and positively correlated with shoot dry weight (Table 5. 2A). Stomatal conductance was strongly and positively correlated with transpiration. There was a strong and positive correlation between total root volume and mean root diameter by both methods (WinRHIZO and X-ray CT). X-ray CT total root volume also strongly correlated with air filled porosity (Table 5. 2A).

The results showed that total dry weight at 30 days after germination was strongly and positively correlated with shoot and root dry weight, volumetric water content, X-ray CT total root volume and shoot length (Table 5. 2B). Stomatal conductance was strongly positively correlated with transpiration while it was negatively correlated with water use efficiency (based on root dry weight) and inWUE (Table 5. 2B). The data also showed an important correlation between each of the shoot and total dry weights with total root volume by X-ray CT of plants grown under the combined effect elevated CO_2 and temperature under water stress conditions in the sandy loam soil type with typical R^2 values of 0.9 to 0.97 (Figure 5.12).

A



B

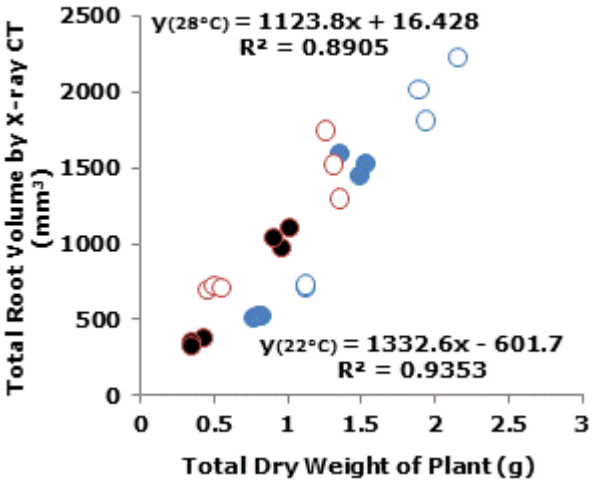


Figure 5.12: Scatter diagram between each of the shoot (A) and total dry weight (B) with total root volume by X-ray CT of Xi 19 wheat variety grown under conditions 400 ● ● and 800 ○ ○ ppm CO_2 at 22 and 28°C, respectively under two different soil moisture contents (75% and 25% FC) in the sandy loam soil 30 days post germination after scanning, at $P< 0.05$, n=3.

Table 5.2: Correlation matrix between all variables (see list of abbreviations) of Xi 19 wheat variety grown under conditions 400 and 800 ppm CO_2 at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination at P< 0.05.

A.

Positive significant correlation between variables	Critical significant value	0.404
Negative significant correlation between variables	Number of observations:	24
No significant correlation between variables		

Key relationship

Plantdwt	**1**	-																	
Psynth	**2**	0.36	-																
RSRatio	**3**	-0.31	-0.53	-															
Root_dwt	**4**	0.50	-0.23	0.64	-														
STomC	**5**	-0.13	-0.03	-0.29	-0.35	-													
Shoot_dwt	**6**	0.93	0.51	-0.63	0.16	-0.01	-												
Transp	**7**	-0.20	-0.19	-0.22	-0.37	0.91	-0.07	-											
VWC	**8**	0.57	0.25	-0.68	-0.17	0.19	0.72	0.15	-										
WRDiam	**9**	-0.08	-0.53	0.75	0.62	-0.59	-0.35	-0.53	-0.46	-									
WRRootvol	**10**	0.11	-0.35	0.65	0.67	-0.62	-0.15	-0.58	-0.32	0.96	-								
WUE_R	**11**	0.13	0.02	0.03	0.22	-0.53	0.05	-0.71	-0.10	0.28	0.27	-							
WUE_SH	**12**	0.39	0.33	-0.29	0.08	-0.58	0.42	-0.76	0.26	0.17	0.27	0.75	-						
WUE_T	**13**	0.32	0.27	-0.18	0.14	-0.61	0.31	-0.81	0.14	0.21	0.28	0.86	0.98	-					
X_rayAvgDiam	**14**	-0.07	-0.44	0.52	0.44	-0.41	-0.26	-0.47	-0.30	0.83	0.80	0.40	0.28	0.32	-				
X_ray_RootVol	**15**	0.13	-0.36	0.43	0.50	-0.66	-0.06	-0.67	-0.14	0.78	0.80	0.54	0.48	0.52	0.82	-			
iwuepc	**16**	0.23	0.45	-0.11	0.07	-0.75	0.23	-0.80	0.01	0.19	0.31	0.49	0.71	0.69	0.14	0.42	-		
iwuept	**17**	0.15	0.45	-0.16	0.00	-0.65	0.17	-0.79	0.00	0.15	0.25	0.60	0.77	0.77	0.16	0.39	0.96	-	
porosity_sp	**18**	-0.44	0.15	-0.13	-0.49	0.65	-0.30	0.69	-0.31	-0.59	-0.68	-0.52	-0.68	-0.66	-0.55	-0.80	-0.48	-0.46	-
reg_p_ht	**19**	0.61	0.63	-0.67	-0.12	0.02	0.75	-0.12	0.74	-0.52	-0.35	0.09	0.40	0.31	-0.40	-0.20	0.27	0.27	-0.22	-
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**

B.

Plantdwt	1	-																	
Psynth	2	0.44	-																
RSRatio	3	-0.23	-0.47	-															
Root_dwt	4	0.96	0.35	0.04	-														
STomC	5	-0.20	0.13	-0.34	-0.31	-													
Shoot_dwt	6	0.99	0.48	-0.34	0.92	-0.15	-												
Transp	7	-0.41	0.28	-0.42	-0.54	0.89	-0.34	-											
VWC	8	0.80	0.60	-0.63	0.65	0.29	0.85	0.13	-										
WRDiam	9	-0.10	-0.49	0.87	0.13	-0.63	-0.21	-0.68	-0.62	-									
WRRootvol	10	0.96	0.30	0.01	0.98	-0.32	0.92	-0.56	0.64	0.16	-								
WUE_R	11	0.44	-0.01	0.54	0.62	-0.84	0.35	-0.85	-0.13	0.73	0.60	-							
WUE_SH	12	0.79	0.24	0.16	0.86	-0.60	0.74	-0.68	0.33	0.37	0.87	0.84	-						
WUE_T	13	0.70	0.15	0.36	0.82	-0.65	0.62	-0.73	0.18	0.53	0.82	0.92	0.97	-					
X_rayAvgDiam	14	-0.24	-0.37	0.59	-0.08	-0.77	-0.31	-0.59	-0.69	0.74	-0.08	0.64	0.28	0.39	-				
X_ray_RootVol	15	0.91	0.58	-0.54	0.78	-0.07	0.95	-0.19	0.91	-0.42	0.78	0.17	0.59	0.43	-0.40	-			
iwuepc	16	0.34	0.36	0.24	0.44	-0.80	0.28	-0.62	-0.06	0.44	0.42	0.83	0.69	0.74	0.58	0.22	-		
iwuept	17	0.57	0.11	0.42	0.72	-0.77	0.49	-0.83	0.06	0.57	0.70	0.94	0.85	0.91	0.49	0.32	0.84	-	
porosity_sp	18	-0.63	0.00	0.31	-0.56	-0.33	-0.65	0.05	-0.67	0.30	-0.59	0.13	-0.28	-0.18	0.58	-0.55	0.34	-0.05	-
reg_p_ht	19	0.86	0.32	-0.17	0.84	-0.32	0.84	-0.52	0.64	0.00	0.86	0.49	0.74	0.67	-0.20	0.79	0.42	0.63	-0.52	-
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**

Positive significant correlation between variables	**Critical significant value** 0.404
Negative significant correlation between variables	**Number of observations:** 24
No significant correlation between variables	

Key relationship

Table 5.3: P values for all variables by ANOVA of Xi 19 wheat variety grown under conditions 400 and 800 ppm CO_2 at 22 and 28°C under two different soil moisture contents (75% and 25% FC) in the sandy loam soil after 14 (A) and 30 (B) days after germination after scanning.

A.

Measurements/Treatment	Water	CO_2	Temp.	Water*CO_2* Temp.
Total root volume (X-ray)	P> 0.05	P> 0.05	P< 0.001	P> 0.05
Mean root diameter (X-ray)	P< 0.05	P> 0.05	P< 0.001	P> 0.05
Total root volume (WinRHIZO)	P< 0.001	P< 0.05	P< 0.001	P< 0.01
Mean root diameter (WinRHIZO)	P< 0.001	P< 0.001	P< 0.001	P< 0.001
Photosynthesis	P< 0.01	P< 0.001	P< 0.01	P> 0.05
Stomatal conductance	P< 0.05	P< 0.001	P< 0.001	P> 0.05
Transpiration	P< 0.001	P< 0.001	P< 0.001	P> 0.05
inWUE	P> 0.05	P< 0.001	P= 0.001	P> 0.05
iWUE	P> 0.05	P< 0.001	P< 0.001	P> 0.05
WUE (Shoot dry weight)	P< 0.05	P< 0.001	P< 0.001	P> 0.05
WUE (Root dry weight)	P> 0.05	P< 0.05	P< 0.001	P> 0.05
WUE (Total dry weight)	P> 0.05	P< 0.001	P< 0.001	P> 0.05
Shoot dry weight	P< 0.001	P< 0.05	P> 0.05	P< 0.05
Root dry weight	P> 0.05	P> 0.05	P< 0.05	P> 0.05
Shoot length	P< 0.001	P< 0.001	P> 0.05	P> 0.05
Total dry weight	P< 0.01	P> 0.05	P> 0.05	P> 0.05
Root to shoot ratio	P< 0.001	P< 0.001	P< 0.01	P< 0.001
Air filled porosity	P< 0.001	P> 0.05	P< 0.001	P> 0.05
Volumetric water content	P< 0.001	P> 0.05	P> 0.05	P< 0.01

	Significant p values
	Non-significant p values

B.

Measurements/Treatment	Water	CO_2	Temp.	Water*CO_2* Temp.
Total root volume (X-ray CT)	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Mean root diameter (X-ray CT)	P< 0.001	P< 0.001	P< 0.05	P> 0.05
Total root volume (WinRHIZO)	P< 0.001	P< 0.001	P< 0.001	P< 0.05
Mean root diameter (WinRRHIZO)	P< 0.001	P< 0.001	P< 0.001	P= 0.001
Photosynthesis	P< 0.001	P< 0.001	P< 0.05	P> 0.05
Stomatal conductance	P< 0.001	P< 0.001	P< 0.001	P< 0.05
Transpiration	P< 0.001	P< 0.001	P< 0.001	P> 0.05
inWUE	P> 0.05	P< 0.001	P< 0.001	P> 0.05
iWUE	P> 0.05	P< 0.001	P< 0.001	P< 0.01
WUE (Shoot dry weight)	P< 0.001	P< 0.001	P< 0.001	P< 0.01
WUE (Root dry weight)	P< 0.001	P< 0.001	P< 0.001	P> 0.05
WUE (Total dry weight)	P< 0.05	P< 0.001	P< 0.001	P> 0.05
Shoot dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Root dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Shoot length	P< 0.001	P< 0.05	P< 0.01	P> 0.05
Total dry weight	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Root to shoot ratio	P< 0.001	P> 0.05	P< 0.001	P> 0.05
Air filled porosity	P< 0.001	P< 0.001	P< 0.001	P> 0.05
Volumetric water content	P< 0.001	P> 0.05	P> 0.05	P> 0.05

	Significant p values
	Non-significant p values

5.4 Discussion

Drought and high temperature are significant environmental factors that restrict plant photosynthesis and yield, while elevated CO_2 can stimulate plant growth and photosynthesis. A number of studies have examined the combined effects of elevated CO_2 and high temperature or elevated CO_2 and water deficit, but none have investigated the combined effects of these variables on shoot and root growth in wheat. Therefore, it is very important to investigate the combined effect of elevated CO_2 and temperature on wheat root and shoot growth under water stress conditions.

5.4.1 Total root volume and mean root diameter

Water stress increased total root volume at ambient and high temperature at both CO_2 levels (400 and 800 ppm) for the short term experiment (14 days) whereas, it significantly decreased total root volume during for the long term experiment (30 days). The reasons for this may be related to the partitioning of resources according to phenology, with more allocation to shoots as the plants mature, this is probably due to the plants being root-bound (the root system does not have enough space to grow). Or it could be due to root length and root branching decreasing under elevated CO_2 leading to decrease in root biomass (Benlloch-Gonzalez et al., 2014).

Total root volume during 30 days was significantly improved by elevated CO_2 under ambient and high temperature, but the positive

effect of elevated CO_2 was less when the plants were grown under high temperature. Elevated CO_2 enhanced both roots volume and shoot growth at both temperatures compared with ambient CO_2. These findings support the hypothesis that elevated CO_2 enhance root architecture systems but not preferentially over shoot growth as has been previously reported elevated CO_2 leads to enhance root growth (Rogers et al., 1994, Rogers et al., 1995). See section 5.4.3 for further discussion on the effect of elevated CO_2 effect of elevated CO_2 on shoot and root growth.

The results also showed that elevated CO_2 significantly increased mean root diameter of Xi 19 during 30 days after germination in comparison to ambient CO_2. It has been found that roots become more numerous, longer, thicker, and faster growing in crops exposed to high CO_2 with increased root length in many plant species (Madhu and Hatfield, 2013).

5.4.2 Photosynthesis measurements

Elevated CO_2 with ambient and high temperature increased significantly photosynthesis for both time periods (14 and 30 days after germination). However, stomatal conductance and transpiration decreased significantly under high CO_2 levels as commonly observed. Higher photosynthesis was most likely due to high Rubisco activity and particularly a higher carboxylase to oxygenase activity (Lieman-Hurwitz et al., 2003) caused by a higher rate of CO_2 transfer and hence Ci.

However decreases in stomatal conductivity and transpiration caused by partial stomatal closure under elevated CO_2 could have been offset by increased leaf area and vapour pressure deficits (VPDs) caused by increased leaf temperature (Prasad et al., 2005). However the higher leaf temperature in this experiment did not result in adverse VPDs (data not shown).

Higher temperature significantly enhanced photosynthesis. The higher temperatures used in this experiment was much closer to the optimal temperature for photosynthesis in this variety as shown in Figure 5.4. This is probably due to the activities of Calvin cycle and electron transport with temperature-dependent limitations to enzyme activity (e.g., Rubisco). This is despite the enhanced oxygenation of Rubisco that would normally be observed in C3 plants at temperatures higher than 25 °C and suggests that photosynthesis in this variety is adapted to high temperatures. Importantly higher temperatures were also associated with higher values of stomatal conductance (Song et al., 2014). This may have helped to overcome the higher ratios of oxygenation to carboxylation expected at higher temperatures. High CO_2 commonly decreases stomatal conductance, while high temperature increases stomatal conductivity and transpiration due to an increased vapour pressure gradient between leaf surface and atmosphere. It would therefore be expected that drought will be more severe at higher temperatures, although that was not observed in this experiment suggesting that the water deficit was not as severe as

anticipated. This is probably due to the required water that was added to the plants every day to reach the required levels of water stress. Consequently, a combination of elevated CO_2 and temperature could give variable effects (Prasad et al., 2005).

Instantaneous and agronomic water use efficiency also improve under elevated CO_2 (Rogers et al., 1995, Drake et al., 1997, Garcia et al., 1998, Long et al., 2004, Palta et al., 2009). This could be due to reduced stomatal conductance, which reduces water use, and due to increased photosynthesis rates and increased biomass accumulation (Miglietta et al., 1996, Garcia et al., 1998, Bunce, 2000).

5.4.3 Shoot and root characteristics

Roots of water-stressed plants during 14 days grown with elevated CO_2 produced significantly higher root dry mass than roots of well-watered plants grown with ambient CO_2. The present data are in agreement with Benlloch-Gonzalez et al. (2014) and Chaudhuri et al. (1990). These responses in root growth and morphology led to elevated CO_2 and decreased soil moisture content and support the hypothesis that plants grown in a high CO_2 environment may better compensate for soil water stress conditions (Wechsung et al., 1999, Schenk and Jackson, 2002). An adaptation mechanism of plants to water stress occurs by physiological and biochemical changes in the plant (Akıncı and Lösel, 2006). The general consensus is that photosynthesis and C allocation to plant roots increases as CO_2 increases which leads to an increase in

the above and below biomass (Del Castillo et al., 1989, Norby et al., 1992, Rogers et al., 1994).

The 30 day experiment showed that elevated CO_2 enhanced shoot and root biomass accumulation under both ambient and high temperature, but the positive effect of elevated CO_2 on biomass was reduced with increasing temperature. This is probably due to high temperature restricting the positive effect of elevated CO_2 on shoot and root growth. It has been reported that root and shoot growth was improved by elevated CO_2 under ambient and high temperature, but the positive effect of elevated CO_2 was less when plants were grown under high temperature (Benlloch-Gonzalez et al., 2014). It has also been reported that high CO_2 concentration often stimulates the growth of shoots and roots, but the question remains whether elevated CO_2 affects root and shoot growth proportionally (Rogers et al., 1995).

Root to shoot ratio (dry mass) should help as a measurement of the preferential allocation of C to roots or shoots (Madhu and Hatfield, 2013). In this study, under high temperature and water stress elevated CO_2 significantly increased root system architecture and shoot growth compared to root to shoot ratio. This could be due to the nutrient amount within the soil, (Norby, 1987) concluded that limiting nutrients may promote partitioning to the roots and assimilate partitioning under high CO_2 may depend on nutrient status (Lawlor and Mitchell, 1991). Madhu and Hatfield (2013) suggested to quantify the nutrient levels in

the soil when interpreting root to shoot ratio responses to elevated CO_2. It has also been reported that different factors could be associated with root to shoot ratio including shifts in nutrient availability (Madhu and Hatfield, 2013), water uptake (Shangguan et al., 2004), light exposure (Nagel et al., 2015) and CO_2 concentrations (Jia et al., 2011). Our results are in line with that findings in Larrea tridentate, a desert herb (Obrist and Arnone 2003), tall grasses (Mo et al., 1992), four native chalk grassland herbs (Ferris and Taylor, 1993), and winter wheat (Chaudhuri et al., 1990). It has been stated in a review on root to shoot ratio of crops as influenced by CO_2 that 60% of studies on crops show a positive response on root to shoot ratio, 37% show a negative response, and 3% show no response (Rogers et al., 1995). Allen et al. (1988) reported that soybean grown under increased CO_2 maintained a similar partitioning of C into their respective components. Similar results were reported by (Baxter et al., 1994) in grasses, but roots often exhibit the greatest relative dry weight gain under high CO_2 (Hocking and Meyer, 1991). The root to shoot ratio response to elevated levels of CO_2 differed among species and growth conditions (Ferris and Taylor, 1993). Further studies are needed to investigate the effect of elevated CO_2, high temperature and water stress on root to shoot ratio of early plant growth until the harvesting time to confirm positive effect of elevated CO_2 on root to shoot ratio at all plant growth stages.

5.4.4 Water use efficiency

In this study, the data showed that increased CO_2 levels from 400 to 800 ppm led to increased water use efficiency and these are in agreement with that reported by Tyree and Alexander (1993). This could be due to decreased stomatal conductance and transpiration and increased photosynthesis at elevated CO_2. It was also found that elevated CO_2 increased plant growth (both the above-and below ground) and improved plant water relations (reduces transpiration and increases WUE) (Prior et al., 2011). Increased water use efficiency due to decreased stomatal conductance at high CO_2 can be offset by increased temperature (Jones et al., 1985, Allen et al., 1985, Allen, 2000). Polley (2002) concluded that yield benefits of improved water use efficiency at high CO_2 are eroded due to decreased harvest index at high temperatures. Therefore, if global temperatures increase with increasing CO_2, future crop water use will increase especially in arid subtropical and tropical regions where air temperatures are already high.

5.5 Conclusions

It can be concluded that total root volume and mean root diameter increased significantly in response to water stress at both CO_2 levels after 14 days under both temperatures whereas, after 30 days the root volume decreased significantly. Total root volume, mean root diameter, shoot and root dry weights at 22°C were significantly greater than those at 28°C. Elevated CO_2 combined with ambient and high temperature increased significantly photosynthesis while the stomatal conductance and transpiration decreased significantly. Photosynthesis parameters at 28°C were significantly greater than at 22°C. Water use efficiency for 800 ppm CO_2 was significantly greater than 400 ppm. There is a need to carry out more research that focuses on plant growth under the same conditions (elevated CO_2, temperature and water stress) to consider variations over longer timescales than considered here.

6.0 The effect of repeated soil wetting and drying cycles on shoot and root growth of wheat under elevated temperature

6.1 Introduction

The data reported in chapter four indicated that Istabraq was the most drought resistant wheat variety compared with Hereward and Xi 19. Therefore, Istabraq was chosen to investigate the responses of root and shoot growth of wheat to repeated soil wetting and drying irrigation in two different soil textures (sandy and clay) during early stage of vegetative growth which is more indicative of a natural wetting pattern in the field regardless of whether the water is via precipitation or irrigation. Efficient use of water by irrigation is becoming increasingly important (Shahnazari et al., 2005). With regards to the intensity and the duration of stress, plant response to water scarcity differs (Farooq et al., 2009). Constant or slowly imposed water stress often inhibits photosynthesis for long periods resulting from stomatal closure (Saccardy et al., 1996) and consequently reduces plant height, biomass, and leaf area (Maseda and Fernández, 2006). With time, development of a larger root system can exploit a greater volume of soil, consequently increasing the potential for water absorption. Furthermore, increases in root to shoot ratio proportionally reduce the evaporation area of shoots relative to the absorptive area of roots (Silva et al., 2012). Development of novel water saving irrigation

techniques represents another option for increasing water use efficiency (Shahnazari et al., 2005).

Changes in climate (rainfall patterns) and/or resource management (irrigation quotas) mean that future crops, either unintentionally or deliberately, will receive deficit irrigation (DI, less water than crop ET), necessarily drying the soil, limiting leaf expansion and gas exchange and, consequently, yield (Dodd, 2009). Decreasing water resources and limited clean water reservoirs will make it difficult to satisfy food requirements in the future as 70%, and in some places 90% of the available water resources is used in food production (Mao et al., 2003). To manage with the water shortage, it is essential to adopt water-saving agriculture counter measures (Mao et al., 2003).

Many studies have focussed on the effect of wetting and drying cycles on wheat (Whalley et al., 2006, Whitmore and Whalley, 2009, Whitmore et al., 2011, Silva et al., 2012), but no study has considered the influence of repeated wetting and drying cycles on shoot and root growth in different soil textures. Therefore, this chapter examined the effect of repeated soil wetting and drying cycles on wheat growth, photosynthesis, stomatal conductance and transpiration and the interaction between root growth and photosynthesis in different soil textures. It was hypothesized that repeated wetting and drying cycles would reduce the effect of drought on root and shoot growth of wheat

in comparison to a more constant water treatment i.e. water content maintained at a specific matric potential or % of field capacity .

6.2 Materials and methods

6.2.1 Sample preparation and X-ray μCT

A Newport series loamy sand and a Worcester series clay soil were prepared and packed as before (chapter three) but using soil columns (20cm height × 5cm diameter). Three replicates were prepared for each soil type and treatment combination to give a total of 24 columns. All columns were then water-saturated and drained for 2 days (to reach to a notional field capacity which is hypothesised to represent soil moisture content after free drainage had ceased) and then to reach to different levels of field capacity (75%, 75-50%, 75-25%, and 50-25% FC). At 75% FC all plants were kept at the same moisture content during the whole experiment. Whereas for the 75-50% and 75-25% FC treatments they were left to drain down to the calculated water contents appropriate for 50% and 25% FC, respectively after which they had water added to the plants to return to 75% FC. For the 50-25% FC the plants were grown at 50% FC and left to drain to 25% FC. An average of the time of irrigation and number of cycling for all these water treatments were shown in Table 6.1. Details of the exact volumetric water content, matric potentials and other relevant soil properties are given in Table 6.2. Seeds of Istabraq were germinated for 48 hrs before being planted 5 mm below the soil surface. Thereafter, they were placed in a growth room under conditions of 28/22°C day/night with a 16 hrs photoperiod, at 50% relative humidity. All columns were placed in a transpired propagator to

maintain high relative humidity levels during germination and seedling growth. They were weighed daily and sufficient water was added to maintain soil moisture content close to the four pre-determined moisture contents (75%, 75-50%, 75-25%, and 50-25% FC) as wetting and drying cycles. These columns were scanned at day 30 after germination using a Phoenix V|tome|x m Computed Tomography X-ray µCT scanner set at 150 kV and 160 µA, and an image averaging of 3. Pixel/voxel resolution was set at 56 µm and each scan took 76 min to complete. For each column, 2160 image projections were captured on all sampling dates; each volume had a size of approx. 46GB. Thereafter, these images were reconstructed using VG StudioMAX (Volume graphics).

6.2.2 Image processing and analysis

Root systems were non-destructively segmented from the grey-scale µCT images using the Region Growing selection tool in VG StudioMAX® 2.0 software as described by Tracy et al. (2012). Region Growing classifies voxels in a certain grey-value range from a starting seed point. Tolerance values were adjusted to ensure that only root material was included in the growing region of interest (ROI) from the original seed points. The root systems segmented from the µCT image data were used for quantitative determination of root volume and mean diameter. The mean root diameter was measured by the distance measurement tool. As the study was concerned with the interaction

between roots and soil, the soil pore characteristics were also measured in addition to root measurements by using the calibration wizard and volume analyser tools in VGStudioMAX®.

6.2.3 Plant measurements

Photosynthesis, stomatal conductance and transpiration were measured the day after scanning by infrared gas analysis (LI-6400XT Portable Photosynthesis System, Licor, Illinois). The settings were the same as mentioned above (second experiment, chapter four).

6.2.4 Statistical analysis

The results were analysed by general analysis of variance (ANOVA) including water, soil types and their interaction as explanatory variables using Genstat 15.1. In addition, the correlation between variables was used by summary statistics using Genstat 17 for analysing data. Finally, the results were also analysed using principal component by multivariate analysis (biplot).

Table 6.1: Time of irrigation at four different water treatments (75%, 75-50%, 75-25%, and 50-25% FC) and number of wetting and drying cycles in the loamy sand and the clay soil types.

Water Treatment	Loamy sand (day)	Clay (day)	No. of wetting and drying cycles in loamy sand	No. of wetting and drying cycles in clay
75% FC	1	2	30	15
75-50% FC	2	4	15	8
75-25% FC	4	6	8	4
50-25% FC	3	4	10	8

Table 6.2: The values of volumetric water content, matric potential (ψ)(-kPa) at different water content (100%, 75%, 50% and 25% FC), the percentage of the sand, silt, the clay, Organic Matter by Loss on Ignition, pH and nutrient content for the loamy sand and the clay soils in Newport and Worcester.

Soil Properties	Loamy Sand	Clay
VWC ($g.g^{-1}$) at 100%FC	0.31	0.38
VWC ($g.g^{-1}$) at 75%FC	0.23	0.28
VWC ($g.g^{-1}$) at 50%FC	0.16	0.19
VWC ($g.g^{-1}$) at 25%FC	0.08	0.09
Ψ (-kPa) at 100%FC	20	80
Ψ (-kPa) at 75%FC	50	400
Ψ (-kPa) at 50%FC	200	1300
Ψ (-kPa) at 25%FC	1000	1500
% sand	87	34
% silt	9	19
% clay	4	47
Organic Matter by Loss on Ignition (%)	4.1	7.9
pH	8	7
Carbon (mg/kg soil)	17374	28609
Nitrogen (mg/kg soil)	1572	3002
Phosphorus (mg/kg soil)	199	240
Potassium (mg/kg soil)	4088	7298
Magnesium (mg/kg soil)	1535	3735
Sulphur (mg/kg soil)	41	52
Sodium (mg/kg soil)	595	689
Calcium (mg/kg soil)	888	1738

6.3 Results

6.3.1 Root characteristics

6.3.1.1 Total root volume

The root volume by X-ray CT and WinRHIZO® analysis at 30 days post germination significantly decreased when subjected to increased water stress conditions as compared to 75% FC in both soil types (the loamy sand and clay) (P_{water}< 0.001, $P_{soil*water}$ < 0.001) (Figure 6.1 and 6.2). WinRHIZO® analysis and X-ray CT revealed that total root volume in the loamy sand (6814 and 1410 mm^3, respectively) was significantly greater than the clay (4164 and 963 mm^3) (P_{soil}< 0.001) (Figure 6.1 and 6.2). The p-values for all variables at 30 days after germination of Istabraq are shown in Table 6.4.

6.3.1.2 Mean root diameter

Mean root diameter by X-ray CT and WinRHIZO® analysis 30 days post germination decreased significantly with increasing water stress in the loamy sand and the clay, except in the clay soil type where it increased between the 75-25% FC to 50-25% FC treatments (P_{water}< 0.001, $P_{soil*water}$< 0.001) (Figure 6.1 and Figure 6.2). X-ray CT and WinRHIZO® analysis also revealed that mean root diameter in the loamy sand was significantly greater than the clay, except at 75% FC which was lower (P_{soil}<0.001) (Figure 6.1 and 6.2).

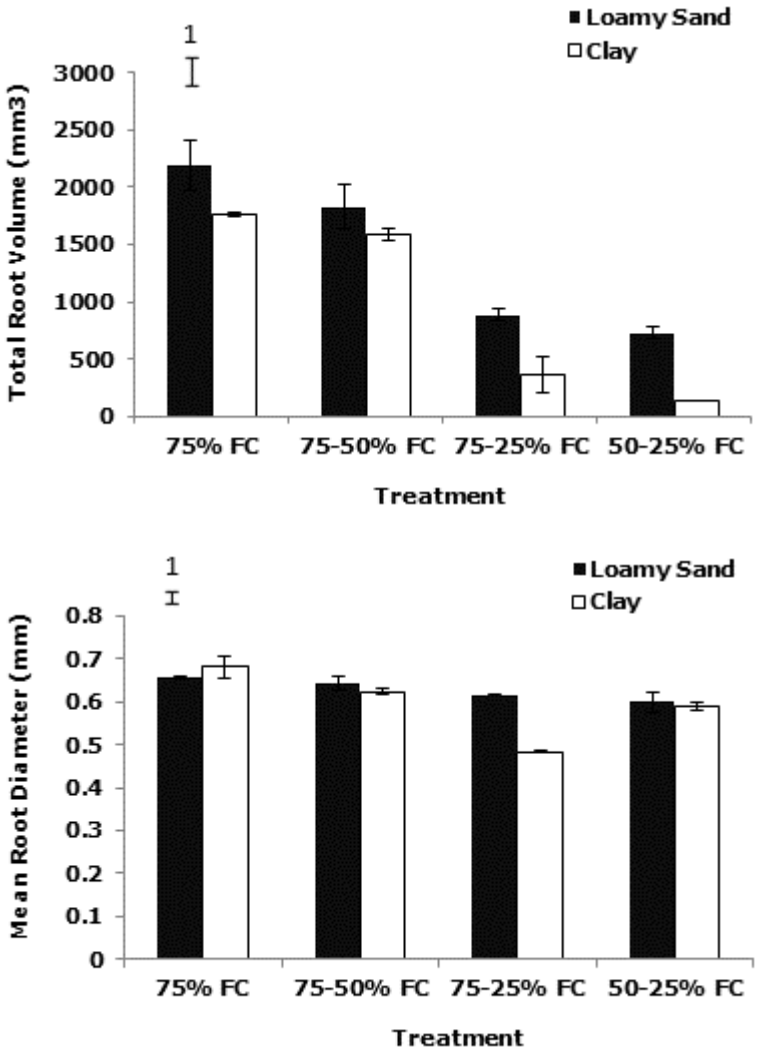


Figure 6.1: Total volume and mean diameter of root by using X-ray CT of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on total root volume and mean root diameter at P< 0.001. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

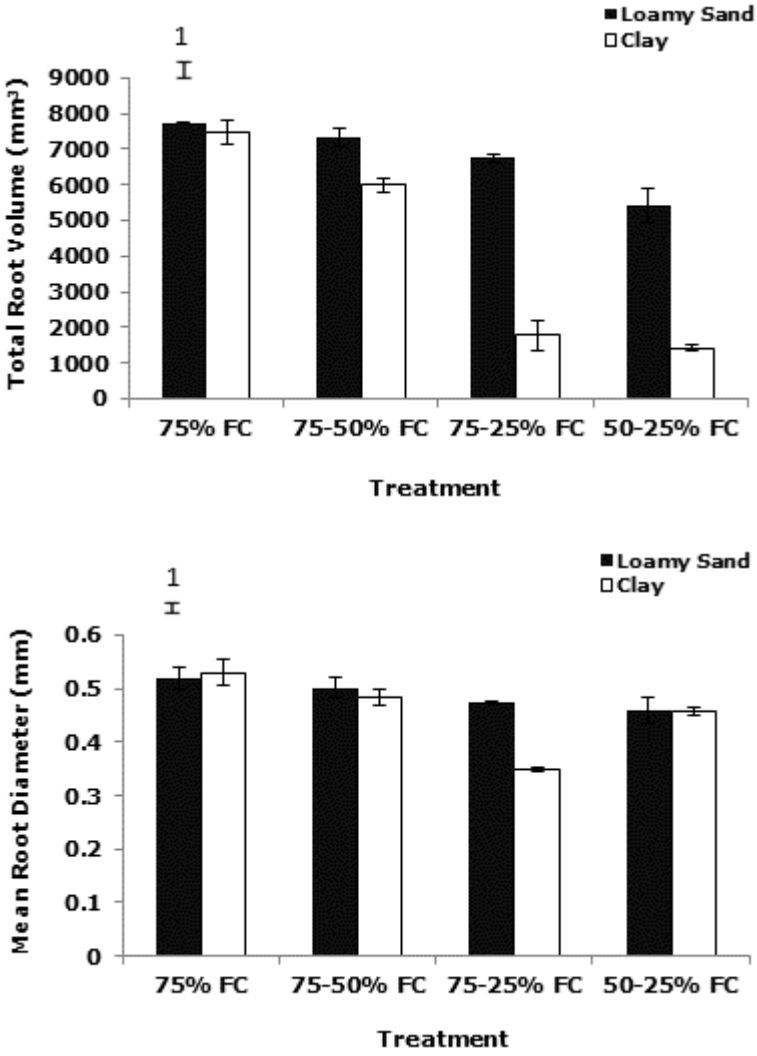


Figure 6.2: Total volume and mean diameter of root by using WinRHIZO of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on total root volume and mean root diameter at $P< 0.001$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.1.3 Comparison of root imaging methods

An example of images of root architecture for both methods is shown in Figure 6.3 and 6.4. The main roots decreased significantly due to water stress in both soil types in both measurement approaches. It was also found (Figure 6.3, 6.4) that there was a significant difference between soil textures (loamy sand and clay) especially at 75-25% FC and 50-25% FC water treatments.

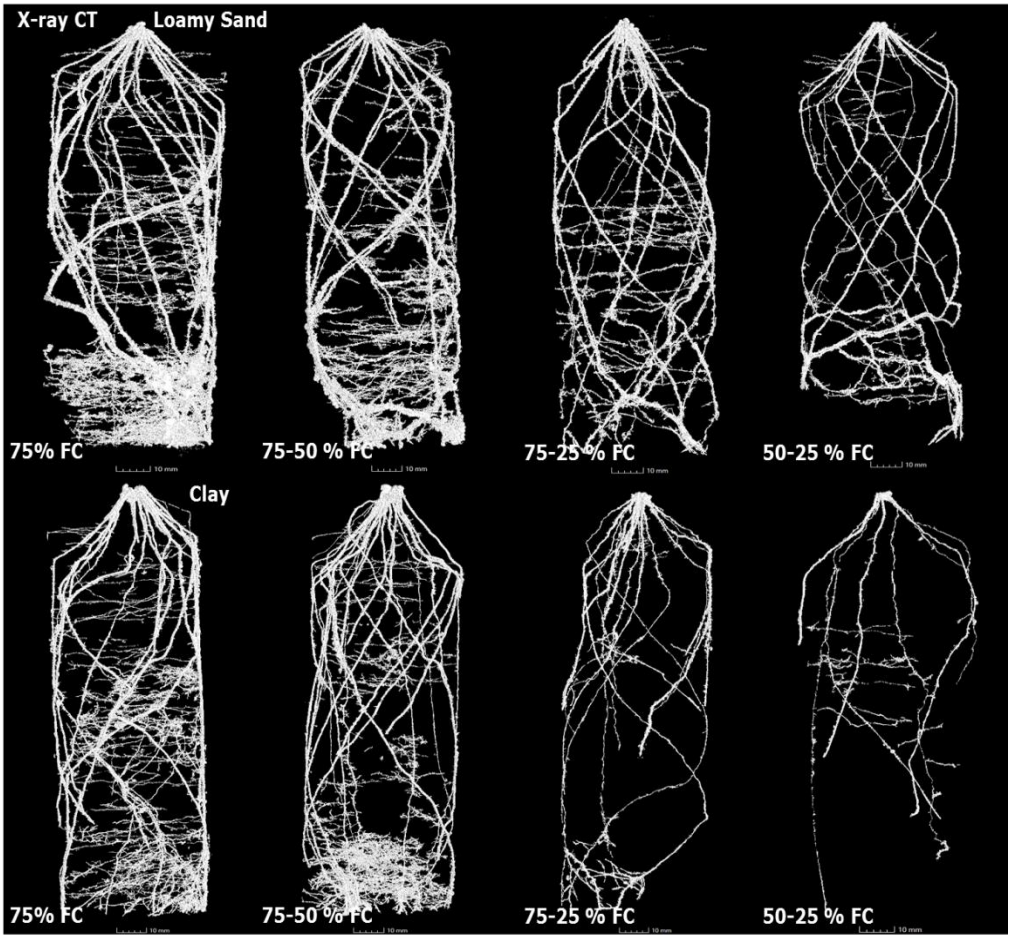


Figure 6.3: CT images of the roots of Istabraq wheat variety at 30 day after germination which were grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Scale bars represent 10mm.

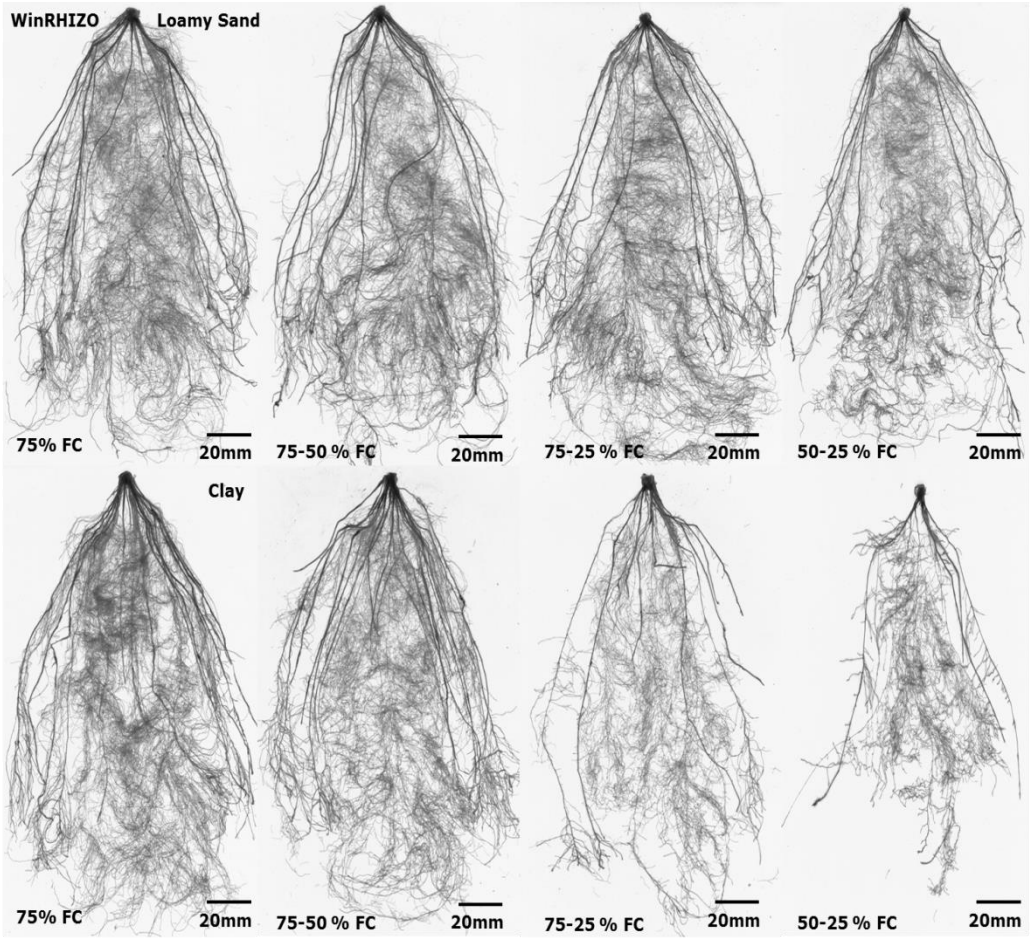


Figure 6.4: WinRHIZO images of root systems of Istabraq wheat variety after extraction and analysis using WinRHIZO® equipment at 30 day post germination after scanning which were grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types. Scale bar represents 20mm.

6.3.2 Photosynthesis, stomatal conductance and transpiration

6.3.2.1 Photosynthesis

To assess the effect of repeated soil wetting and drying cycles on photosynthesis, stomatal conductance and transpiration the same technique described above (4.2.2) was used for measuring these parameters. The data revealed that photosynthesis for Istabraq 30

days after germination decreased significantly with decreasing soil moisture content in the loamy sand and the clay soil types (P_{water}< 0.001). Photosynthesis in the clay (22.74 $\mu mol.m^{-2}.s^{-1}$) was significantly greater than the loamy sand (20.61 $\mu mol.m^{-2}.s^{-1}$) (P_{soil}< 0.001, $P_{soil*water}$> 0.05) (Figure 6.5).

6.3.2.2 Stomatal conductance and Transpiration

The data showed that stomatal conductance and transpiration for Istabraq 30 days after germination reduced significantly with reducing soil moisture content in both soil types (P_{water}< 0.001). Stomatal conductance and transpiration in the loamy sand was 228.7 $mmol.m^{-2}.s^{-1}$ and 5.66 $\mu mol.m^{-2}.s^{-1}$, respectively which were significantly higher than 4.80 $mmol.m^{-2}.s^{-1}$ and 192.2 $\mu mol.m^{-2}.s^{-1}$ recorded in the clay soil type (P_{soil}< 0.05, $P_{soil*water}$> 0.05) (Figure 6.5).

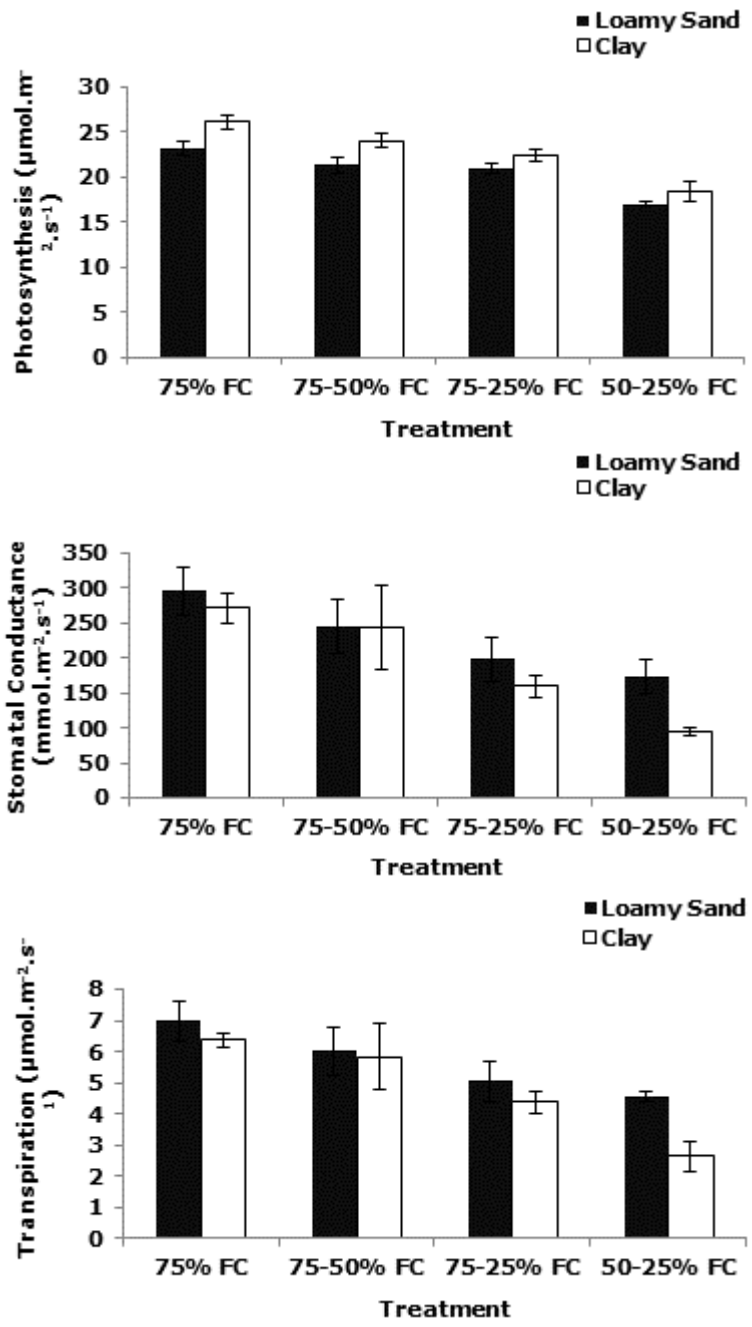


Figure 6.5: Photosynthesis, stomatal conductance and transpiration of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on photosynthesis, stomatal conductance and transpiration. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.2.3 Intrinsic and instantaneous water use efficiency (photosynthesis /stomatal conductance and photosynthesis/transpiration) (inWUE and iWUE)

The results showed that inWUE and iWUE for Istabraq 30 days post germination increased significantly with increasing water stress in the loamy sand from 75% FC to 75-25% FC, while from 75-25% FC to 50-25% FC decreased. While it increased significantly with increasing water stress from 75% FC to 50-25% FC in the clay soil type (P_{water}< 0.001). inWUE and iWUE 0.13 and 5.11, respectively in the clay was significantly greater than the loamy sand (0.09 and 3.72) (P_{soil}< 0.001, $P_{soil*water}$< 0.001) (Figure 6.6).

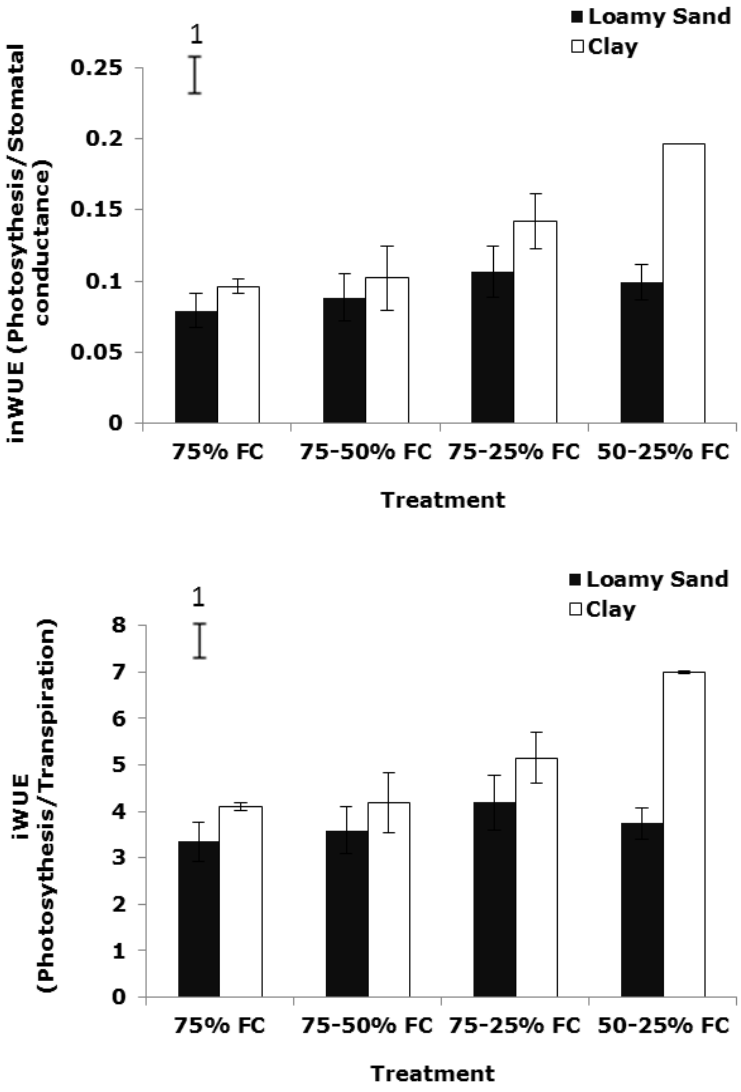


Figure 6.6. Intrinsic and instantaneous water use efficiency of Istabraq wheat variety were grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on intrinsic and instantaneous water use efficiency at P< 0.001. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.3 Combined shoot and root characteristics

6.3.3.1 Shoot and root dry weight

To study the effect of repeated wetting and drying cycles on shoot and root growth of Istabraq 30 days after germination, shoot and root dry weight was measured. The data showed that shoot and root dry weight decreased significantly with increasing water stress in both soil types (P_{water}< 0.001). Shoot and root dry weight in the clay were 36% and 39%, respectively, significantly lower than the loamy sand (P_{soil}< 0.001, $P_{soil*water}$< 0.001) (Figure 6.8). Examples images of leaves of Istabraq grown under different moisture content (75%, 75-50%, 75-25%, and 50-25% FC) in the sandy loam and the clay soil types 30 days after germination are shown in Figure 6.7.

6.3.3.2 Total dry weight

Total dry weight of plant was calculated to estimate the influence of repeated wetting and drying cycles on plant growth of Istabraq 30 days after germination. The data showed that total dry weight of Istabraq decreased significantly with decreasing soil water content in both soil types (P_{water}< 0.001). Total dry weight in loamy sand (2.02g) was significantly greater than the clay (1.26 g) (P_{soil}< 0.001, $P_{soil*water}$< 0.001) (Figure 6.8).

6.3.3.3 Shoot length

To examine the impact of repeated wetting and drying cycles on shoot growth of Istabraq 30 days after germination, shoot length was measured. The data showed that shoot length decreased significantly with decreasing soil moisture content from 33.5 and 30.8 cm in the loamy sand and clay, respectively at 75% FC to 28.3 and 18.7 cm at 50-25% FC ($P_{water}< 0.001$, ($P_{soil*water}< 0.05$). Shoot length in the clay was 18% less than the loamy sand ($P_{soil}< 0.001$) (Figure 6.8).

6.3.3.4 Root to shoot ratio

Root to shoot ratio (dry weight) of plant was calculated to assess the responses of root and shoot growth to the effect of repeated wetting and drying cycles of Istabraq 30 days after germination. The data showed that root to shoot ratio for Istabraq increased significantly with decreasing soil moisture content in the loamy sand while in the clay it decreased significantly from 75% FC to 75-50% FC and then increased dramatically to 75-25% FC before decreasing in the 50-25% FC treatment ($P_{water}< 0.001$). Root to shoot ratio in the clay was significantly greater than the loamy sand, except at 50-25% FC which was lower ($P_{soil}< 0.05$, $P_{soil*water}< 0.001$) (Figure 6.8).

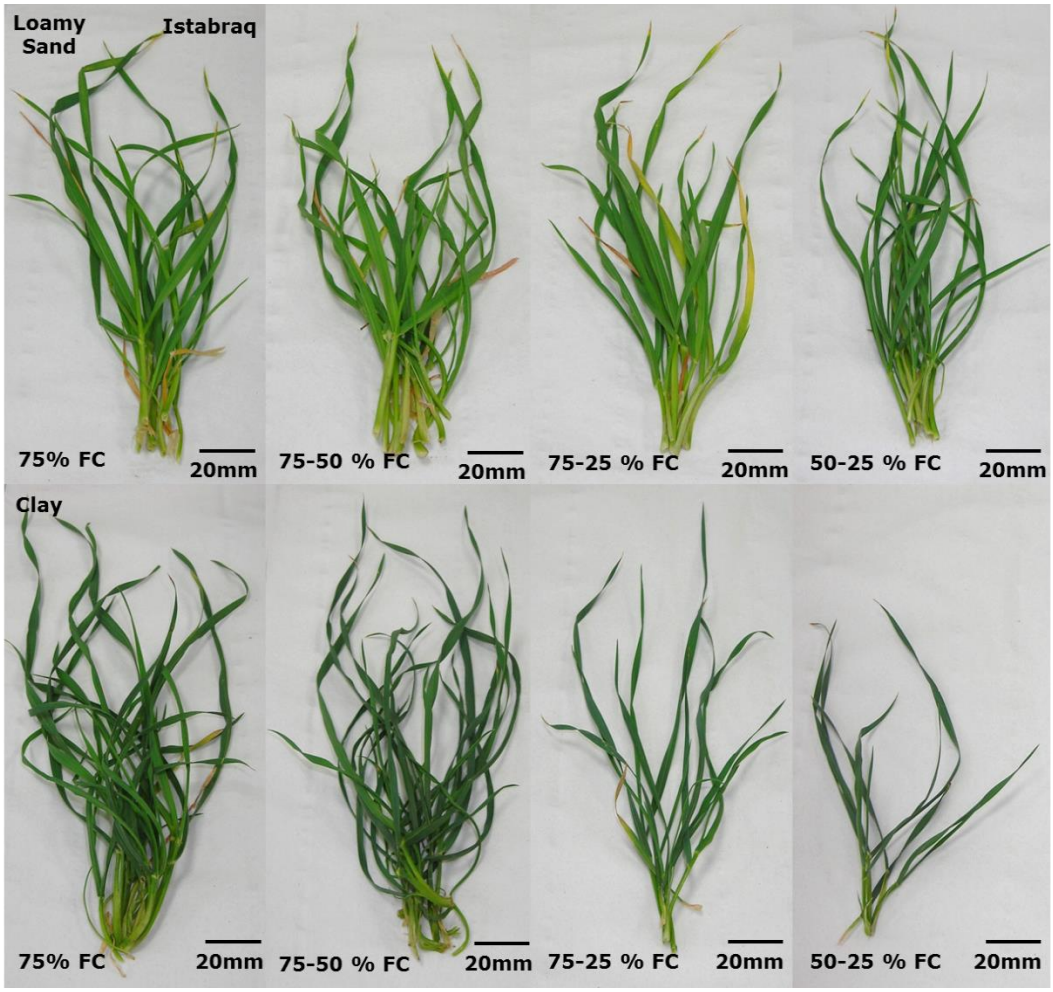


Figure 6.7: Leaves of Istabraq wheat variety were grown under conditions 28°C and four different moisture contents levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Scale bar represents 20mm.

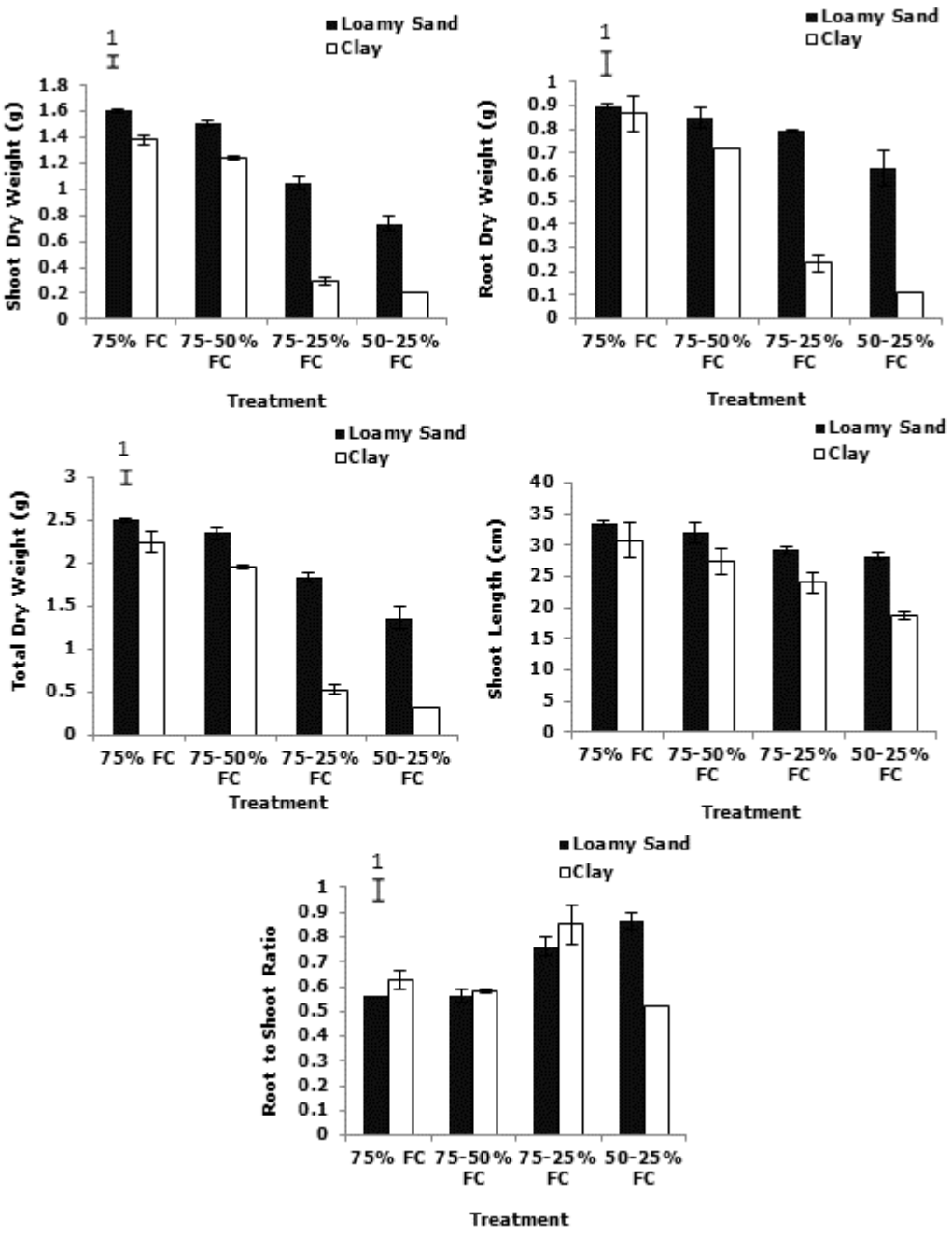


Figure 6.8: Shoot, root and total dry weight of plant, shoot length and root to shoot ratio of Istabraq wheat variety were grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days post germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on shoot, root and total dry weight at P< 0.001, shoot length and root to shoot ratio at P< 0.001. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.4 Water use efficiency

6.3.4.1 Water use efficiency (shoot and root dry weight) (SH and R.D.WT)

To study the effect of repeated wetting and drying cycles on water use efficiency of Istabraq 30 days after germination, water use efficiency was determined of each of the shoot, root and total dry weight per unit of water lost through transpiration. The results showed that water use efficiency (based on shoot and root dry weight) increased significantly with decreasing soil water content in the loamy sand from 75% FC to 75-25% FC and then decreased to 50-25% FC. Whereas, in the clay soil type, it decreased significantly with increasing water stress ($P_{water(sh.d.wt)}< 0.001$, $P_{water(r.d.wt)}< 0.001$). Water use efficiency (SH and R.D.WT) in the loamy sand was significantly greater than the clay, except at 75% FC (R.D.WT) which was lower ($P_{soil}< 0.001$, $P_{soil*water}< 0.001$) (Figure 6.9).

6.3.4.2 Water use efficiency (total dry weight)

The results showed that water use efficiency (total dry weight) increased significantly with decreasing soil water content in the loamy sand from 75% FC to 75-25% FC and then decreased, whereas, it decreased significantly with increasing water stress from 75% FC to 50-25% FC in the clay soil type ($P_{water}< 0.001$). Water use efficiency in the loamy sand was significantly greater than the clay ($P_{soil}< 0.001$, $P_{soil*water}< 0.001$) (Figure 6.9).

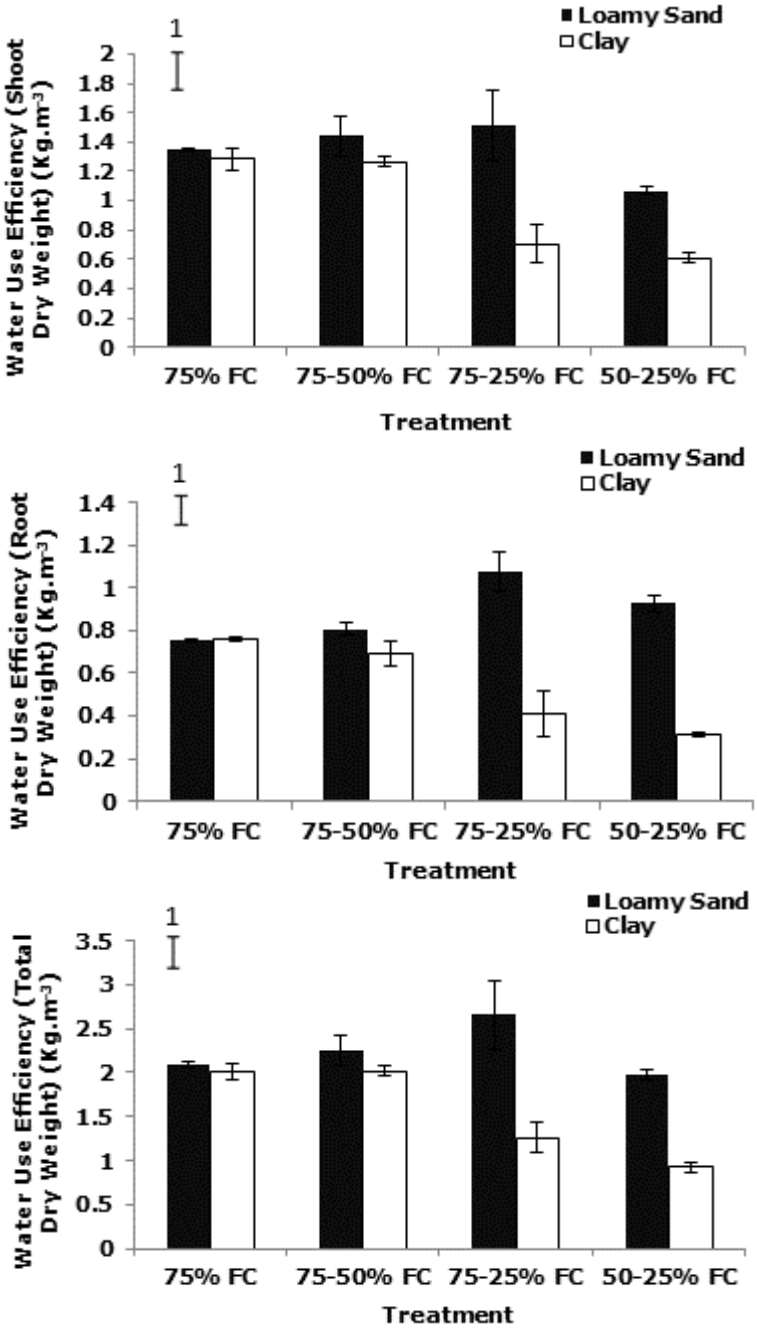


Figure 6.9: Water use efficiency (based on shoot, root and total dry weight) of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on water use efficiency (based on shoot, root and total dry weight) at $P < 0.001$. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.5 Soil characteristics

6.3.5.1 Volumetric water content

Volumetric water content 30 days after germination was 0.23, 0.17, 0.09, and 0.09 $g.g^{-1}$ at 75%, 75-50%, 75-25% and 50-25% FC, respectively in the loamy sand soil while it was 0.26, 0.22, 0.18 and 0.15 $g.g^{-1}$ at 75%, 75-50%, 75-25% and 50-25% FC, respectively in the clay soil (P_{water}< 0.001). Volumetric water content in the clay soil (0.20 $cm^3.cm^{-3}$) was significantly greater than the loamy sand (0.14 $cm^3.cm^{-3}$) (P_{soil}< 0.001, $P_{soil*water}$< 0.05) (Figure 6.10).

6.3.5.2 Air filled porosity

The X-ray CT images used to examine the root systems architecture could also be used to reveal the soil air filled pore space (for the given spatial resolution). Air filled porosity increased significantly with decreasing soil moisture content in the loamy sand and the clay soil types from 30.6% and 20.1 at 75% FC to 36.7% and 25.6% at 50-25% FC respectively (P_{water}< 0.001). Air filled porosity in loamy sand (33.4%) soil was dramatically greater than the clay (22.9%) (P_{soil}< 0.001) (Figure 6.10).

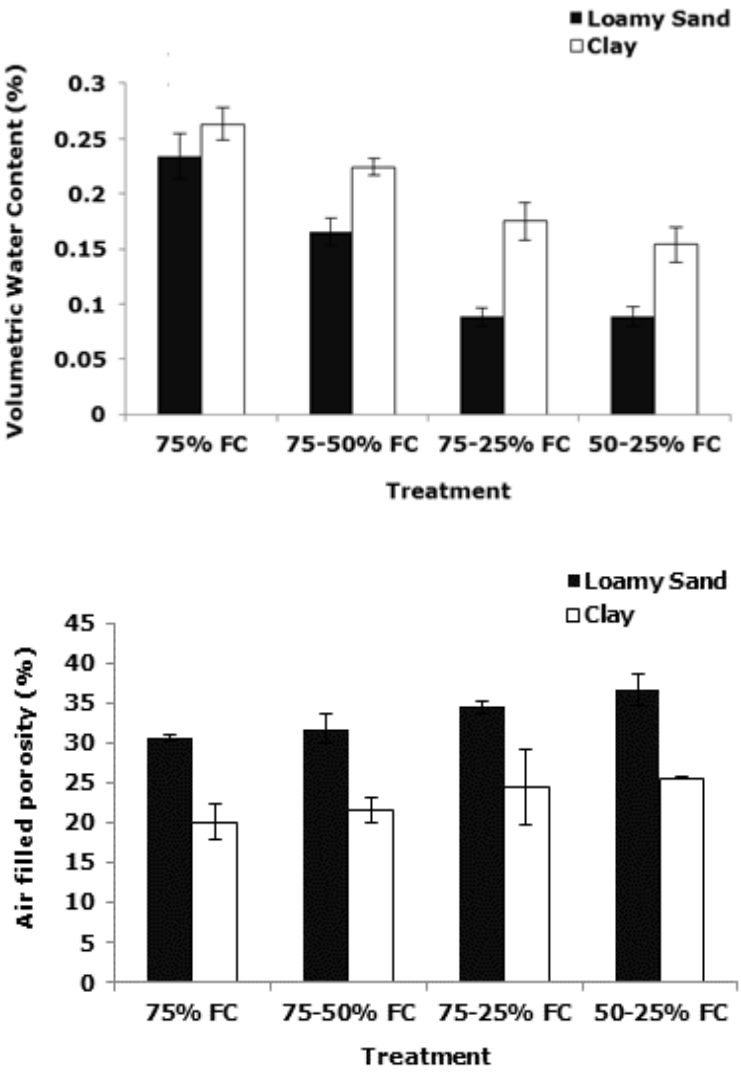


Figure 6.10: Volumetric water content and air filled porosity of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types 30 days after germination after scanning. Bars indicate means ± S.D (n=3). General analysis of variance (ANOVA) showed soil x water interaction on volumetric water content and air filled porosity. Vertical bar (1) represents standard errors of difference (SED) between means where interaction is significant.

6.3.6 Correlation matrix analysis

Total, shoot and root dry weight, stomatal conductance, transpiration, water use efficiency (based on shoot and total dry weight), X-ray CT total root volume and shoot length were positively and significantly correlated with each other. While there was a negative correlation between those variables and each of the inWUE and iWUE. Some important and interesting correlations between shoot and root variables are highlighted in Figure 6.11 and 6.12. The data showed that total dry weight was strongly, positively correlated with total root volume by X-ray CT of Istabraq with the typical R^2 value of 0.86 in loamy sand and 0.98 clay soil types (Figure 6.11). It was also found that shoot dry weight was strongly, positively correlated with total root volume by X-ray CT (R^2= 0.9-0.99 in loamy sand and clay soils, respectively) and root dry weight (R^2= 0.84-0.99). The correlation between these variables was stronger in the clay soil type compared to the loamy sand, except for the correlation between shoot dry weight and root dry weight in loamy sand which was stronger.

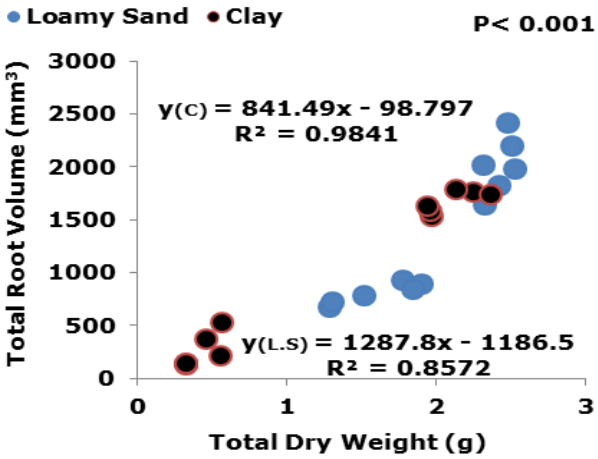
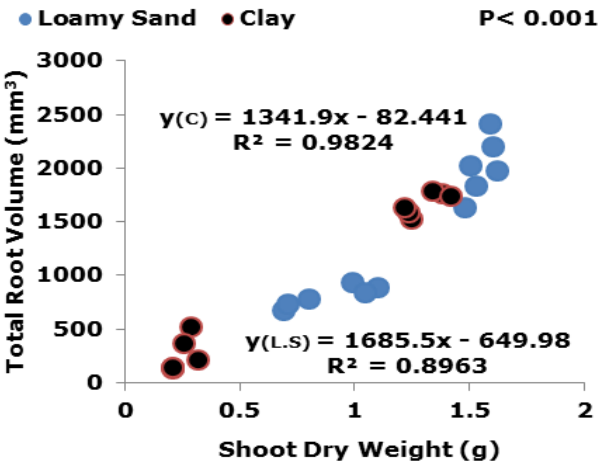


Figure 6.11: Scatter diagram between total dry weight and total root volume by X-ray CT of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand (L.S) and the clay (C) soil types after 30 days post germination after scanning. P-values refer to linear regression analysis between total dry weight and total root volume by X-ray CT.

A



B

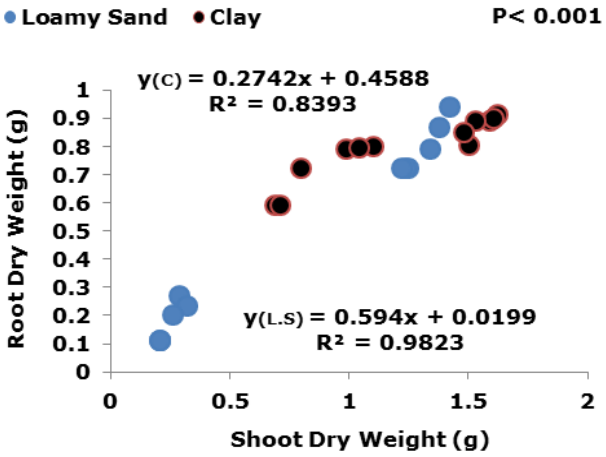


Figure 6.12: Scatter diagram between (shoot dry weight and total root volume by X-ray CT) (A) and (shoot dry weight and root dry weight) (B) of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand (L.S) and the clay (C) soil types 30 days after germination after scanning. P-values refer to linear regression analysis between between (shoot dry weight and total root volume by X-ray CT) (A) and (shoot dry weight and root dry weight) (B).

Table 6.3: Correlation matrix between all variables (see list of Abbreviations) of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types after 30 days post germination after scanning at P< 0.05.

Positive significant correlation between variables	Critical significant value	0.404
Negative significant correlation between variables	Number of observations:	24
No significant correlation between variables		

Key relationship

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Plantdwt	1	-																		
Psynth	2	0.51	-																	
RSRatio	3	-0.32	-0.29	-																
Root_dwt	4	0.98	0.44	-0.15	-															
STomC	5	0.87	0.64	-0.28	0.82	-														
Shoot_dwt	6	0.99	0.54	-0.40	0.95	0.88	-													
Transp	7	0.87	0.63	-0.22	0.83	0.99	0.88	-												
VWC	8	0.38	0.81	-0.55	0.25	0.55	0.45	0.52	-											
WRDiam	9	0.78	0.29	-0.59	0.74	0.60	0.78	0.55	0.35	-										
WRRootvol	10	0.98	0.41	-0.21	0.99	0.82	0.95	0.83	0.25	0.79	-									
WUE_R	11	0.68	-0.01	0.26	0.79	0.46	0.60	0.49	-0.32	0.48	0.79	-								
WUE_SH	12	0.88	0.34	-0.19	0.90	0.72	0.86	0.73	0.10	0.63	0.90	0.82	-							
WUE_T	13	0.80	0.20	0.04	0.86	0.61	0.75	0.64	-0.10	0.51	0.85	0.92	0.97	-						
X_rayAvgDiam	14	0.78	0.34	-0.58	0.74	0.61	0.79	0.57	0.37	0.94	0.80	0.49	0.67	0.54	-					
X_ray_RootVol	15	0.94	0.61	-0.46	0.86	0.91	0.96	0.91	0.61	0.74	0.87	0.44	0.73	0.59	0.74	-				
iwuepc	16	-0.86	-0.36	-0.03	-0.88	-0.89	-0.83	-0.91	-0.20	-0.50	-0.87	-0.73	-0.79	-0.78	-0.51	-0.80	-			
iwuept	17	-0.81	-0.27	-0.11	-0.85	-0.84	-0.78	-0.88	-0.12	-0.44	-0.84	-0.74	-0.77	-0.78	-0.44	-0.75	0.99	-		
porosity_sp	18	0.10	-0.65	0.30	0.19	-0.05	0.05	-0.01	-0.72	0.02	0.21	0.54	0.29	0.42	0.04	-0.08	-0.27	-0.35	-	
reg_p_ht	19	0.90	0.41	-0.05	0.91	0.83	0.88	0.86	0.25	0.58	0.91	0.72	0.80	0.77	0.63	0.84	-0.89	-0.89	0.27	-

Table 6.4: P values for all measurements by ANOVA of Istabraq wheat variety grown under conditions 28°C and four different moisture content levels (75%, 75-50%, 75-25%, and 50-25% FC) in the loamy sand and the clay soil types after 30 days post germination after scanning.

Measurements/Treatment	Soil	Water	Soil*Water
Total root volume (X-ray)	P< 0.001	P< 0.001	P> 0.05
Mean root diameter (X-ray)	P< 0.001	P< 0.001	P< 0.001
Total root volume (WinRHIZO)	P< 0.001	P< 0.001	P< 0.001
Mean root diameter (WinRHIZO)	P< 0.001	P< 0.001	P< 0.001
Photosynthesis	P< 0.001	P< 0.001	P> 0.05
Stomatal conductance	P< 0.05	P< 0.001	P> 0.05
Transpiration	P< 0.01	P< 0.001	P> 0.05
inWUE	P< 0.01	P< 0.001	P< 0.001
iWUE	P< 0.01	P= 0.001	P< 0.001
WUE (Shoot dry weight)	P< 0.001	P< 0.001	P< 0.001
WUE (Root dry weight)	P< 0.001	P= 0.001	P< 0.001
WUE (Total dry weight)	P< 0.001	P< 0.001	P< 0.001
Shoot dry weight	P< 0.001	P< 0.001	P< 0.001
Root dry weight	P< 0.001	P< 0.001	P< 0.001
Shoot length	P< 0.001	P< 0.001	P< 0.05
Total dry weight	P< 0.001	P< 0.001	P< 0.001
Root to shoot ratio	P< 0.05	P< 0.001	P< 0.001
Air filled porosity	P< 0.001	P< 0.001	P> 0.05
Volumetric water content	P< 0.001	P< 0.001	P> 0.05

	Significant p values
	Non-significant p values

6.4 Discussion

6.4.1 Root and shoot characteristics

In order to better understand the influence of repeated wetting and drying irrigation on root and shoot growth in different soil textures (sandy and clay) at vegetative growth stages the effect of wetting and drying irrigation on root and shoot growth of wheat was investigated. Significant differences between soil types were apparent for all root and shoot characteristics examined with the soil*water interactions also significant for total root volume, mean root diameter, shoot and total dry weight. The results revealed that high water stress has a significant negative effect on total root volume in both soil types. Total root volume at severe water stress (50%-25% FC) decreased significantly in both soil types, in comparison to the 75% FC treatment. This could be due to the effect of water stress on soil nutrient availability, as it has been showed that water stress affect both plant water uptake and the activity of soil microbes involved in nutrient cycling (Dodd et al., 2015), or it could be due to increased soil hardness and strength as mentioned before in chapter three. Plants grown in the loamy sand typically exhibited greater growth than in the clay soil under wetting and drying cycles. This also probably due to soil strength as strong soil restricts access of root system to water and nutrients and decrease plants growth (Clark et al., 2003).

It this study, the data found that the effect of the soil wetting and drying irrigation (fluctuation on water regime) (75%-50% FC and

50%-25% FC) on total root volume was reduced in comparison to that found under single drought conditions in chapter four (75%, 50%, and 25% FC). This difference could be due to repeated wetting and drying irrigation which enhanced wheat growth. It has been stated that soil drying not only limits root water uptake which can perturb shoot water status, but also alters root synthesis of phytohormones and their transport to shoots to regulate leaf growth and gas exchange. Re-wetting the soil rapidly restores leaf water potential and leaf growth (minutes to hours), but gas exchange recovers more slowly (hours to days), probably mediated by sustained changes in root to shoot phytohormonal signalling (Dodd et al., 2015). In addition, it has been reported that soil wetting and drying of the rootzone substantially improves crop yields compared with maintaining fixed wet and dry zones or conventional deficit irrigation (Dodd et al., 2015). Root to shoot ratio increased due to soil drying and wetting irrigation in sandy soil (Silva et al., 2012, Liang et al., 2002). This increase means shoot growth was significantly and severely affected than root growth which indicates the proportion of dry matter allocated to above-ground was decreased in comparison to roots (Boutraa et al. (2010). There were significant positive relationships between shoot dry weight and total root volume and root dry weight. Therefore, decreasing shoot growth is likely to be related to increased root growth. These findings are in line with existing work suggesting that the growth and development of the shoot of the whole plant strongly relied on the concurrent and unrestricted development of the root system

(Jeschke et al., 1997, Werner et al., 2010, Xu et al., 2009). In plants growing under dry conditions, root system development is usually less inhibited than shoot growth. Maintenance of root growth during water deficit is obviously important to maintain an adequate plant water supply (Adda et al., 2005).

6.4.2 Photosynthesis measurements

To assess the effect of repeated soil wetting and drying irrigation on photosynthesis parameters such as photosynthesis, stomatal conductance and transpiration infrared gas analysis was used. The present study showed that photosynthesis and stomatal conductance and transpiration were significantly influenced by increasing water stress via the soil wetting and drying irrigation. In moisture stressed plants a decrease in photosynthesis can result through stomatal closure and impairments in photochemical and/or biochemical reactions (Tang et al., 2002, Yu et al., 2009). The decline in photosynthetic activity among plants under several stresses has been cited in other studies as one of the main causes of reduced growth and productivity (Djekoun and Ykhlef, 1996, Wang et al., 1997). This decrease can be influenced by stomatal effects that results in low diffusion and CO_2 fixation (Miyashita et al., 2005) and/or non-stomatal effects.

Photosynthesis in the clay was significantly greater than in the loamy sand while stomatal conductance and transpiration was the converse. Also photosynthesis at early growth stage under repeated soil wetting and drying irrigation (mild and severe deficit irrigation)

was improved greatly in comparison with single drought in chapter four. (Meng et al., 2003) have reported that regulated deficit irrigation at early growth stage can subsequently enhance plants photosynthesis in the late growth stage. Leaf photosynthetic rate during anthesis was improved due to regulated deficit irrigation at early growth stages that led to increased water use efficiency (Ma et al., 2016).

Intrinsic and instantaneous water use efficiency increased significantly by increasing water stress, except in the clay where intrinsic water use efficiency decreased from 75-25% FC to 50-25% FC. This could be due to reduced stomatal conductance, which led to reduce water use, and due to increased photosynthesis rates (Miglietta et al., 1996, Garcia et al., 1998, Bunce, 2000). iWUE was negatively correlated with WUE. This could be due to the time in the cycle when the licor measurements were made.

6.4.3 Water use efficiency

The effect of repeated soil wetting and drying irrigation on water use efficiency of Istabraq 30 days after germination was determined by calculating dry weight of each of the shoot, root and total per unit of water lost through transpiration. Water use efficiency (shoot, root and total dry weight) in loamy sand soil was significantly increased by enhanced water stress conditions while in the clay it decreased significantly. This could be due to shoot and root dry weight because there was significant positive relationship between them. These results are in agreement with Kang et al. (2002) who investigated

the effects of limited irrigation on yield and water use efficiency of winter wheat and found out high values of grain yield, WUE, and harvest index were obtained under mild water deficit conditions at the early growth stages, in addition to a further soil water depletion at the physiological maturity to harvest stage. Also mild water deficit applied at the early stages of growth was shown to enhance the level of drought resistance later in the life cycle and consequently maintain (Liu et al., 2006a) or even increase plant yields (Cui et al., 2009b, Xue et al., 2006). It has been stated that repeated soil wetting and drying cycles in some extreme cases can save water up to 50 % with a minimal impact on crop yield (Xie et al., 2012, Li et al., 2010). Repeated soil wetting and drying irrigation at early growth stages of plant is not only important for water saving irrigation but also for improving root and shoot growth (Dodd et al., 2015).

6.5 Conclusions

Total root volume, root and shoot dry weight, photosynthesis, stomatal conductance, and transpiration were significantly decreased due to repeated soil wetting and drying cycles in both soil types. The measurements in loamy sand soil were significantly greater than the clay soil, except photosynthesis was lower. The results suggest that an optimal matric potential for plant growth for enhanced root and shoot growth is around –50 and –400 kPa for loamy sand and the clay respectively, although it is important to note that these values are approximated. The reported data show how repeated soil wetting and drying irrigation under high temperature affect the growth of wheat's root and shoot with some considerable effects reported. Repeated soil wetting and drying cycles at (75%-50% FC and 50%-25% FC) of Istabraq improved photosynthesis measurements, roots and shoots growth and water use efficiency in comparison to that found under different water stress levels (75%, 50%, and 25% FC). This revealed that repeated artificial soil wetting and drying cycles is not only important for water saving irrigation but also for improving root and shoot growth. Repeated soil wetting and drying irrigation may be accepted by farmers to cope with water scarcity in wheat production. Taken together the work presented here suggest that 75%-50% FC is probably the most realistic water treatment for shoot and root growth of Istabraq in both soil types for UK weather conditions.

7.0 General discussions and conclusions

The main aim of this study was to quantify responses of roots and shoots growth of different wheat varieties under variations in soil water, temperature and CO_2 concentrations. The responses of root and shoot growth to repeated wetting and drying cycles in different soil textures was also investigated.

7.1 Responses of root system architecture to water stress, high temperature and different CO_2 levels

Drought is a threat which is likely to become more pressing with the predicted future global increase in temperature. Increases in atmospheric CO_2 concentration and temperature and associated with future climates are expected to affect wheat growth and yield. The ability of wheat to adapt to these changes is closely related to the response of the root system. The response of root system architecture of different wheat varieties in different soil types (sandy and clay) to water stress, high temperature and different CO_2 levels was investigated in this study. The data revealed that important root characteristics for Zebedee, Hereward, Xi 19 and Istabraq are negatively affected by increases in water stress. For example, total root volume decreased significantly at severe water stress (25% FC) in the sandy and the clay soil types. It was expected that the plants grow better in the clay soil than the sandy soil due to the clay soils typically having greater nutrient availability and increased water retention. However, the soil moisture content negatively affected plant growth in the clay more than in the sandy soil. This could be

due to the formation of large and observable cracks in soil with low water content under high temperature. These cracks have a negative effect on plant growth, can rupture roots and allow even more water to evaporate. Another influencing factor might be the increased soil hardness and strength (Whalley et al., 2006, Whalley et al., 2008) in the clay soil. As soil moisture content decreases, soil strength increases leading to a decrease in root elongation rate due to increasing resistance of the soil particles displacement. Strong soil can restrict access of root system to water and nutrients (Clark et al., 2003) and decrease plants growth. It is also worth noting that the soils in the study were not field structured, but sieved and packed into columns. As a result, the clay soil sieved to <2mm contained a larger portion of macropores than might exist in the field (not including cracks) which may have contributed to enhanced drainage than in the sand soil. Or this could be due to the capability of the tested varieties to grow much better in light soil than in heavy textured soil but the reason for this issue still not clear. However, the results in this study revealed that Hereward, Xi 19 and Istabraq wheat varieties did not grow very well at very high water stress level in the clay soil. The reason for that could be due to the volumetric water content at 25% FC was 0.13 $g.g^{-1}$ in clay soil type which equates to a matric potential lower than of wilting point (-1500 kPa) by reading from the water release curve (Figure 2.1 and 2.2). Consequently, plants growth was more affected in the clay soil type. Istabraq typically had higher total root volume than Xi 19 and

Hereward and even Zebedee. This implied Istabraq was more drought-resistant than others wheat varieties.

The hypothesised field capacity based on two days drainage after saturation (100% FC), almost certainly had a negative effect on plant growth due to excessive soil moisture at this water stress level. This led to a decrease in shoot and root growth of wheat. The high water content (100% FC) at this stage leads plants to hypoxia and anoxia as oxygen is needed in the soil for roots to respire (Yavas et al., 2012). The optimal water content (%FC) for the maximum shoot and root growth of Zebedee wheat variety was 75% FC in comparison to other water stress levels (100%, 50% and 25%).

The combined effect of elevated CO_2, temperature under water stress on total root volume of Xi 19 was determined. This was chosen to study the combined effect of these environmental conditions on root architecture system. The data showed that total root volume increased significantly under water stress at ambient and high temperature at both CO_2 levels (400 and 800 ppm) during 14 days. Whereas, during 30 days the total root volume decreased significantly with increasing water stress. This could be due to the partitioning of resources according to phenology, with more allocation to shoots as the plants mature. This is possibly due to the plants being root-bound as discussed in chapter five. Total root volume during 30 days improved significantly by elevated CO_2 under both temperatures, but the positive effect of elevated CO_2 was less

when the plants were grown under high temperature. These data support the hypothesis that elevated CO_2 enhance root architecture development, possibly by the provision of extra photosynthate, as demonstrated by the photosynthesis measurements in chapter five. Increased photosynthesis at elevated CO_2 tends to enhance belowground C input, fine-root growth (Curtis and Wang, 1998; Pendall et al., 2005) and root biomass (Allen et al., 2000; Pregitzer et al., 2000; Wan et al., 2004). These data are also in line with the results that were reported by Benlloch-Gonzalez et al. (2014) who investigated that high temperature reduces the positive effect of elevated CO_2 on wheat root growth and found elevated CO_2 decreased significantly root length and root branching which lead to decrease root biomass.

Repeated soil wetting and drying irrigation on root system architecture of Istabraq (was chosen as more drought-resistant than others wheat varieties due to the results found in chapter four) wheat variety under two different soil textures was also examined. The data found that severe water stress treatment (fluctuation on water regime) (50%-25% FC) also significantly and adversely affected the total root volume in the sandy and the clay soil types. This is largely due to soil hardness and strength as discussed before in more detail in chapter three. Or it is could be due to the effect of water deficit on the plant water uptake (Dodd et al., 2015). The effect of the water stress (75%-50% FC and 50%-25% FC) on total root volume of Istabraq wheat variety was reduced in comparison to

that found under single drought (75%, 50%, and 25% FC) in chapter four. The difference could be due to wetting and drying irrigation which enhanced wheat growth (Dodd et al., 2015). In addition, the data also showed that root growth was greater in the sandy soil than in the clay under repeated soil wetting and drying irrigation. The difference is likely due to the soil strength; it has been reported that plants growth was reduced in strong soil due to a combination of poor root penetration restricting access to water (Whalley et al., 2006, Whalley et al., 2008). A significant positive relationship between shoot dry weight and total root volume and root dry weight under the repeated soil wetting and drying irrigation of Istabraq under two different soil textures was found. This means that a decrease in shoot growth was compensated for by increased root growth. A decrease in shoots is an important mechanism for crops to survive while consuming less water (Kang et al., 2002).

The effect of water stress on mean root diameter of wheat varieties Zebedee, Hereward, Xi 19 and Istabraq at vegetative growth stages was also investigated. The results found that Zebedee had thicker root diameter in the sandy soil than in the clay while it was greater in clay soil type for Hereward, Xi 19 and Istabraq. An increase in mean root diameter could be due to the bulk density (1.24 $g.cm^{-3}$); it has been found that mean root diameter in tomato (*Solanum lycopersicum* L.) was thicker in the sandy soil than the clay under bulk density of 1.2-1.3 $g.cm^{-3}$ while it was greater in clay under 1.4-1.6 $g.cm^{-3}$ bulk density (Tracy et al., 2013). It has also been showed

that the effect of soil texture on root growth in compacted soil is partly attributable to the availability of nutrient (White, 2006). Therefore, at very early (seedling) growth stages (three weeks), when plants are still primarily dependent on nutrients, increased soil bulk density represents the main obstacle that influences the plant growth. As the effects of bulk density and soil type on root growth are strongly connected, consideration of only one of these variables within experiments cannot adequately inform research regarding the effects of soil physical properties on root growth (Alameda and Villar, 2012).

A sharp increase of mean root diameter from 100% to 75% FC was observed in this study while it was slightly increased from 75 to 25% FC. This is more likely due to water stress. However, the reason for the thicker root diameter in the clay soil for Hereward, Xi 19 and Istabraq could be due to gap formation between the roots and soil led to a reduction of mean root diameter in the sandy soil by shrinkage (Carminati et al., 2013). The data in this study are in agreement with that reported by Aggarwal et al. (2006) who found a greater root diameter with drier soil conditions. In addition, Muñoz-Romero et al. (2010) reported that the greatest root diameters were observed during very early growth stages and/or stem elongation. However, their results concluded that root diameter decreases under drought conditions and from the stem elongation stage onward. The smaller root diameters under water

stress can be due to the greater resistance to penetration (Muñoz-Romero et al., 2010).

In general, Istabraq at vegetative growth stages under water stress conditions had a larger root system than Hereward and Xi 19 and even Zebedee. This could be due to Istabraq having a larger root diameter than others (Figure 4.1 and 4.2) as discussed before in more detail in chapter three (the relationship between root thickness and root branching). Thick root diameter tends to increase root branches that enhance the capacity of water absorption (Ingram et al., 1994), and then increased root system architecture which increases photosynthesis. Therefore, it appears that Istabraq was more drought-resistant than the others tested. Further studies are needed to quantify root diameters and to investigate the effect of water stress on these wheat varieties over longer growth periods than considered here.

The combined effect of elevated CO_2, temperature under water stress on mean root diameter of Xi 19 was also determined. The results also showed that elevated CO_2 increased significantly the mean root diameter of Xi 19 during 30 days after germination in comparison to ambient CO_2. It has been found that roots become more numerous, longer, thicker, and faster growing in crops exposed to high CO_2 with increased root length in many plant species (Madhu and Hatfield, 2013). It has also been found that soybean plants grown under elevated CO_2 exhibited a 27% increase in root diameter, stele diameter (23%), and cortex width (28%) in

the root hair zone (Rogers et al., 1992). It has also been found that root growth differences between ambient and elevated CO_2 occurred in the upper layer of soil and concluded that high levels of CO_2 compensate for restriction in growth of wheat roots by drought (Chaudhuri et al., 1990).

7.2 Responses of photosynthesis, stomatal conductance and transpiration to water stress, high temperature and different CO_2 levels

Temperature and drought are the major abiotic stresses that induce severe cellular damage in plant species, including crop plants. Fluctuations in temperature occur naturally during plant growth and reproduction. However, extreme variations during hot summers can damage the intermolecular interactions needed for proper growth, thus impairing plant development (Bita and Gerats, 2013). The increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves cause significantly yield losses with great risks for future global food security (Christensen and Christensen, 2007). Increase in temperature and decrease in soil moisture content influenced photosynthetic rate, stomatal conductance, transpiration rate, CO_2 concentration and stomatal resistance, hence, final plant growth. In this study, the effect of water stress, high temperature and elevated CO_2 on photosynthesis, stomatal conductance and transpiration of different wheat varieties Zebedee, Hereward, Xi 19 and Istabraq at early growth stages were investigated. The results showed that photosynthesis, stomatal conductance and transpiration were

markedly and adversely affected by severe water stress of wheat varieties Zebedee, Hereward, Xi 19 and Istabraq. Higher photosynthesis and stomatal conductance for Zebedee were shown in the clay soil under water deficit conditions, which were greater than in the sandy soil. However, many studies have been undertaken on the impact of water stress on wheat growth on one soil type but no single study, as yet, to account for different soil types. Therefore, the reason for the differences reported in this study with regards to photosynthesis parameters in these two soil types is unknown but it was expected to be greater in clay than sandy soil due to the clay soil type having higher nutrient and water availability than sandy. Whereas, these parameters in the sandy soil were greater than in the clay for Hereward and Xi 19, except at 75% FC where photosynthesis and stomatal conductance were lower for Hereward and also at 75% and 50 % FC for Istabraq. This variation could be due to more photosynthate relatively consumed at night (via respiration) of these varieties of wheat in comparison with day time carbon gain, and consequently decreasing plant growth and productivity under water stress conditions (Wu and Bao, 2011). Or it could be due to the water stress which significantly reduces crop photosynthesis (Shao et al., 2005, Huang et al., 2009, Zheng et al., 2010). Although other non-stomatal effects on photosynthesis exist, based on the degree of water stress (Yu et al., 2009, Santos et al., 2009), partial stomatal closure is seen at early stage of drought when slight or mild water deficiency occurs (Santos et al., 2009, Yu et al., 2009, Souza et al., 2010). These data are in agreement with

Ahmed et al. (2012) who conclude that a reduction in soil moisture content affected the photosynthesis rate, stomatal conductance and then transpiration rate. Reduction of leaf elongation rate and stomatal closure is considered as important response of plants to water shortage to decrease water consumption through transpiration (Tatar and Gevrek, 2008).

Photosynthesis, stomatal conductance and transpiration were significantly influenced by increasing water stress. In moisture stressed plants a decrease of photosynthesis can occur as a result of stomatal closure and impairments in photochemical and/or biochemical reactions (Tang et al., 2002, Yu et al., 2009). The decline in photosynthetic activity among plant under several stresses was cited by other studies as one of the main causes of reduced growth and productivity (Djekoun and Ykhlef, 1996, Wang et al., 1997). The decrease can be affected by stomatal effects that results in low diffusion and CO_2 fixation (Miyashita et al., 2005) and/or non-stomatal effects. Separation of biochemical effects from stomatal effects was not an objective of this thesis and so this was not carried out. However it is possible to do this using methods such as A versus Ci analysis (Sharkey et al., 2007).

Different varieties of wheat showed a contrasting photosynthetic trend at 50% FC. The highest value of photosynthesis for all wheat varieties was at 50% FC, except of that in the clay soil type where it was at 75% FC for Hereward. This study showed that a larger proportion of photosynthates were allocated to the root, while

higher root to shoot ratio has been considered as one of the mechanisms involved in the adaptation of plants to drought (Turner, 1997). It is established that reduced photosynthesis associated with water stress often reduces the growth and yield of plant (Chaves et al., 2002). The ratio of root/shoot showed significant variation with water supply and with the three genotypes that had different sensitivities to water deficit. Higher R/S has been considered as one of the mechanisms involved in the adaptation of plants to drought (Turner, 1997).

The results of this study also found that elevated CO_2 under 22°C and 28°C significantly increased the photosynthetic rate measured at 14 and 30 days post germination. However, stomatal conductance and transpiration decreased significantly. These results are supported by Juan et al. (2007), who examined the effect of elevated CO_2 concentration on the growth and water use of tomato seedlings. They reported that elevated CO_2 significantly increased photosynthesis and decreased stomatal conductance and transpiration. Higher photosynthesis under high CO_2 is likely due to high Rubisco activity and specifically a higher carboxylase to oxygenase activity ratio (Lieman-Hurwitz et al., 2003, Ainsworth and Long, 2005). However, decreases in stomatal conductivity and transpiration at high CO_2 caused by partial stomatal closure were more than offset by increased photosynthesis associated with leaf area (and possibly vapour pressure deficits in the high temperature treatments) (Prasad et al., 2005).

In this study, photosynthesis was significantly enhanced under high temperature. It is largely suggested to be due to Calvin cycle activity (largely Rubisco and stomatal conductance) (Song et al., 2014). This is consistent with the optimal temperatures for photosynthesis and stomatal conductance (chapter 5). High CO_2 tends to decrease stomatal conductance via a reduction in aperture while high temperature increases stomatal conductivity and transpiration due an increased vapour pressure gradient between leaf surface and atmosphere (Bounoua et al., 1999, Chwin-Ming and Heilman, 1991). Therefore, a combination of elevated CO_2 and temperature could give variable effects (Prasad et al., 2005) but the tendency will be for one to offset the other.

To support the above observations, instantaneous and agronomic water use efficiency was also stimulated under elevated CO_2 (Rogers et al., 1995, Drake et al., 1997, Garcia et al., 1998, Long et al., 2004, Palta et al., 2009). This is likely due to reduced stomatal conductance leading to a decrease in water use coupled increased photosynthesis rates and increased biomass accumulation (Miglietta et al., 1996, Garcia et al., 1998, Bunce, 2000).

inWUE and iWUE increased significantly by increasing water deficit, except in the clay soil where inWUE decreased from 75-25% FC to 50-25% FC. The results showed a diverse trend between inWUE and stomatal conductance, and iWUE and transpiration. Therefore, decreasing and increasing of inWUE and iWUE is largely due to stomatal conductance and transpiration, respectively and/or

photosynthesis as discussed above in more detail in chapter three. Although plant intrinsic water use efficiency (inWUE) shows a physiological response of plants to the concentration of CO_2 in the atmosphere, it does not provide direct information about actual water use efficiency. The values of iWUE, and, especially, transpiration efficiency, can be more readily associated to the plant growth rate than the inWUE (Feng, 1999).

7.3 Responses of shoot and root characteristics to water stress, high temperature and different CO_2 levels

In this study, root and shoot characteristics of different wheat varieties (Zebedee, Hereward, Xi 19 and Istabraq) were also determined under the effect of water deficit, high temperature and elevated CO_2. Root to shoot ratio is one of several ratios that give estimates of the dry matter distribution between the different plant organs (Hunt, 1990). It is a measure of the distribution of dry matter between the root and the shoot systems and it is a good indicator for effects on root and shoot dry weights. The results in this study showed root to shoot ratio of these wheat varieties increased with increasing water stress. Boutraa et al. (2010) also found root to shoot ratio increased under mild water stress (50% FC) demonstrating that shoot dry weight was severely affected compared to the root system. This increase in root to shoot ratio indicates that the proportion of dry matter allocated to shoots was decreased compared to the roots. Root to shoot ratio for Zebedee in the clay under water deficit was significantly greater than in the

sandy soil, while it was lower for Hereward, Xi 19 and Istabraq. This means that for dry climates genotypes with a well-developed root system there might be a stronger drought resistance (Wu and Bao, 2011). Similarly, Ali et al. (2009) found water stress increases root to shoot ratio as found in this study. In contrast, Khan et al. (2010) found that root growth is less affected than shoot by water stress. In general, root to shoot ratio increases under water stress, although the effect of root growth by water stress is minimal (Khan et al., 2010). This is probably due to more suppression of shoot than root growth under water stress condition. The reason for increased root to shoot ratio might be due to restricted supply of water and nutrients as discussed before in chapter three (Khan et al., 2010).

During 14 days, higher root dry mass was produced by plants' roots grown at elevated CO_2 than plants grown at ambient CO_2. These findings are in line with Benlloch-Gonzalez et al. (2014) and Chaudhuri et al. (1990). They reported that roots of water-stressed plants grown under elevated CO_2 produced significantly higher root dry weight than those of well-watered plants grown with ambient CO_2 of wheat. Root growth and morphology responses led to elevated CO_2 and decreased soil water content which support the hypothesis that plants grown in a high CO_2 environment might be able to compensate better to soil water stress conditions (Wechsung et al., 1999, Schenk and Jackson, 2002). An adaptation mechanism

of plants to drought condition happens by physiological and biochemical changes in the plant.

During 30 days, elevated CO_2 enhanced shoot and root biomass accumulation under ambient and high temperatures (Juan et al., 2007), but the positive effect of elevated CO_2 on biomass was reduced when plants were grown under high temperature. This is probably due to high temperature restricting the positive effect of elevated CO_2 on shoot and root biomass (Benlloch-Gonzalez et al., 2014). High temperature induces the closing of stomata that tends to decrease the positive effect of elevated CO_2 on plant growth. The results are also in agreement with that reported by Mitchell et al. (1993). These results are also in line with that reported by Pilumwonga et al. (2007), who studied the effects of temperature and elevated CO_2 on shoot and root growth of peanut (Arachis hypogaea L.) during the seedling growth stage.

The response of wheat roots growth to elevated CO_2 was variable. The data revealed enhanced root characteristics under ambient CO_2 at 14 days which disagrees with Benlloch-Gonzalez et al. (2014). This difference could be due to the short time period used in this study which was not enough to develop the effect of the environmental condition on the root system. Or due to root length and root branching dramatically decreasing under elevated CO_2 leading to decrease root biomass (Benlloch-Gonzalez et al., 2014). Other studies have observed a greater root biomass under high CO_2

(Chaudhuri et al., 1990, Gifford et al., 1985, Acock et al., 1990, Weigel et al., 1994, Barnes et al., 1995).

A high CO_2 concentration often motivates shoot and root growth of plants, but the question remains whether elevated CO_2 influences root and shoot growth proportionally (Rogers et al., 1995). The general consensus is that photosynthesis and C allocation to plant roots increases due to increasing of CO_2 which leads to an increase in above and below biomass (Del Castillo et al., 1989, Norby et al., 1992, Rogers et al., 1994).

Root to shoot ratio (dry mass) could help as a measurement of the preferential C allocation to roots or shoots (Madhu and Hatfield, 2013). In the present study, elevated CO_2 under high temperature and water stress significantly increased root system architecture and shoot growth compared to root to shoot ratio. This probably due to the amount of nutrient in the soil (Norby, 1987). The root to shoot ratio response to elevated levels of CO_2 differed among species and growth conditions (Ferris and Taylor, 1993). Further studies are required to investigate the effect of elevated CO_2, high temperature and water stress on root to shoot ratio of early plant growth until the harvesting time to confirm positive effects of elevated CO_2 on root to shoot ratio at all plant growth stages.

Reduction in shoot growth could be due to root growth because a correlation analysis showed a significant positive relationship between shoot dry weight and total root volume and root dry weight ($r_{(SH.D.WT,X\text{-}ray.T.R.V)}$=0.96 and $r_{(SH.D.WT,\ R.D.WT)}$= 0.95, respectively)

(Table 6.2). Root system development of plants growing under dry conditions is usually less inhibited than shoot growth. Maintenance of root growth is noticeably important during water deficit to maintain an adequate water supply to plant (Adda et al., 2005).

7.4 Responses of water use efficiency to water stress, high temperature and different CO_2 levels

The effect of water stress, high temperature and elevated CO_2 on water use efficiency (based on shoot, root and total dry weight of plant) of different wheat varieties at vegetative growth stages was also determined. The results showed that water use efficiency (WUE) for Istabraq was significantly greater than Xi 19, Hereward, and Zebedee grown under moisture stress conditions. This is probably due to a greater shoot and root dry weights and the water consumed during the plant growth of Istabraq than other three varieties of wheat. Or this could be due to decreased stomatal conductance and transpiration and/or increased photosynthesis lead to increase water use efficiency as discussed before in details in chapter three and four. Or probably could be due to lower root to shoot ratio (dry weight) of Istabraq observed higher water use efficiency in comparison to other wheat varieties. The results in this study are in contrast to Boutraa et al. (2010) findings who used different wheat varieties under different levels of water stress (80%, 50% and 30% FC) and found out that there was no significant effect of the moderate water stress on WUE of any of the wheat cultivars examined in their study. Whereas, at severe water stress (30% FC)

WUE reduced greatly in Hab-Ahmar and Sindy-2, but in Sindy-1 and Al-gaimi were not influenced. A number of studies used different wheat cultivars found that WUE was decreased in the presence of water stress (Shangguan et al., 2000, El Hafid et al., 1998b).

The data also showed increased CO_2 level from 400 to 800 ppm led to an increase in water use efficiency, such results are also reported by Tyree and Alexander (1993). This could be due to dry weight of plant which under elevated CO_2 was significantly greater than the ambient CO_2 at both temperatures (ambient and high temperatures) and combined with the significantly higher inWUE and iWUE. Elevated CO_2 increases plant growth (above-and below-ground) and improves plant water relations (reduces transpiration and increases WUE) (Prior et al., 2011). Increased water use efficiency due to decreased stomatal conductance at high CO_2 (Juan et al., 2007) were offset by increased temperature (Jones et al., 1985, Allen et al., 1985, Allen, 2000). Polley (2002) concluded that yield benefits of improved water use efficiency at high CO_2 are eroded due to a decreased harvest index at high temperatures. However this effect was not observed in this study where substantial increases in inWUE and iWUE were observed, probably due to the fact that the leaf temperatures in the growth room were not substantially affected. Therefore, if global temperatures increase with increasing CO_2, future crop water use will increase especially in arid subtropical and tropical regions where air temperatures are already high.

Water use efficiency of Istabraq in loamy sand was significantly increased under wetting and drying conditions while in the clay it decreased significantly. This could be due to plant dry weight which has a significant positive correlation with water use efficiency (Table 6.2) and combined with the significantly higher inWUE and iWUE. These results are in agreement with Kang et al. (2002) who examined the effects of limited irrigation on water use efficiency and yield of winter wheat variety and found out high values of WUE, grain yield and harvest index were obtained under mild water deficit conditions at the early vegetative growth stages, in addition to a further soil water depletion at the physiological maturity to harvest stage. It has also been found that the mild water deficit applied at the early stages of growth was shown to enhance the level of drought resistance later in the life cycle and consequently maintain (Liu et al., 2006a) or even increase plant yields (Cui et al., 2009b, Xue et al., 2006). It has been stated that in some extreme cases repeated soil wetting and drying irrigation can save water up to 50 % with a minimal effect on the yield of crop (Xie et al., 2012, Li et al., 2010). Repeated soil wetting and drying irrigation at early growth stages of plant is not only important for water saving irrigation but also for improving root and shoot growth (Dodd et al., 2015). Interestingly, it was also found that repeated wetting and drying cycles at (75%-50% FC and 50%-25% FC) of Istabraq improved photosynthesis measurements, roots and shoots growth and water use efficiency in comparison to that found under different water stress levels (75%, 50%, and 25% FC). This showed that

repeated soil wetting and drying irrigation at early growth stages (vegetative growth stages) of plant is not only important for water saving irrigation but also for enhancing root and shoot growth (Dodd et al., 2015).

7.5 Conclusions

The key conclusions of this research can be summarised as follows:

1. Total root volume decreased significantly with decreasing soil water content independently for the wheat varieties Zebedee, Hereward, Xi 19 and Istabraq and in combination with elevated CO_2 and temperature (for Xi 19). Whereas, it increased significantly at both CO_2 levels (400 and 800 ppm) at both temperatures (22°C and 28°C) 14 days after germination.
2. Mean root diameter increased significantly with increasing water stress independently and in combination with elevated CO_2 and temperature. Whereas, it decreased significantly under repeated soil wetting and drying irrigation. Total root volume and mean root diameter at 400 ppm CO_2 growth was significantly greater than at 800 ppm CO_2 growth at 14 days, while it was significantly lower at 30 days. Total root volume, mean root diameter, and shoot and root dry weights at 22°C were significantly greater than those at 28°C. Shoot and root characteristics were significantly greater in sandy than clay soil type.
3. Photosynthesis, stomatal conductance and transpiration decreased significantly with increasing water stress for Zebedee in both soil types and Hereward in clay. However, photosynthesis parameters for Hereward, Xi 19 and Istabraq wheat varieties increased with decreasing soil moisture content in the sandy loam and the clay soil types from the low water stress (75% FC)

level to the medium (50% FC) and then decreased to high water stress level (25% FC).

4. Photosynthesis measurements under water stress condition of Zebedee in the clay loam were significantly greater than in the sandy loam soil. These same measurements for Hereward and Xi 19 in the sandy soil were significantly greater than in the clay soil except at 75% FC where the photosynthesis, stomatal conductance were lower for Hereward and also at 75% and 50 % FC for Istabraq.
5. The optimal matric potential for both roots and shoots growth of Zebedee was approximately -10 kPa in the sandy loam and -150 kPa the clay loam soil at 75% FC. However, the optimal matric potential for photosynthesis and stomatal conductance and transpiration was -5 kPa in the sandy loam and -30 kPa the clay loam soil at 100% FC. The notional 100% FC in this experiment was most likely too wet for optimal growth conditions. However, the impact of water stress on the precise root system architecture is not well known. In this study the root architecture in sandy soil was much better than in clay which was surprising and converse to our hypothesis. Therefore, further experiments are required to explore this further including examining the response of drought resistant varieties to the same moisture stress conditions.
6. Under water stress conditions, Hereward had higher photosynthesis than Xi 19, Istabraq and Zebedee in the sandy loam while in the clay Istabraq was significantly greater than the

others. Istabraq had a more developed root system than Xi 19 and Hereward and even Zebedee in both soil types. This suggests that Istabraq was more drought-resistant than the other wheat varieties especially in the clay soil. Taken together the data presented here suggest while there is some variation between the varieties in terms of shoot and root characteristics especially under mild and moderate water stress, Istabraq was more drought-resistant than the other three varieties.

7. Elevated CO_2 combined with ambient and high temperature increased photosynthesis, while the stomatal conductance and transpiration decreased. Photosynthesis measurements at 28°C were significantly greater than at 22°C. Water use efficiency for 800 ppm CO_2 was significantly greater than 400 ppm. There is a need for more research to focus on plants growth under the same conditions (elevated CO_2, temperature and water stress) over longer timescales than considered here.
8. Under repeated soil wetting and drying irrigation, important root characteristics (total root volume, mean root diameter), shoot and root dry weight, and stomatal conductance and transpiration were significantly greater in loamy sand than clay soil type. However, photosynthesis was greater in clay soil type. The optimal matric potential for shoot and root characteristics and photosynthesis parameters were approximately -50 kPa in loamy sand and -400 kPa in the clay soil type. This study is important as it shows how repeated soil wetting and drying irrigation under high temperature affect the growth of wheat's root and shoot.

Repeated wetting and drying cycles at (75%-50% FC and 50%-25% FC) of Istabraq improved photosynthesis measurements, root and shoot growth and water use efficiency in comparison to that found under single drought conditions (75%, 50%, and 25% FC). This revealed that repeated wetting and drying cycles is not only important for water saving irrigation but also for improving root and shoot growth. Repeated wetting and drying irrigation may assist farmers coping with water scarcity in wheat production. This would help to overcome the food security challenge. Taken together the work presented here suggest that 75%-50% FC is probably the most preferable water status for shoot and root growth of Istabraq in both soil types for the typical UK weather condition.

7.6 Future work

The future work in this area of research should consider the following:

1. Further studies are required to examine the effect of higher temperature degrees than that was used on the root and shoot growth of the different wheat varieties (Zebedee, Hereward, Xi 19, and Istabraq) under water stress conditions based on climate change projections.
2. There is a need to carry out more studies that focus on plants grown under the combined effects of elevated CO_2, temperature and water stress and over longer periods. It is also necessary to consider different wheat varieties.
3. Additional studies are needed to investigate the responses of root and shoot growth of different wheat varieties to repeated wetting and drying irrigation in other soil types especially silt dominated soils as not considered here.
4. It is also necessary to study the influence of partial irrigation on root and shoot growth of the same wheat varieties (Zebedee, Hereward, Xi 19, Istabraq) in the laboratory and field.

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