Exploring relationships between root architecture and growth of UK wheats (*Triticum aestivum* L.)

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Declaration

I hereby declare that, unless where otherwise credited, the contents of this thesis are my own original work, and have not been previously submitted for award of a higher degree elsewhere.

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June 2017

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Abstract

On Australian soils, where water is only available at depth during the grain filling period, growing deep rooting cultivars increased wheat yields by approximately 15%. Seedling root angle is associated with adult root system depth in Australian field-grown wheat cultivars (*Triticum aestivum* L.), but it is not yet known whether selection for root angle and rooting depth could benefit UK wheats. A soil-based, 3D, basket pot screen identified distinct root angle phenotypes (ranging from 35° to 52° from the vertical) among a selection of UK commercial cultivars. These phenotypes were generally stable despite varying soil water content, soil strength, and aerial temperature, but two cultivars showed significant changes in root angle in response to soil moisture. Cultivars Istabraq and Battalion displayed contrasting shallow and deep root angles, respectively. In fully factorial pot trials, with contrasting vertical soil moisture gradients and terminal drought treatments, genotype did not significantly affect early growth and physiology of young (tillering) wheat plants, but there were significant interactions with moisture gradient and water availability in some treatments. Under soil drying, ABA-mediated stomatal closure maintained Ψ_{leaf} in Battalion, whereas Ψ_{leaf} decreased in Istabraq in the absence of changes in foliar ABA concentrations. This suggests contrasting water use strategies (isohydric Battalion and anisohydric Istabraq) are responsible for the observed physiological differences, rather than root angle. Field trials investigated whether these laboratory observations were correlated with root system development and yield of mature field-grown wheat. Soil cores taken from experimental wheat plots at Rothamsted Research's Woburn farm were split at 10 cm intervals, and the number and positioning of roots and bio-pores at the exposed faces counted. Roots were washed from core sections to measure root length density by depth. Root length density declined with soil depth in all cultivars, and was not associated with seedling root angle phenotype, nor did yield correlate with root system traits. In the drier year only, Battalion had the most roots in the surface soil layers, which experienced greater soil drying under Battalion than under other cultivars. These studies contribute to the growing body of research on root system ideotypes in rainfed temperate climates, by identifying genetic variation in seminal root angle in high performing commercial wheat cultivars. While seedling root angle was not correlated with yield in field trials, it was associated with ability of roots to penetrate hard soils and access water in deeper strata of the soil profile. These traits would be especially beneficial during the grain-filling phase, and may potentially increase UK wheat yields during dry summers.

Publications arising from this work:

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Chapter 1: General Introduction

1.1 Introduction

The Food and Agriculture Organisation of the United Nations (FAO) (2002) has defined food security as:

> "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life."

Food security can be addressed at different scales, and can describe access to food at an individual, community, national, or even global scale. To better understand the multiple facets that are involved in food security (or lack thereof), the problem can be broken down into key four issues (FAO 2002):

- Physical availability of food (is there enough?);
- Access to food (is there sufficient infrastructure in place so that both people and food can easily reach markets; can people afford to buy the available food?);
- Food utilisation (is there sufficient knowledge of how to prepare a variety of foods, and is there access to a diverse diet for suitable nutrition?);
- Resilience of the systems and factors described above over time (factors such as seasonal variation, political instability, changes in income, and food price).

Achieving food security on the global scale has become one of the most pressing problems facing the continued growth of the human population. It is predicted that by 2030, food production worldwide will have to increase by 50% on current rates to provide all people with enough food for good nutrition (Beddington 2010). Alongside increasing energy production by 50% and fresh water availability by 30%, these are obstacles that must be overcome while simultaneously coping with, or mitigating, effects of climate change. This scenario is now commonly referred to as 'the perfect storm' (see Figure 1.1).

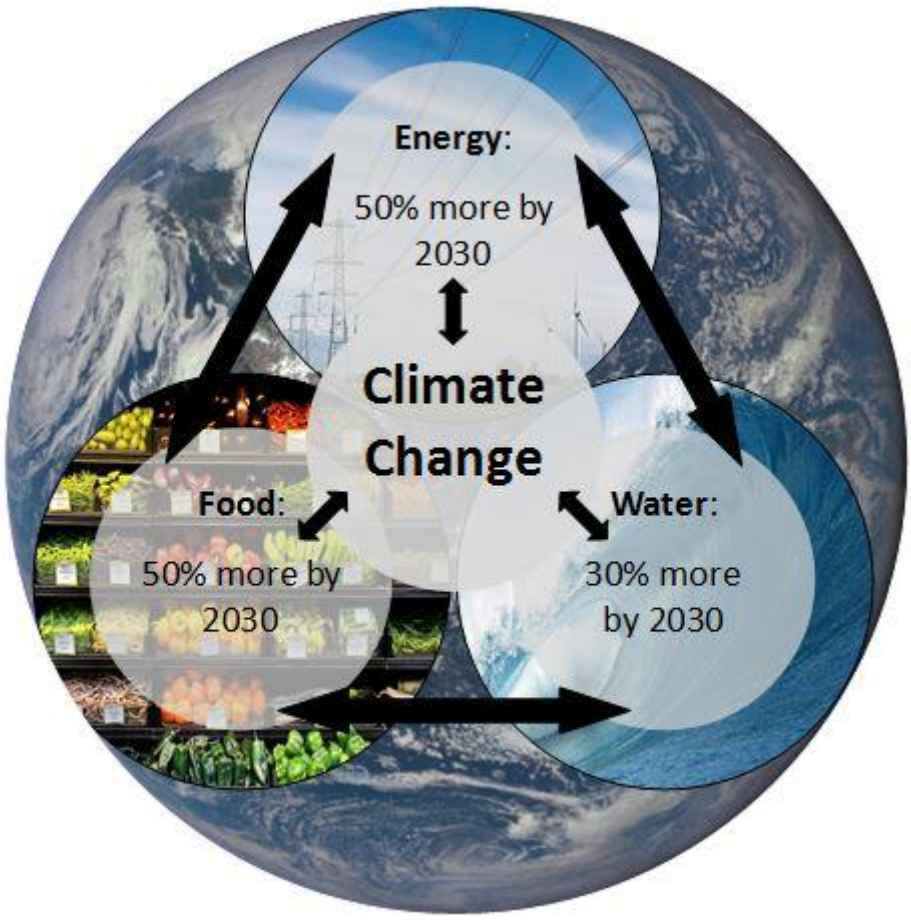


Figure 1.1: Illustration adapted from Maslin (2013) highlighting the interrelated problems of Beddington's (2010) 'perfect storm'. Not included in this illustration is increasing global population, which is the primary driver of global energy demand, food consumption and fresh water usage, and contributes to (and is affected by) climate change.

As shown in Figure 1.1, these issues are all interrelated. Water and energy are both currently overused resources, but remain crucial for agricultural production even under ideal growing conditions. In arid regions, both are used extensively to irrigate crops. Even in a temperate climate, lack of water can limit crop production, with up to 10% of the UK's potential wheat production being lost through insufficient soil water (Dodd *et al*. 2011). More generally, 20% of UK crops encounter drought, often coinciding with grain filling (King *et al*. 2003). Further demands on energy and water within agriculture include use of both to power and maintain machinery, and to process crops during and after harvest. Similarly, they are both vital to transport and store food products efficiently, especially in hotter climates or when a product is likely to spoil quickly. The production of nitrogen fertilisers through the Haber-Bosch process, while allowing for significant increases in yields through increased nutrient uptake, requires intensive energy inputs and is still largely fuelled by non-renewable resources (Townsend and Howarth 2010).

Climate change could have a potentially catastrophic effect on agricultural production worldwide. There are many forecasts of what could happen as global temperatures increase, but it is a consistent prediction that patterns of rainfall will change, with already drought prone areas becoming more at risk of arid conditions and desertification (Matawal and Maton 2013). A warmer climate caused by increased global temperature may benefit some regions at high latitudes by extending the growing season. Other regions will experience dramatic changes that may limit crop productivity, such as influx of new pests and pathogens (Gregory *et al*. 2009). Coastal flooding events may become more common, increasing chances of crops being negatively affected by saline conditions.

Predicted effects of climate change in the UK

In years to come, global climate change is predicted to affect the UK climate by increasing average temperatures during summer, which may allow an increased variety of crops to be grown, better seasonal growth and improved yields of crops already produced. Areas which were once too cold or damp for certain crops may find that conditions become more favourable, as other crops suffer and become less reliable in other regions where conditions become less suitable. For example, 23% – 26% of the increase in potato yields in Scotland since 1960 is thought to be attributable to climate change (Gregory and Marshall 2012). However, shifts in UK species' (and cultivars') range boundaries may also introduce new crop pests (Gregory *et al*. 2009). Climate change is also expected to cause noticeable differences in expected seasonal weather patterns. Many models predict greater extreme weather events, such heavier and more frequent rainstorms especially during winter months in the UK (Fowler *et al*. 2005), with a corresponding increased chance of floods following intense prolonged rainfall (Schaller *et al*. 2016). In addition to increased risk of heavy winter rains and flooding, some models project that long summer droughts may become less frequent, but the risk of intense short-term summer droughts will increase, especially in England and Wales (Blenkinsop and Fowler 2007, Burke *et al*. 2010). This could affect UK wheat crops at both ends of the growing cycle, by making it difficult to time the drilling of seed beds due to inclement weather (Oleson *et al*. 2011), and by causing yield losses through lack of water during grain-filling (Foulkes *et al*. 2002). Summer rains can also negatively impact on harvest, by damaging and reducing the quality of the harvested crops, or even resulting in the complete loss of a crop, if the ground becomes so waterlogged that the

harvest machinery cannot be brought into the field on time (Why is rain such a problem at harvest? 2014).

As one of the UK's major crops, there has been much interest in the potential impacts of climate change on wheat yields. In the south east of the UK, which produces some of the country's highest yields, hotter and drier summers could shorten the grain-filling phase, therefore decreasing wheat yields (Farooq *et al.* 2011, Newton *et al*. 2011). High temperatures (> 30 °C) can even cause sterility in wheat ears without being lethal to the plant, thereby restricting yield (Porter and Gawith 1999, Farooq *et al*. 2011). Amongst 21 UK wheat cultivars, yields under droughts (through use of rain-out shelters) were positively correlated with ability to extract deep water, although there was a large amount of variation (Ober et al. 2014). It could be hypothesised that the cultivars that best maintained yields under drought had root systems that maintained water uptake during an extended period of soil drying. In areas with terminal drought, this would usually be a deep root system that can exploit stored soil water (Wasson *et al.* 2012). In the UK, where droughts may not last the entire summer and in season rainfall is possible (or even the norm, depending on region), it may be better to have a root system that is more reactive to the local environment, and plastic in its development. Investing heavily in deep roots when they are not necessary may also impose a yield penalty through inefficient use of carbon resources and assimilates (Wasson *et al*. 2012).

Wheat and its consumption around the world

Wheat is a part of the staple diet across the world, being grown and eaten in countries as culturally and climatically diverse as New Zealand, the UK, India, and Uganda (to name but a few). It is an almost ubiquitous grain used in a diverse range of foodstuffs including breads, pasta, noodles and baby food. To give just one example of the importance of wheat production on the global scale: in 2010, severe droughts in Russia led the country's wheat yield being 33% lower than expected (Wegren 2011). Russia is the world's fourth largest exporter of wheat. The prospect of a shortfall in domestically produced wheat meant that Russia did not export any wheat in 2010, prioritising the needs of their own citizens and the country's livestock industry (Wegren 2011). With a grain surplus from the previous year, Russia did not actually experience a shortage of wheat, but this did not stop price rises in bread and other wheat products in the country (Wegren 2011).

The effects of the Russian drought were also felt outside the country, as wheat prices on global markets rose to a 22-month high (BBC 2010a). Grain purchasers from south east Asia bought up grain on the open market, speculating on further increases in the price of wheat (BBC 2010a). After the Russian government blocked its own producers from selling grains including wheat outside of the country, the markets were balanced out by a higher than average harvest in the USA (BBC 2010b). However, this did not prevent increased wheat and bread prices from becoming a contributing factor to riots in Mozambique later that same year (Nhate *et al*. 2013). In the following year, China also experienced severe drought and significant agricultural losses, which resulted in the further reduction of grain exports available to the world market. In many Middle

East countries (amongst them Egypt, Libya and Syria, all net-importers of wheat), prices for basic food commodities rose. This may have played a part in the 'Arab Spring' revolutions in many countries in the region (Sternberg 2012); the long-term humanitarian and political consequences of the Arab Spring are still unfolding today. Although the UK did not experience a wheat shortage, UK consumers felt the effects of the Russian drought and grain ban as a small increase in the price of bread and other wheat products resulted from the global increase in the price of wheat (BBC 2010b).

This example demonstrates two points. Firstly, wheat is produced and consumed globally, forming a staple diet for the human population as well as being used as feed for livestock. Secondly, wheat is economically important on the global scale, and there are far reaching financial impacts of higher or lower than anticipated yields, even in countries that do not experience changes in grain supply.

Wheat in the UK

UK wheat yields are generally high by global standards, and have in some years been record breaking (Guinness World Records 2015). This is partly brought about by intensive farming methods using large amounts of resources such as fuel for agricultural machinery, and nitrogen and phosphorus based fertilisers. In 2014, the UK used 243 kg of fertiliser per hectare of arable land, compared with a global average of 138 kg per hectare (The World Bank Group, 2017). Arguably, the current system is not sustainable. Most farm machinery requires non-renewable fossil fuel resources, and over use of fertilisers increases air pollution and causes eutrophication of water

ways (Mueller *et al*. 2014). Rather than increasing yields through further resource use, some have proposed maintaining current yields with fewer resources (Bennet *et al*. 2014) and utilising more sympathetic agricultural techniques to prevent further environmental damage from farming. One of the questions raised in this philosophy is the issue of land sharing *versus* land sparing (Phalan *et al*. 2011). Land sharing takes the approach of less intensive agriculture with fewer inputs over a larger area, whereas the land sparing approach would protect some land from any inputs, at the expense of other areas which would be intensely farmed, with much higher resource use, but on (theoretically) a smaller area. In the context of this study, this poses the question: is it better to concentrate on maintaining wheat yields in countries that already have relatively high yields, or to try to boost meagre yields in arid regions?

1.2 History of wheat improvement

The origins of wheat

There is archeaobotanical evidence of wheat domestication in the Middle East dating back to approximately 8000 – 7000 B.C. (Nesbitt and Samuel 1998). These early wheats were derived from emmer (*Triticum dicoccoides*) and einkorn (*T. boeoticum*) wheats, for which some landraces still persist and provide possible sources of genetic material for breeding trials and crop improvement (Charmet 2011), along with other wild and relic progenitor grains (Reif *et al*. 2005). Using new material from other species can introduce entirely new traits to wheat (*T. aestivum*), which has limited genetic diversity. For example, incorporating the narrow, deep root system of spelt (*T.*

spelta) into wheat varieties could aid adaptation to hot and dry environments by improving water extraction by the root system (Xie *et al*. 2017).

Modern wheat was produced by hybridising the domesticated tetraploid *T. dicoccum* (the progenitor of durum wheat *T. durum*) and wild diploid wheat *T. tauschii* to make the hexaploid *T. aestivum*, commonly known as wheat (Charmet 2011). The hexaploid genetic structure of modern wheat places limitations on conventional breeding by phenotypic selection (Langridge and Fleury 2011), as target genes may be located on one of the three pairs of chromosomes or scattered across multiple locations in the genome. Breeding programmes selecting for genes with major effects, which have clear effects on the phenotype, are more effective than breeding programmes targeting complex traits controlled by additive gene effects (Langridge and Fleury 2011). To identify genes responsible for these additive traits, it is often necessary to grow large mapping populations of recombinant inbred lines (RILs) to map out the positions of sections of DNA (quantitative trait loci (QTLs) in the genome that are likely to contain genes that control target phenotypes (Börner *et al*. 2002). Once genes have been identified and located, modern genetic and molecular breeding techniques may be able transfer a desired trait into a high-yielding variety, with a speed and accuracy not possible using conventional breeding.

While genetic modification could potentially speed up the breeding process, conventional wheat breeding has still produced some impressive yield increases, especially in the developing world. The 'Green Revolution' saw dramatic improvements in wheat yields through the introduction of dwarf cultivars from the 1960s through to the 1970s and 1980s (Khush 1999). Dwarf wheats are gibberellin

insensitive and do not grow as tall as non-dwarf cultivars. Reducing the stem height increases the harvest index (HI), which is the ratio of grain weight to total plant weight (Sinclair 1998). A secondary benefit of dwarfing genes is the decreased risk of yield losses through lodging (Berry *et al*. 2004). In recent years though, there has been considerable stagnation in year-on-year yield gains (Ray *et al* 2012). This is particularly true in north west Europe, including the UK (Oleson *et al*. 2011), but overall, 37% of wheat growing regions around the world are experiencing yield stagnation (Ray *et al*. 2012).

A conceptual framework to understand how different processes contribute to water-limited crop yields is the Passioura Identity (Passioura 1983), which indicates that yield is the product of water used (WU), water use efficiency (WUE) and harvest Index (HI). Thus, any increase in HI should be expected to increase yields, assuming no change in water use and WUE. In contrast, even a mild drought can result in yield gaps when the crop fails to reach the full yield potential; because, as previously discussed, seasonal water use can be limited by periods of drought even in the relatively wet UK climate.

More recently, 'stay green' phenotypes have also become popular, especially in hot dry countries. These were developed by selecting for physiological traits such as maintaining green leaf area and prolonging photosynthetic activity of the flag leaf (Chen *et al*. 2010), allowing longer periods of water uptake during the grain-filling phase. 'Stay green' is not the only aboveground trait currently of interest to breeders for wheat improvement. Many breeders are looking to improve yield associated traits such as the water soluble carbohydrate content in the stem (Shearman *et al*. 2005), or

improving the efficiency of nutrient use and efficiency of photosynthesis (Foulkes *et al.* 2009). However, there is some evidence to suggest that modern European wheat cultivars are close to meeting the hypothetical maximal HI (Shearman *et al*. 2005). Some researchers have turned to molecular approaches, such as increasing the efficiency of Rubisco enzymes and photosynthesis (Parry *et al*. 2013), to find new ways of increasing yields. However, as conventional breeding techniques exhaust new aboveground traits for wheat yield improvements, scientists and breeders are increasingly looking below ground to the root system, for the next major leap in crop improvement and yield increase.

1.3 Importance of roots in modern crop improvements

All root systems are inherently plastic and flexible in their morphology and physiology, a necessary adaptation for a sessile organism that must exploit the environment in which it grows (Hodge 2004). This plasticity makes developing a root system ideotype difficult, because even under ideal conditions it is highly unlikely that two plants of the same genotype will develop truly identical root systems (Malamy 2005). It is possible though, to identify patterns of root system architecture (RSA) in genotypes; these patterns can be consistent root system traits under all environments, or the consistent development of distinct phenotypes under certain conditions. The disposition towards a particular pattern of root growth is often genetically controlled (de Dorlodot *et al*. 2007); if found to bestow a benefit to either the individual plant or whole crop then a root trait could be of interest to crop breeders.

Seeds imbibe water to trigger the germination response (Wuest and Lutcher 2012), provided that local conditions including temperature, oxygen availability, and low levels of illumination are suitable for seedling growth (Bewley and Black 1982). Seeds contain some nutrient resources usually in the form of a mix of proteins, phytin, carbohydrates, and lipids, to support early growth and root and shoot formation (Bewley and Black 1978). In most plants, once seed resources are exhausted, the roots take up all the nutrients and water required by the plant. Seed weight is important because it indicates the amount of reserves available to the developing seedling (Bouaziz and Hicks 1990). Wheat seeds lose much of their starting weight during germination as they are hypogeous and endospermic (Bewley and Back 1978). Hypogeous seeds germinate with the cotyledon below the ground while the epicotyl elongates above the cotyledon to reach the soil surface. Endospermic means that seed reserves are not stored in the cotyledons, but in endosperm within the seed. Higher levels of reserves are positively correlated with germination success (Khan 2004), seedling vigour and establishment (Stanton 1984). Thus, greater seed weight at planting is correlated with higher yields in the mature plant (Knott and Talukdar 1971, Ries and Everson 1973, Bewley and Black 1978, Stanton 1984).

After germinating, the wheat root system comprises the primary root (which grows at a steep angle almost vertically into the soil) and the subsequent seminal roots which sprout from the base of the seed (see Figure 1.2). Later, once the wheat plant begins tillering, nodal roots form at the base of the tillers. Nodal roots are thicker and generally shallower in the soil profile than the seminal root system (Araki and Iijima 2001). Wheat seminal roots can survive the whole lifespan of the crop (Manschadi *et al*. 2008) and are believed to be very important in accessing stored water deep in the

soil profile during the grain-filling stage (Palta *et al*. 2011), making them critical in determining grain yield in water-limited conditions.

Roots play an important role in the functioning of a healthy terrestrial plant by providing anchorage against both upward and sideways forces acting on the stem (Fitter 2002, Berry *et al* 2007). For smaller plants, like grasses and cereals, the upwards force experienced because of grazing activity from large herbivores can be as relevant to the plant as the sideways forces that plants experience under windy conditions. To improve the strength of anchorage from the root zone and prevent the plant being overturned (where the root system is ripped from the soil, also known as root lodging), it is necessary either to increase the structural rooting depth or to increase the horizontal spread of roots at the structural rooting depth (Berry *et al*. 2007). The latter runs counter to the root ideotype for improved water uptake, wherein a steeper angle of root growth and a corresponding narrow spread of roots is deemed to be the most efficient RSA. Under UK conditions, now and in the future, the traits desired when breeding for an ideal root system may vary greatly, depending on the nature of the most severe threat to UK wheat yields.

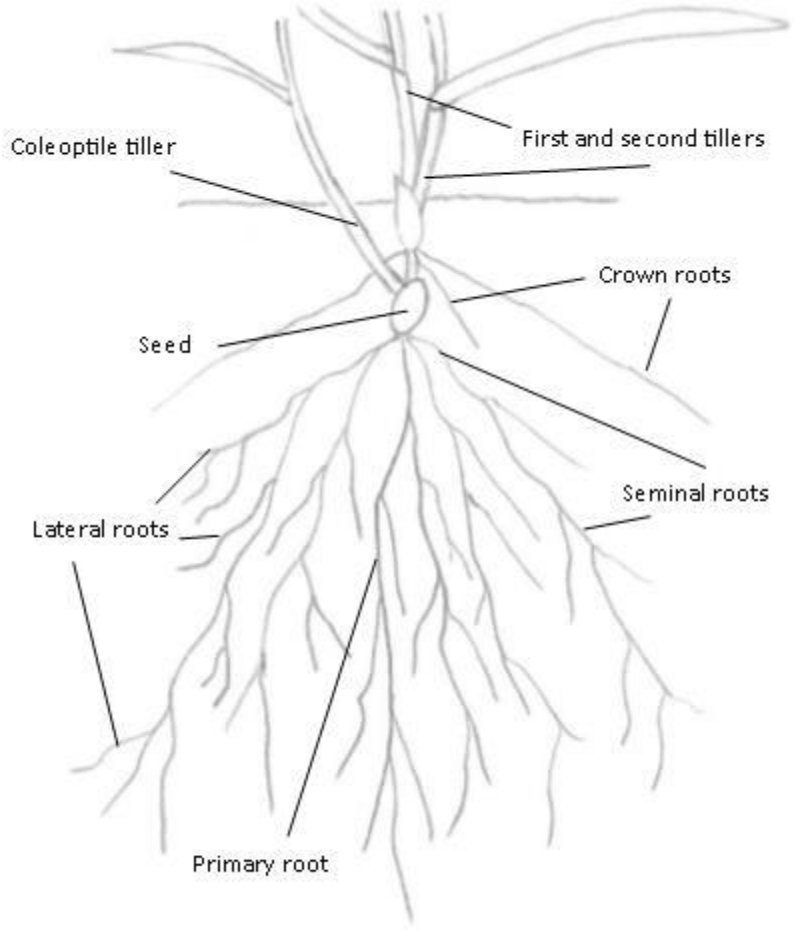


Figure 1.2: Diagram of the wheat root system (not to scale), at the start of the tillering and crown root growth stages, based on Oswalt *et al*. (1959). Wheat is a monocot cereal, with a typical fibrous root system. By contrast, dicot root systems typically consist of a prominent taproot, from which lateral roots grow to form the root system (Klepper 1991).

When dwarf varieties were originally introduced, there was concern that the dwarfing genes would also affect the root system, decreasing root mass (Manske and Vlek 2002). QTL studies found that genes controlling root traits and genes controlling shoot traits can be co-located on chromosomes. Within a mapping population (comprised of progeny from crossing cultivars (cvs.) Avalon and Cadenza), genes controlling the number of seminal root axes and those controlling plant height were co-located on three chromosomes (Bai *et al*. 2013). However, the same study found many other root and shoot genes were independent. Furthermore, a study on 44 cultivars found that

while yields increased with dwarfing genes, absolute root length density did not significantly change (Manske 1997). In trials comparing seedling root growth between tall, semi-dwarf and dwarf wheats; root lengths of the tall and semi-dwarf genotypes were similar, but root length of the dwarf varieties varied significantly depending of the growth medium used (Wojciechowski *et al*. 2009). Since these plants were not grown beyond the first stages of tillering, the shorter dwarf roots may have caught up with the semi-dwarf and tall root systems later in the growing season, had the experiment lasted longer. Greater differences in root dry weight than in root length in near isogenic lines (NILs) of spring wheat (Miralles *et al.* 1997), suggested that dwarf wheats have higher root mass per root unit length; possibly because the root system acts as an alternative carbon sink for photoassimilates that are no longer stored in the stem. Although the evidence is not conclusive, it appears that dwarfing genes can affect root system traits.

Direction of root growth is controlled by the interaction of several tropisms (Porterfield 2002), the most prominent of which are gravitropism, growth/movement as a response to gravity (Oyanagi 1994, Araki and Iijima 2001), hydrotropism, growth/movement as a response to moisture (Oyanagi *et al*. 1995, Eapen *et al*. 2005), oxytropism, growth/movement as a response to the presence of oxygen (Porterfield and Musgrave 1998, Colombi *et al*. 2017), and thigmotropism, growth/movement as a response to touch stimuli (Massa and Gilroy 2003). In addition to these major tropisms, plant roots can also sense and react to other environmental stimuli including salinity (Galvan-Ampudia and Testerink 2011), nutrient availability (Liao *et al*. 2001), and soil temperature (Onderdonk and Ketcheson 1973). In all these examples, these environmental stimuli can affect the direction of root growth, causing individual roots

to grow away from their usual direction of growth, and at a different angle relative to the vertical. The precise molecular mechanisms governing many of these root tropisms are yet to be determined. Roots can also display other reactions to soil stimuli, such as root proliferation (Hodge 2004).

As well as influencing angles of root growth, local sensing of the environment by roots can trigger responses in the aboveground plant tissues through root-to-shoot signalling. Information on local soil and climatic conditions sensed by the roots is reported to the shoot through plant hormones, such as ABA and ethylene (or possibly their precursors or conjugates) (Bacon *et al*. 2002, Sauter *et al*. 2002, Shabala *et al*. 2016). In some species, the stomata open and close in response to changes in xylem tension (Christmann *et al*. 2007), but in other species the stomatal movements seem independent of leaf water status (Wilkinson and Davies 2002). This suggests that it is more than just water availability alone that causes a drought response in plants. Plant water deficits increase root, shoot and foliar ABA levels, which are integral to the soil drying response; this has led to ABA being called the drought stress hormone. The most commonly accepted model to explain how ABA controls the drought response can be summarised as:

1. Root tips sense soil drying, thus inducing root ABA synthesis. This response is amplified by an associated reduction of ABA catabolism in the roots.
2. The ABA enters the xylem in the roots and is transported through the stem to the leaves.
3. Once in the leaf tissue, the ABA enters the guard cells and causes the stomata to close, preserving leaf water potential (Wilkinson and Davies 2002).

More recently, an alternative model for root to shoot ABA signalling has been put forward, to explain plant reaction to water stress:

1. Reduced root water status in drying soil lowers whole plant water potential, through the internal water column in the xylem.
2. The sudden decrease in leaf water potential triggers ABA biosynthesis in the leaf, closing the stomata.
3. ABA synthesised in the leaves is transported to the roots, promoting root growth and increasing access to water in unexploited soil (McAdam *et al*. 2016).

There is evidence in the literature to support both models, and there is still much debate regarding the precise mechanisms by which either of these responses would be enacted. It is possible that the conflicting evidence is due to between-species differences in hormone signalling mechanisms, but even within one species (tomato, *Lycopersicon esculentum*) there have been reports of behaviour supporting both paradigms. Sobeih *et al*. (2004) and Dodd (2007) describe partial rootzone drying (PRD) experiments, during which leaf water status is maintained despite significant changes in stomatal conductance between well-watered and PRD treatments (Sobeih *et al*. 2004). PRD also had a significant effect on the amount of ABA in the root xylem (Dodd 2007); as the soil moisture content decreased root ABA concentrations increased exponentially. In contrast, grafting experiments using ABA-deficient mutants of tomato have found that regardless of the ability of the root system to produce ABA, under water stress the stomata will close (Holbrook *et al*. 2002). Using

isotope-labelled ABA, Manzi *et al*. (2015) reported movement of ABA from leaf tissue to the root system under drought. These conflicting examples suggest that the exact nature of ABA signalling under water stress may be situational, but under either paradigm (or both) there is a clear association of increased ABA concentration in both the root and the shoot tissues under soil drying.

Roots can sense pockets with high concentrations of essential nutrients and water in soil and proliferate into these pockets to maximise uptake of limited resources (Hodge 2004, see Figure 1.3). In the technical sense, root proliferation is the initiation of new lateral roots on a local scale, but this term has also been applied to elongation of individual roots, total root length, overall root production, and initiation of other lateral roots at the whole root system scale (Hodge 2004), resulting in many studies reporting root proliferation in reaction to a stimulus in the soil environment. High levels of phosphate (PO_4^{3-}), ammonium (NH_4^+), and nitrate (NO_3^-) caused dramatic root proliferation in barley, although there was no evidence of proliferation in response to potassium (K) (Drew 1975). The benefit of root proliferation in patches containing high concentrations of immobile nutrients such as PO_4^{3-} is self-explanatory; it is less apparent why roots will proliferate in patches of mobile nutrients NO_3^- which can leach away (Hodge 2004). Arguably, plants would benefit from a more regular distribution of lateral roots when mobile nutrients are present in high concentrations, to better acquire the mobile resource as it leaches through the soil (King *et al*. 2003).

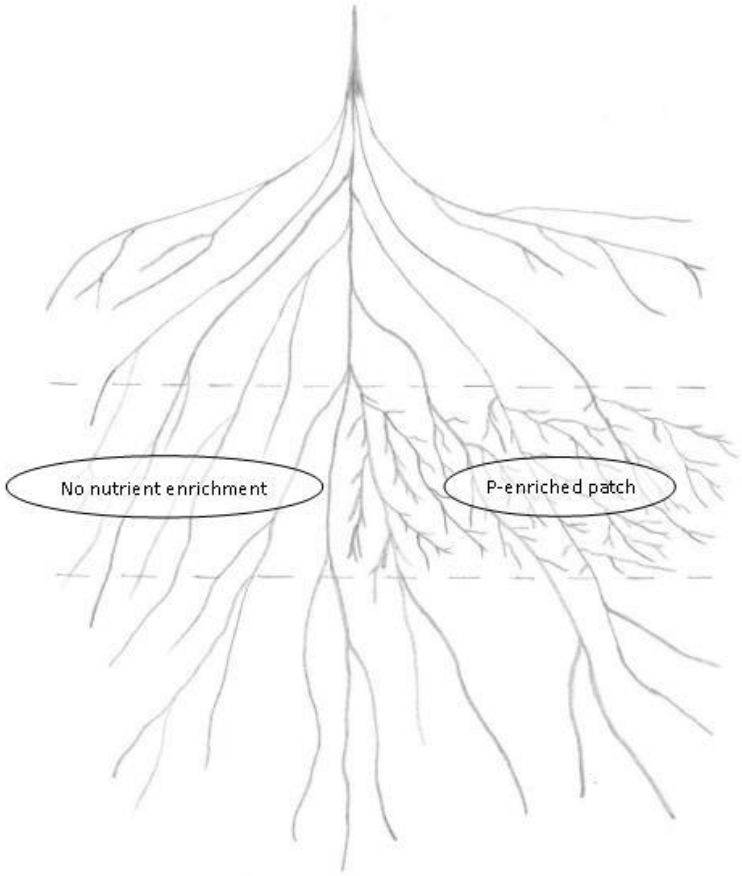


Figure 1.3: Diagram of root proliferation in a patch of nutrient rich (in this case phosphorus enriched, as an example) soil. On the left hand side of the root system, the middle layer of soil is not nutrient rich and shows no proliferation. On the right hand side, there is a patch of high P content, and the roots proliferate to exploit this resource. Based on experiments of Drew (1975).

Control of root proliferation is also an important aspect of RSA improvement; root proliferation in surface soil layers would benefit the plant in the short term by increasing nutrient access, but in the long term may leave the plant at risk of having a greater proportion of its root system in drying soil. Analysis of a system modelling the impact of root system distribution on UK winter wheat yields suggested that a deeper root system to access water, with an even distribution of lateral roots throughout the profile, could significantly improve yields by improving the efficiency of root system resource acquisition, and by reducing the pressure of inter-root competition (King *et*

al. (2003). Root proliferation is a reaction to localised soil conditions, and any genetic propensity to root proliferation is unlikely to be obvious in the first couple of weeks of root growth, due to the lack of lateral root growth in this period. The potential for areas of root proliferation remains an important consideration if investigating root distribution with depth in field-grown crops.

1.4 Root improvement targets

Until recently, directly selecting for root traits in breeding programmes has been difficult. As such, any root improvements have been incidental, and associated with more obvious aboveground traits that could easily be distinguished and selected for. In maize, increased biomass at the point of kernel number determination was a major factor in yield increases. Biomass was increased, in part, through selection for improved resource acquisition traits, such as leaf angle and higher leaf area index (LAI); both traits are easily selected for through canopy measurements. However, it was subsequently discovered that deeper root angles had also been selected for over time, despite belowground traits not being assessed (Hammer *et al*. 2009).

Shallow root systems were identified as improving phosphorus (P) uptake in some crops, notably in maize (*Zea mays*), common bean (*Phaseolus vulgaris*), and soybean (*Glycine max*) (Lynch 2011). This is because most immobile nutrients are most prevalent in the upper soil profile, even if water is scarce. Despite this observation, most current root improvement projects are based on the hypothesis that 'steep, cheap and deep' (SCD) root systems offer the highest potential benefits to crops under water limiting conditions (Lynch 2013). While primarily proposed for maize in water-

limited soils, the SCD ideotype is hypothesised to be largely beneficial to other monocotyledons (including wheat) and probably dicotyledons. The key components of the SCD ideotype are primary and seminal roots that grow at a steep angle going deep into the soil, which allows better penetration of hard soil layers (Lynch *et al*. 2014), access to deeper strata and greater exploration of the soil profile, increasing likelihood of roots finding resources. 'Cheap' roots have a reduced cortical respiratory burden, due to formation of root aerenchyma and/or fewer cortical root cells. Cheap root systems also have a reduced number of roots, but these are generally thicker and longer (Lynch and Wojciechowski 2015), again reflecting a more efficient investment of resources to the roots. In wheat grown in arid conditions there is certainly a focus of steep and deep to access water stored in soil profile to improve water uptake during grain filling (Passioura 1983, Manschadi *et al.* 2010, Rogers and Benfey 2015).

Most previous studies discussing the importance of root angle to plant growth grew wheat in arid and/or tropical regions (Oyanagi 1994; Manschadi *et al*. 2006; Manschadi *et al*. 2008; Wasson *et al*. 2012), and the ideotypes for such environments are likely to differ from one better suited to the more temperate UK climate. In regions where late season droughts are likely, narrow root angles improve access to water stored deeper in the soil profile at the grain filling stage, which allows the crop to allocate more resources to the grains, as well as extending the period of time over which grains are filled, thus increasing yield (Manschadi *et al*. 2006, Wasson *et al*. 2012). These 'deep' rooting genotypes have seedling root angle phenotypes of around 28° - 36° from the vertical, whereas 'shallow' genotypes may have average root angles of around 50° from the vertical (Oyanagi 1994, Manschadi *et al*. 2008). Vertical gradients in soil moisture in the UK may differ from those in arid regions, as

more frequent rainfalls over extended periods will re-wet the soil surface layers (although this water could be rapidly lost again by soil evaporation).

When this thesis commenced, no studies had examined differences in seminal root angle in UK wheats, nor had the impact of root angle on wheat crops grown in wet, temperate climates been explored. It has been proposed that vigorous deep root systems in dry environments might disadvantage wheat crops in very dry regions because they may reach the deep stored water too early in the growing season (pre-anthesis), thus exhausting the stored water supply during the vegetative growth phase and leaving an inadequate water supply during grain-filling (Palta *et al*. 2011). However, in a more temperate system with in-season rainfall, a deep vigorous root system may be far more efficient at exploiting soil water throughout the soil profile, without the risk of depleting all stored water too early.

Despite the difficulty in translating laboratory observations into predictions for field root system phenotypes due to highly variable climatic and soil constraints, some previous trials have shown encouraging results suggesting that controlled environment and modelling experiments can provide useful information with which to select genotypes for further study and breeding potential. For example, Manschadi *et al*. (2006) combined root angle data produced by laboratory-based phenotyping with the APSIM agricultural model (Keating *et al*. 2003) to estimate how a high-yielding variety of wheat, Hartog, would perform compared to a hypothetical variety that had Hartog's aboveground properties, but the deep-rooting traits of the drought-adapted variety SeriM82. One of the most notable differences in root traits between the two varieties is the lateral spread of the root zone. Although rooting depth at anthesis did

not differ between the two varieties, Hartog had a higher proportion of roots in the upper layers of the soil profile, whereas SeriM82 had proportionally more roots deep in the soil profile. Having roots deep in the soil profile during anthesis and grain-filling allows greater water uptake; Manschadi *et al*. (2006) estimated that utilising drought-adapted RSA traits, such as narrow rooting angle and high relative root density at depth, could increase yields in areas of southern Queensland, Australia, by 14.5% on average during seasons with water deficit. This prediction is consistent with the results of field trials conducted in another region of Australia with similar soils and climate. Kirkegaard *et al*. (2007) found that the crop's WUE of subsoil water during the grain-filling period was 59 kg per hectare, per millimetre of extra water. Manschadi *et al*. (2006) predicted post-anthesis average water use efficiency (WUE) of approximately 55 kg per hectare for each millimetre of water taken up during the grain-filling period. The relative proportions of the root systems in the different layers of the soil profile have a greater impact on soil water use than rooting depth alone.

1.5 Roots and crop management

By breeding for root systems of particular shapes and/or structures, it may be possible to exploit either established patterns of soil moisture within the soil profile, or known plant responses and interactions with the soil. Roots near the soil surface are more likely to experience evaporative water loss and soil drying, and so are more likely to synthesise the drought hormone ABA (Saradadevi *et al*. 2016). Selectively drying some regions of the root zone, while wetting others, can increase yields in many crops by increasing HI. This is the basis of the partial root zone drying (PRD) technique that

has been applied worldwide to a variety of crops and has often been found to improve both the water use efficiency (WUE) and the quality of the crop (Davies *et al*. 2011).

PRD usually applies lateral soil moisture heterogeneity, although the presence of vertical soil moisture gradients under a crop could also be seen as a form of PRD. However, roots of bean plants growing within vertical soil moisture gradient had homogeneous root ABA concentrations irrespective of root position in the soil profile and soil localised water content (Puértolas *et al*. 2013). In contrast, most studies of split-root (PRD) plants found pronounced variation in root ABA concentration according to local soil moisture conditions (eg. Khalil & Grace 1993). This strongly suggests that controlling the direction of water gradients in the soil may provide farmers with a way to control root accumulation of ABA, according to the needs of their crop.

In the UK, where wheat crops are largely dependent on seasonal rainfall, a vigorous deep growing root system is likely to have a greater beneficial effect on grain yields than in arid environments, as there is a much lower risk of depleting all stored soil water before the grain filling period (Palta *et al*. 2011). While there are likely to be large stores of water deeper in the soil profile, having some roots distributed near the surface will allow the plant to exploit areas of higher nutrient concentrations and seasonal rainfall (see Figure 1.4).

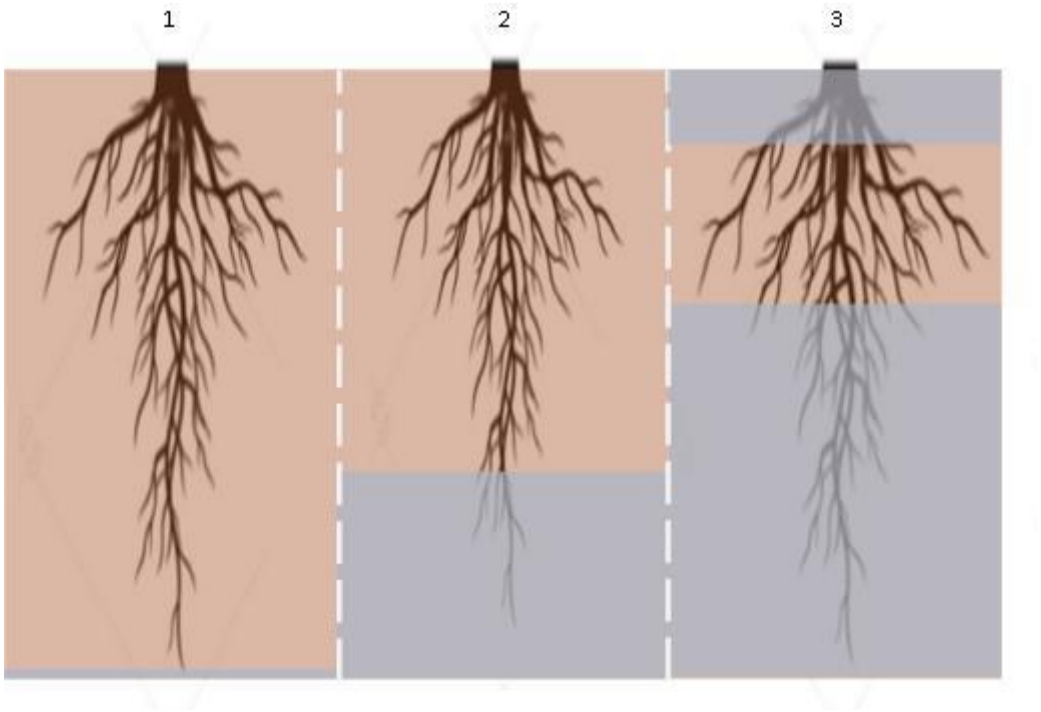


Figure 1.4: Three alternative rooting phenotypes and soil moisture profiles, as described above. Root zone 1 demonstrates a vigorous root system on a water-depleted (e.g. Australian) soil: late in the growth phase there is no water left. Root zone 2 shows the Australian ideotype on the same soil as 1: here the roots only reach the stored water during the grain-filling phase. Root zone 3 is a hypothetical vigorous root system in a wetter UK soil: where water is less readily depleted at depth, and there is a chance of in season rainfall wetting the surface layers.

1.6 Summary

General Conclusions

Although historically underutilised as a target for crop improvement, roots are fast becoming a burgeoning area of plant physiology research. As such, much of the published literature is recent and ideas and trends are constantly evolving. In particular, novel root phenotyping techniques are suggested and adapted many times, for both laboratory and field use.

Roots are necessarily highly plastic in their development, because root environments can differ extensively in (to name just a few examples) ecology, soil texture, soil water content, particle density, temperature, and many other factors individually and in combination (Hodge 2004). This can obscure genotypic differences and phenotype trends. Laboratory phenotyping trials may identify target traits that do not transfer well to the field, and theoretical yield improvements may not materialise. In the field, many environmental variables differ from controlled laboratory conditions, and the trait may not be expressed in the field as it is in the laboratory. Moreover, since roots are so inherently plastic in their morphology and physiology, there is a high likelihood of unexpected genotype x environment interactions. Alternatively, the trait may still be expressed, but under the field conditions confer no benefit. Therefore, when targeting genotypic and phenotypic trends in the laboratory, especially if working with traits in young plants to improve qualities in the adult plant (e.g. yield), it is important to test these traits under field conditions as well.

The RSA traits that have been highlighted as being of potential interest to wheat breeders include root angle, branching patterns, root number (both seminal and nodal), and rooting depth. Some of these are difficult to study in detail in a laboratory environment since pots or other containers restrict wheat roots, which otherwise can grow deeper than 2 m in the field (Thorup-Kristensen *et al*. 2009). However, seedling root angle can be measured early in plant development and is a promising avenue of research since it is a good indicator of a cultivar's suitability for dry regions with deep stored water (Oyanagi 1994, Manschadi *et al*. 2006, 2008, 2010). There has been little, if any, literature examining whether seedling root angle can affect wheat crops grown in a wetter, more temperate climate.

Availability of literature

When this thesis commenced, there was a general lack of focus in published literature on root angle effects on UK wheat cultivars, with most papers reporting root angle as an incidental measure (e.g. Whalley *et al*. 2013), if at all. However, root angle studies had been published for other cereals: seminal root angles of durum wheat (Sangunieti *et al*. 2007) and temperate barleys (Bengough *et al*. 2004) had been phenotyped. In the time since this thesis began the importance of seedling root angle and its relationship with the adult root system has been subject to many further experiments, and publications have become more abundant (reviewed in Chapter 2). Many recent publications focus on steep, deep and cheap roots, as proposed by Lynch (2013) to be useful in regions prone to drought stress. The ideotypes for seedling and adult root systems in temperate wheat crops that receive summer rainfall still receive far less attention. One of the purposes of this study is to address this gap in the literature.

Scope of project and justification

This thesis focuses primarily on UK wheat varieties, specifically a small selection of commercially and genetically interesting cultivars grown on the experimental farms at Rothamsted Research, Bedfordshire, UK. Previous work on root traits among these cultivars means that they have been reasonably well-characterised (eg. Whalley et al. 2013; Jin *et al*. 2015), and there is access to experimental field plots of these genotypes. Most of these cultivars were selected due to their position on the list of

high performing cultivars (around 2011), although some were also chosen for their potential genetic contrasts, such as the tall and dwarf lines of cv. Mercia.

The main aims of this work are to:

- Develop a suitable model system to phenotype cultivar differences in root angle (Chapter 2);

- Investigate how variation in root angle affects plant function and performance (Chapters 3 and 4);

- Investigate whether laboratory-observed variation in seedling root angle correlates with soil moisture profiles and root length/density distributions in field soils (Chapter 4);

- Investigate the possibility that seedling root angle correlates with water uptake in field soils and can therefore improve yields in UK wheat (Chapter 4).

Chapter 2: Developing a 3D methodology to determine seminal root angle of wheat seedlings

2.1 Introduction

2.1.1 Root angle phenotyping methods

As covered in Chapter 1, the ecological and agronomic importance of several root traits, including angle of seedling root growth, has become apparent in the last couple of decades, and the methods of investigating these traits have undergone rapid development. The importance of laboratory-based phenotyping screens for quantifying relevant traits within agricultural crops remains a contentious subject. Plants can display a large degree of plasticity in their phenotypes depending on the conditions under which they are grown, and this ability to acclimatise to local conditions may result in laboratory phenotypes being absent when the same genotype is grown under field conditions. This is particularly applicable to plant root systems, which have to be flexible in formation and development to find and exploit patches of high resource concentration in a heterogeneous soil; it is therefore expected that many root characteristics would be dynamic and changeable when growing in unpredictable environments (Hodge 2006). Determining root traits of different wheat varieties requires a phenotyping system with high throughput, which is cheap and easily replicable. Previous trials have used various methods to determine root angle. This chapter explores the previous methods used and determine which is most appropriate for application in this trial. Key criteria for the phenotyping system include:

- its suitability for use on a wheat seminal root system which is small, fibrous, and delicate;

- the ease of use and availability of the equipment integral to the system and any further equipment needed to harvest roots and collect data;

- the length of time taken to set up and run trials;

- spatial requirements, considering there may be limited growth space and a need for replication within trials.

Of the available methods, some are used exclusively in the field, and many more are only really of use in controlled environments, although some are suited to laboratory and field trials. However, they can broadly be split into two separate kinds of method, those that deal with a 2D, flattened root system, or those that give more information on the full 3D shape of the root system. Previous studies conducted using 2D methods include the use of rhizotrons, growing plant roots alongside flat planes (e.g. Bengough *et al*. 2004), or similarly, the use of flat boards that have pins pushed into them, before washing soil away, to record an approximation of where the roots were growing (e.g. Manschadi *et al*. 2008, Thangthong *et al*. 2016). Similar to Bengough *et al*.'s (2004) method described above, the more recent development of a high throughput screen for seedling root angle requires that seedling roots grow against the sides of a transparent plastic pot (Richard *et al.* 2015). One of the major advantages of most of these systems is the potential to analyse several plants very quickly in a high-throughput automated system: conveyer belts and/or rotating platforms can be positioned in front

of cameras allowing automatic image acquisition and transmission to analysis software (Richard *et al*. 2015), thus making them more time efficient. However, there is the risk of root angle being determined by growth along a low-resistance surface, or by restricting root system growth or development in some directions. Arguably this is a potential flaw in any artificial environment, 2D or 3D; however, it is an unavoidable risk in 2D, and only a potential risk in 3D. Only a few root angle phenotyping studies have used soil or similar media, due in part to the difficulties in establishing how roots growing in any experimental system are positioned when surrounded by soil. Of these soil-based trials, many grow roots against a solid wall, either removable or made of a transparent material, and therefore impose artificial conditions in the experimental system that would not be present in the field.

3D methods pioneered by earlier studies include the use of 'basket pots' embedded under the soil surface that allow root growth angle to be recorded as it grows through the mesh (Oyanagi 1994). This gives only a snapshot of where a root was at a certain point in time, although it is generally accepted that while roots may deviate in growth direction around obstacles, once the preferred direction of growth becomes free of obstacles then the root will revert to its original orientation (Onderdonk and Ketcheson 1973, Bandara and Fritton 1986). The basket method can indicate seminal root angle of young wheat plants (Oyanagi 1994). Later studies devised methods for tracking root growth over time, including measurement of root angle. One of the simplest such systems is the 'cigar roll' system, where a seed is germinated in a wrap of damp germination paper, or even blue roll (Zhu *et al*. 2005, Bai *et al*. 2013). The roll can be unwrapped to take regular measurements of roots traits, although in this system root angle can be constrained by the tightness of the roll and density of the

paper used. Both simple cigar rolls systems, and more complex dynamic root measuring experiments, have generally relied on the use of photography to collect image sequences for analysis of RSA. Thus, growing in soil systems was generally not possible as the roots may not be visible; instead these methodologies make use of small containers of agar gel as the growing medium (Iyer-Pascuzzi *et al*. 2010, Clark *et al*. 2011) or more recently, transparent 'soils' (Downie *et al*. 2012). As with some of the 2D methods discussed above (e.g. Richard *et al*. 2015), many of these systems can be at least partially automated to improve efficiency and throughput.

Other recent advances include using magnetic resonance imaging (MRI) (van Dusschoten *et al* 2016) or X-ray computed tomography to produce 3D representations of a root system growing within the soil (Gregory *et al*. 2003, Tracy *et al.* 2010, Mooney *et al*. 2012). However, these methods are both more expensive and more time consuming than a photography-based method, thus decreasing throughput. Depending on the specification of the scanner and the computer used to image the scans, a single scan can take over an hour to produce an image (Tracy *et al*. 2012). Further time is then required to process images for data collection, whether that is through use of software, or by having someone assess the images manually. There are also complex technical issues that go hand-in-hand with complex analysis: for example, a major limitation that must be overcome in applying CT scanning technology to study roots growing in soil is the assignment of variation in differences of the attenuation coefficient while scanning. Soil water, bulk soil, and root material can all attenuate the signal; differentiating between them, as a CT scan image is constructed, is likely to become more sophisticated and accurate with time as researchers develop enhanced software and algorithms to do this task (Tracy *et al*. 2012). To clearly resolve roots

and soil in the scan, soil water content must be closely controlled, thus there is no straightforward method for utilising X-ray computed tomography when the impact of variation in soil water content on root traits is of interest.

Another way of preventing soil or growth media from obscuring roots for imaging is to remove it entirely; some researchers have grown plants hydroponically (Chen *et al*. 2011, Shrestha *et al*. 2014) and aeroponically (Lobet *et al*. 2011) to measure root traits, including relative root mass and root length of plants. However, these systems may not be suitable for measuring root angle, as they do not provide the same mechanical resistance and support as soil (Shrestha *et al*. 2014). A rare study directly comparing phenotyping methods found that root percentage mass was significantly reduced in hydroponically grown rice plants, compared to plants grown in soil in pots or rhizotrons (Shrestha *et al*. 2014).

The phenotyping method used in this thesis research needs to be usable under many different conditions, as well as satisfying the criteria first stated above. After appraising various root phenotyping methods (Table 2.1), the basket pot system devised by Oyanagi (1994) was selected, because it is a simple and inexpensive way to grow plants from seed in a relatively unconstrained 3D system for measuring seminal root angle in soil-grown wheat plants.

Table 2.1: Comparison of phenotyping methods by requirements. The throughput column describes both how quickly the experiment is conducted overall, and estimates the number of samples that can be analysed in one trial. Speed of data collection is a simpler estimate of the time (and effort) that goes into recording data from one individual sample within a trial, using the given method.

Method	**Affordability**	**Throughput**	**Dimension of measurement**	**Speed of data collection per individual sample**
Basket pot (Oyanagi 1994)	Cheap	Medium	3D	Slow
Transparent soil (Downie *et al*. 2012)	Middling	Medium to high	3D	Quick
CT scanning (Mooney *et al.* 2012)	Expensive	Low	3D	Slow
Gel media (Bengough *et al*. 2004/ Iyer-Pascuzzi *et al*. 2010)	Cheap	Medium to high	2D or 3D	Quick

2.1.2 Soil variables and root angle interactions

As sessile organisms, plants must adjust to their local conditions as they cannot move to environments that suit them better when conditions are sub-optimal. There is evidence to suggest that it is not necessarily a specific plant species' or cultivar's intrinsic root system traits that provides a selective advantage in a difficult environment, as much as it may be an intrinsic ability for that root system to respond (Malamy 2005). Roots are plastic in that their pattern of growth is modular and reacts to the local environment (Hodge 2009), but some species and cultivars produce stronger responses to environmental stimuli than others.

Availability of nutrients

Roots proliferate (Figure 1.3) in patches of high nutrient concentration in soils, especially if that nutrient is otherwise scarce in the soil environment. Proliferation, growth of adventitious roots, and dispersion of lateral roots are common responses to patches of nitrate (NO_3^-), ammonium (NH_4^+) and inorganic phosphate, but not potassium (K) (Forde and Lorenzo 2001, Hodge 2004). Modification of root angle in response to nutrient sources is less commonly reported, but there are examples such as some common bean (*Phaseolus vulgaris*) genotypes which are adapted to low phosphorus (P) soils (Lynch and Brown 2001). Root angle in low P conditions was shallower, to maximise foraging opportunities as P concentrations decrease with depth in the soil profile. The same response has been observed in soybean (*Glycine max*) and pea (*Pisum sativum*) (Forde and Lorenzo 2001), and *Arabidopsis thaliana* (Bai *et al*. 2013). Meanwhile, low nitrogen (N) conditions caused maize roots to grow at a steeper angle in the soil (Trachsel *et al*. 2013). The only other nutrient reported to affect root angle is K, which triggered a 'slanting' response in *Arabidopsis thaliana* roots (Julkowska and Testerink 2015), with roots growing deeper when K^+ ions are deficient in the soil. Sodium, calcium, and magnesium ions did not influence rooting angle (Julkowska and Testerink 2015).

Soil water availability

Seedling root angle changes have been observed as a response to moisture gradients (Oyanagi *et al*. 1995). While all wheat genotypes appear to have a specific gravity-set rooting angle (Oyanagi 1994), the angle at which seminal roots grow into the soil can

be altered by introducing strong water potential gradients, inducing a positive hydrotropic response (Oyanagi *et al*. 1995). The extent to which the roots reacted to these gradients appeared genetically controlled, with some genotypes displaying a strong positive response to regions with higher water content, whereas others were relatively unresponsive. The original hypothesis that the unresponsive genotypes were more strongly affected by gravitropism than hydrotropism was not supported by the work of Oyanagi *et al*. (1995), who found that many of the genotypes unresponsive to moisture were similarly unaffected by gravity.

The exact mechanism controlling the hydrotropic root response is not yet fully understood, but there is clearly a genetic component, and it is possible that the mechanism is hormonal in nature. Recent studies on the salinity response (a movement away from highly saline conditions, which may share cross-talk with a hydrotropism response) of *Arabidopsis thaliana* roots, found transcription changes in jasmonic acid (JA) signalling when the plants were starved of potassium ions (K^+) (Julkowska and Testerink 2015). K^+ deficiency decreased rooting angle, and a quantitative trait locus (QTL) analysis of this response revealed one locus corresponding to root angle. Isolating the hydrotropic and halotropic responses can be methodologically challenging, as many previous studies used salt solutions to establish moisture or salt gradients to measure the threshold and/or magnitude of root reaction (Oyanagi *et al.* 1995). For the sake of simplicity, the experiments presented in this chapter will focus only on hydrotropism as a response to the presence of water, without measuring salt concentrations in the soil, but it is worth bearing in mind the possible interaction of this factor.

Temperature

Temperature affects seminal root angle in cereals, with the shallowest rooting angles in maize (*Zea mays* L.) observed at a constant soil temperature of 17 °C, with temperatures higher and lower than this causing deeper seminal root angles (Onderdonk and Ketcheson 1973). A more recent study undertaken by Nagel *et al*. (2009) found that low temperatures (10 °C, compared to a control of 20 °C) reduced the branching angles between primary and lateral roots of oilseed rape (*Brassica napus* L.) seedlings grown in agar-filled Petri dishes, again contributing to deeper angle of root growth across the whole root zone.

Soil strength

Soil strength and soil water content are closely related. Even in pot trials, soils may become significantly stronger as they dry. Previous trials have already established that in addition to influencing root growth angles, increased soil impedance correlated with decreased root elongation and decreased stem elongation (Whalley *et al*. 1999), but there are fewer studies into the effect on rooting angle. In an example of one such study, UK winter wheat plants were grown in sand columns, where the control treatment had light polystyrene blocks on top of the columns, and in impeded columns where a heavy weight was placed on top of the columns to increase impedance of root growth (holes in the blocks and weights allowed stem growth without physical restriction). The penetrometer resistance of the control treatment was approximately 0.19 MPa, whereas in the weighted columns penetrometer resistance was approximately 0.75 MPa. Impeded plants had roots that grew more steeply into the

soil, creating a narrower spread of roots (Jin *et al*. 2015). It is hypothesised that the angle at which roots grow into soil may also influence the penetration of bulk soil and hard pans in field soils (Whalley *et al*. 2013).

2.1.3 Aim of this chapter

The aim of this chapter is to develop a simple soil-based phenotyping system that captures 3D root angle data. The system should be easily replicable, cheap to run, and simple to manipulate, allowing the investigation of root angle under a broad variety of conditions. This set up will then be used to phenotype different wheat cultivars to establish root angle under both optimal and suboptimal soil conditions.

2.2 Methods and Materials

2.2.1 Plant growth

The following experiments were conducted using eight cultivars of wheat (*T. aestivum* L.), which were selected from those grown on Rothamsted Research's Woburn farm, to allow comparison of laboratory and field trials. The cultivars grown at Rothamsted were a mix of commonly grown, high performing, commercial cultivars, and some genotypes of scientific interest. Not all cultivars were used in all experiments; for some earlier experiments only two varieties, Battalion (*Bat*) and Robigus (*Rob*) were used; however, where fewer varieties than all eight are used, justification is given for the use of those specific cultivars.

Table 2.2: List of cultivars (with abbreviations) used throughout this thesis.

Cultivar	**Abbreviation**	**Reason for Inclusion**
Avalon	*Ava*	Parent of a mapping population bred by Rothamsted Research
Battalion	*Bat*	Identified as a 'deep' phenotype through previous experiment
Cadenza	*Cad*	Parent of a mapping population bred by Rothamsted Research
Hystar Hybrid	*Hys*	High performing commercial cultivar
Istabraq	*Ist*	High performing commercial cultivar
Rht 1ba Mercia (tall)	*Tall*	Included for comparison with dwarfing cultivar
Rht 1bc Mercia (dwarf)	*Dwarf*	Included for comparison with a 'tall' cultivar
Robigus	*Rob*	Identified as a shallow phenotype through previous experiment

An excess of unsterilised wheat caryopses were germinated in a Petri dish on a sheet of filter paper (Whatman #1, Maidstone, UK) wetted with distilled water, and then left in the dark at room temperature for 24 hours. After 24 hours, 10 seeds of similar size and development for each genotype were planted in a walk-in controlled environment (CE) room at Lancaster Environment Centre (Lancaster University, United Kingdom). Average day and night temperatures were 24°C and 19°C respectively, with 12 hours of artificial lighting per day/night cycle; daytime light intensity at plant height was approximately 600 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Relative humidity did not vary dramatically during the day/night cycle, averaging approximately 60 %.

Seeds were selected for use in trials by visually assessing their development. Seeds that had failed to germinate were discarded, as were seeds where the early stages of

root formation (where the radicula was visible and longer than approximately 1 mm) were apparent. In selected seeds, the coleorrhiza had broken through the pericarp of the caryopsis, but the radicula was not yet visible. Seeds of a similar size were preferentially chosen from the available supply and transferred to a basket pot system, or to blue roll paper tubes (Kimberly-Clark Professional™ WYPALL™ L20) if measuring rates of root elongation.

Figure 2.1: Photograph of wheat caryopses (*Bat*) taken 24 hours after being left to germinate on filter paper. The seeds on the left already show clear radicula and seminal root development, and were discarded. The seeds on the right have only the coleorrhiza showing through the outer pericarp layer, and were planted.

2.2.2 Basket pots

In these trials, a pre-made pot (Figures 2.2a and 2b) was used as the basket pot. The pots measured 50 mm in diameter at the top, 36 mm in diameter at the base, and 49 mm in height, and were manufactured from black plastic (Teku, Pöppelmann Plastiques, Pöppelmann, France). The sides and base of the pot have regular large holes separated by thin strips of plastic, essentially making them a 3D grid structure. Typically used as a container for hydroponics systems, or to propagate *Arabidopsis thaliana*, these were preferred as they were cheap and readily available, and more resistant to accidental deformation or damage than the custom-built, hemispherical nets used by Oyanagi (1994) and Jin *et al*. (2015).

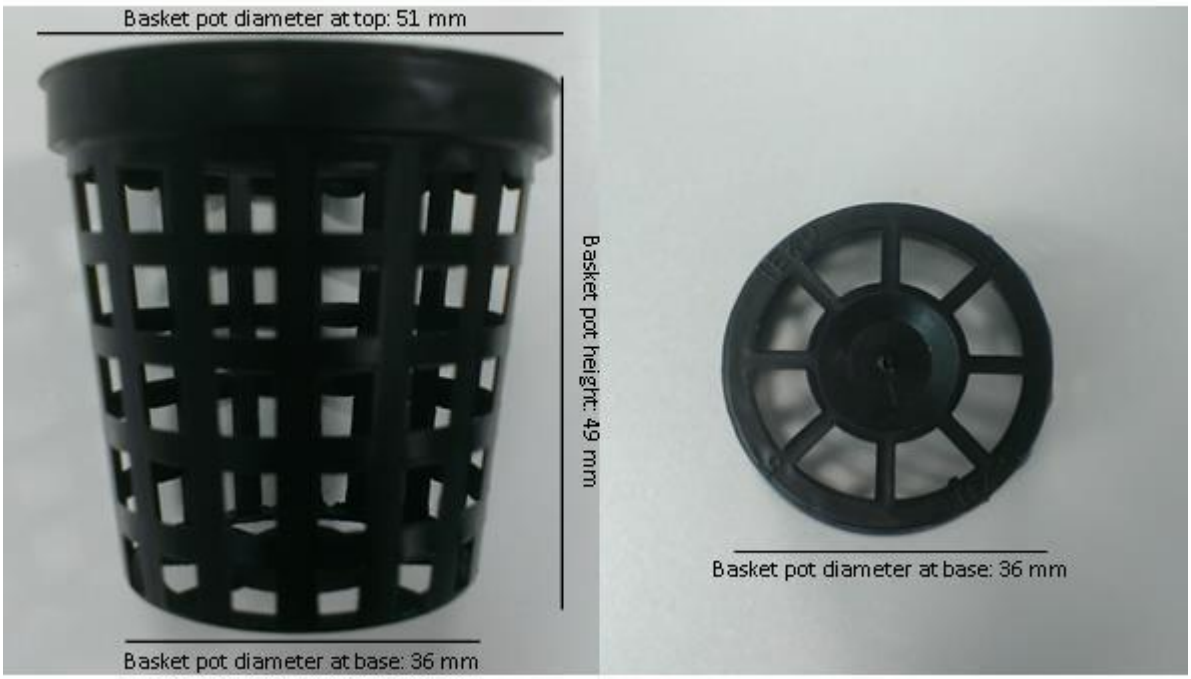


Figure 2.2a, b: Photographs of the basket pots used in this study, a) from the side, and b) from the base. The vertical rows in the mesh are described as columns to distinguish them from the horizontal rows which are described as rows.

Trials used two concentric pots, the inner basket pot and an outer pot; the size of the outer pot was selected for the requirements of a given experiment. All laboratory experiments conducted in this study used the proprietary growing compost John Innes no. 2 (J. Arthur Bower's, William Sinclair Holdings PLC, Lincoln, UK), similar to an organic loam soil (Dodd *et al*. 2010). Regardless of required pot size, the outer pots were filled with untreated John Innes no. 2, and the inner basket pots were filled with John Innes no. 2 sieved using a 4 mm mesh. Sieving removed large particles that could potentially divert the growing root, thereby affecting rooting angle measurement.

The inner pots were filled completely with sieved John Innes no. 2, and the outer pots then filled in around them. 100 ml of tap water was added to pots for well-watered treatments, and the water stressed treatments received only 25 ml. The soil was

allowed to settle and both pots were refilled as necessary, before planting a single pre-germinated wheat seed in the centre of the basket pot at a depth of approximately 5 mm. John Innes no. 2 is designed to support growth of young plants for up to around four weeks. As no trial lasted longer than ten days, no extra nutrients were added.

Pots in this trial, and all further trials in this chapter as detailed below, were arranged on benches under artificial lighting, using a random block configuration.

2.2.3 Determining soil matric potential

Gravimetric soil water content of the upper 6.5 cm of the soil profile was measured daily, as well as at the start and end of every experiment, using an ML2x theta probe (Delta-T Devices, Burwell, UK). Moisture release characteristics were defined in a previous study (Martin-Vertedor and Dodd 2011) by plotting the relationship between matric potential (kPa) and gravimetric water content potential (g g^{-1}), producing the release curve in Figure 2.3. This curve was used to convert gravimetric water content to soil matric potential.

The bulk density of soils can affect the moisture release curve. The experiments of Martin-Vertedor and Dodd (2011) and the experiments described in this chapter were not conducted simultaneously, and therefore it cannot be said with certainty that the same bulk densities were used for both experiments. However, neither study worked at the extremes of bulk density, and it is assumed that the former fell within the average range of bulk densities for John Innes no. 2 (800 – 950 gL^{-1}) (John Innes

Manufacturers Association 2010), and is therefore similar, if not the same as, the bulk density used in the experiments described in this thesis.

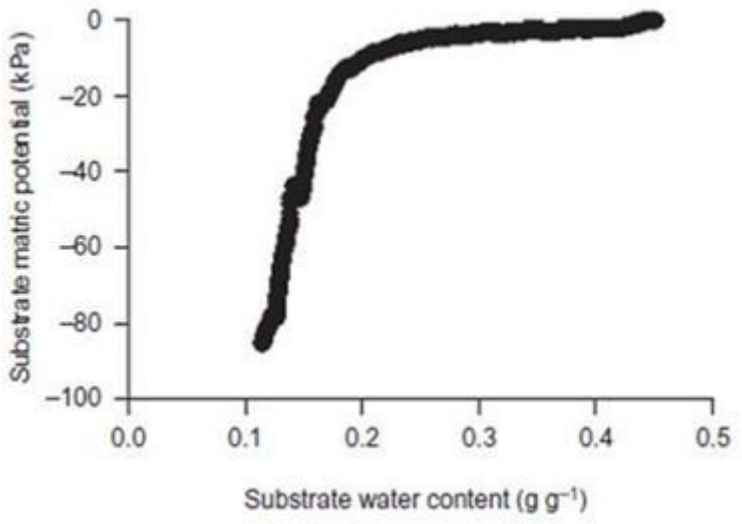


Figure 2.3: Soil moisture release curve for John Innes no. 2 compost (with permission from Martin-Vertedor and Dodd (2011)).

2.2.4 Measuring root angle

Plants were harvested at the second leaf stage, stage 12 in Zadoks code (Zadoks *et al.* 1974), around 8 days after planting. This allowed sufficient time for the seminal roots to emerge from the basket pot and grow into the outer pot.

The outer pots were gently inverted while the basket pots were held securely. Once the outer pot was removed, soil surrounding the basket pot was removed gently by hand, taking care not to damage the seminal roots. It was sometimes necessary to wash the roots while still within the basket pot, to determine which root had emerged through which hole. Once the seed and roots could be seen, the clearly defined crease on the seed lined up with a vertical column boundary of the basket pot (Figure 2.5).

This was considered as 0°, with angle increasing in a clockwise direction around the circumference of the pot. The column of square holes immediately clockwise from the 0° line was column ‘A’, with columns ‘B’ through to ‘P’ proceeding clockwise. Each column contained five vertically aligned holes which were numbered 1 – 5 from top to bottom. If roots emerged from the bottom of the pot then this was labelled as ‘Q’, and the location around the circle was divided into sections from 1 – 8, where Q1 lined up with the A column. Numbers were allocated clockwise, with 1 being the hole at the base, or beginning immediately to the left of, the 0° line. Using these rules, any hole through which a root grew could be uniquely identified (i.e. B3, O4, etc).

The circumference angles were calculated by dividing 360° by the number of grid columns in the net-pot (16 columns), and then halving the resulting value (22.5°/2) to give the centre value of the first column (11.25°). To work out the angle around the circumference of the other columns, multiples of 22.5° were added to 11.25°. The ‘Q’ angles for the bottom of the pot were calculated in a similar fashion, but instead of having 16 columns to use in calculation there were simply eight holes.

Root vertical angles were calculated using trigonometry, taking the centre point of the individual holes as the exact point of emergence. Using the vertical distance to a hole’s centre point from the top of the pot and the horizontal distance to the hole’s centre point from the centre of the pot as known distances (the opposite and the adjacent, respectively), the approximate rooting angle was calculated using the trigonometry rule that the tangent of the angle is equal to the opposite divided by the adjacent. Table 2.3 lists the circumference and vertical angle values used, as calculated using the methods described above.

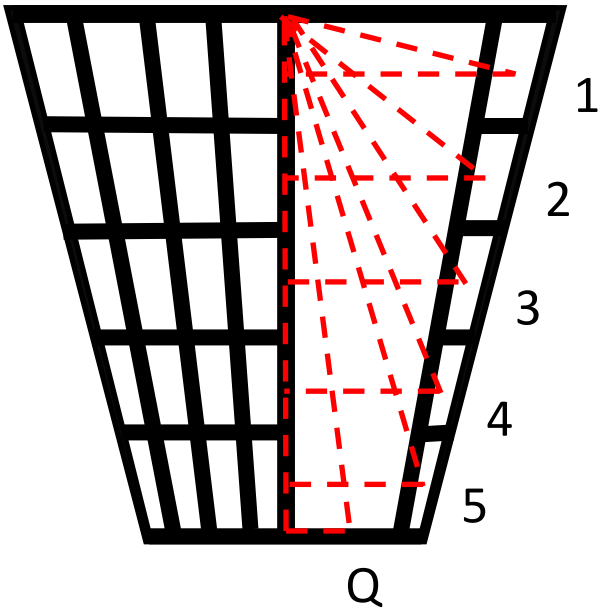


Figure 2.4: Illustration of simple trigonometry technique that allowed calculation of the angle that the root makes with the soil as it emerges from the basket pot; this angle is hereafter referred to as the vertical angle of root growth, and is one of the key traits of interest in this study.

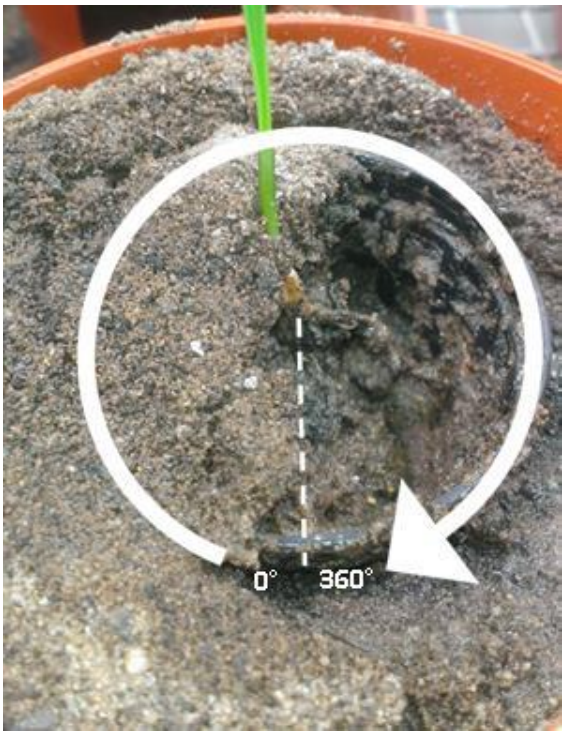


Figure 2.5: The crease of the wheat seed is found by careful excavation of surface soil (illustrated between approximately 180° and 360° on image), and then aligned to the nearest gridline of the basket pot to find 0° around the circumference. The columns are labelled clockwise A, B, C, through to P.

Table 2.3: Compilation of all possible angle values. Circumference angle describes the angle around the circle of the pot clockwise, as viewed from above. Vertical angle describes the angle of the root taken from the side, with 0° being horizontal and 90° being vertical. Q is used to denote when a root emerged from the bottom of the net-pot; in this case the vertical angle is always the same, and there are fewer possible circumference angles.

Column Letter	A	B	C	D	E	F	G	H
Circumference angle (°)	11.25	33.75	56.25	78.75	101.25	123.75	146.25	168.75
Column Letter	I	J	K	L	M	N	O	P
Circumference angle (°)	191.25	213.75	236.25	258.75	281.25	303.75	326.25	348.75
'Q' holes	1	2	3	4	5	6	7	8
Circumference angle (°)	22.5	67.5	112.5	157.5	202.5	247.5	292.5	337.5
Row number	1	2	3	4	5	Q		
Vertical Angle (°)	15.2	29.7	46.5	57.3	64.7	81.5		

2.2.5 Statistical Analysis

Statistical analyses were conducted in SPSS version 22 (IBM Corp, NY, USA). Means were compared using either a T-test or an ANOVA depending on the number of treatments. When the data were not normally distributed, the non-parametric Mann-Whitney U-test or the Kruskal–Wallis test was used instead. Harvest timing was not considered a factor in root angle measurement, and so these data were not normalised.

2.2.6 Experiment Schedule

Experiment 1: Preliminary method trial

Experiment 1 used *Rob* and *Bat*, since a previous study indicated these two genotypes had distinct patterns of root system spread (Whalley *et al*. 2013). Wheat caryopses of both genotypes were prepared as described in section 2.2.1, before 10 seeds of each genotype were planted in basket pots held within an individual 1 L pot (as described in section 2.2.2). After watering at the start of the experiment, the pots were individually weighed to record starting weight. The pots were subsequently watered from the surface every other day to bring them back to their recorded start weight.

To compare root elongation patterns, 8 pre-germinated seeds from each genotype were wrapped in blue roll, and the blue roll tubes placed vertically with the bottom of the seed orientated vertically downwards in an open-topped Perspex rack (length 300 mm, height 150 mm, width 20 mm, see Figure 2.6), which was half-filled with distilled water to keep the blue roll tubes damp throughout and prevent the roots and seed from drying. While there was a concern that the roots may be growing in hypoxic conditions, there was no observed negative effect on root growth, even when they had grown long enough for the tips to be submerged (Figure 2.7). The plants in the blue roll tubes were grown in the same CE room as the basket pots. The roots were unwrapped daily and measured using a transparent 30 cm ruler. This experiment estimated how long it would take the roots to grow through the mesh of the basket pot. Root angle data collection took place a few days after this period to allow for unusually slow growing roots, and also because longer roots were easier to identify

and trace back to their emergence from the basket pot. Root extension rate data support this assumption (see Figure 2.7).

Figure 2.6: Photograph of one rack of assembled blue roll tubes for root elongation growth rate experiments.

Experiment 2.1: Effects of soil water content on seedling root angle

Rob and *Bat* were used again. However, this time two soil water availability treatments were created; half the pots of each genotype received 100 ml of water at the start of the experiment, while the remainder received 25 ml, creating well-watered (pots were at drip point) and dry soil conditions, respectively. To determine the maximum soil water deficit between irrigation events, soil matric potential of the soil in the outer pot was measured daily, approximately 40 mm from the surface, and always immediately before watering, as described in section 2.2.3. Size constraints

prevented measuring soil matric potential inside the inner basket pot, however it is assumed to be similar between inner and outer pots. 10 replicates of each treatment combination were used, as described in the previous experiment.

Experiment 2.2: Measuring soil strength

Throughout the course of the experiment, 6 similarly filled pots (3 well-watered treatments, 3 dry treatments) were set up to investigate the possibility that soil drying was causing soil in the pots to become significantly stronger. To measure soil strength at intervals throughout the experiment, penetrometer resistance was measured with a non-rotating, 2 mm diameter penetrometer which had a 60° cone angle (Whalley *et al*. 2005). Measurements were taken at the start of the experiment, and then before re-watering at 2 and 4 days after planting. The needle was positioned such that the penetrometer was measuring the resistance of the sieved soil inside the basket pot. These measurements were taken on three occasions, as it was assumed that after this time roots had emerged from the basket pot, and so root angle had been set.

Experiment 3: Temperature effects on seedling root angle

The laboratory phenotyping system would ideally present minimal differences when compared to field conditions, so climate-controlled Snijder growth chambers (Snijders Labs, The Netherlands) were used to allow growth of wheat seedlings in low temperature conditions that would more closely reflect ambient soil temperatures when winter wheats are beginning to germinate.

To determine whether soil temperature affected root angle, Experiment 2 was repeated using Snijder growth cabinets set to 2 °C, 6 °C, and 10 °C and the results compared with plants grown in a CE Room with day/night temperatures of 24 °C and 19 °C (22 °C on average) respectively. 10 plants of each genotype were grown for comparison of root angle and set up as stated in section 2.2, under well-watered conditions.

Patterns of root elongation were also compared as described above in Experiment 1, although in this case two racks of each genotype were grown alongside the basket pots in the Snijder cabinets, for a total of 16 plants per genotype, each with 1-3 roots.

Experiment 4: Phenotyping multiple cultivars

Having conducted preliminary laboratory experiments to find a suitable method for screening root angle phenotypes under field-similar conditions, the rest of this chapter is devoted to the phenotype screen itself. The remaining cultivars of interest, *Ava*, *Cad*, *Hys*, *Ist*, *Dwarf*, and *Tall*, were grown in multiple repeated trials, with 10 replicates per trial, as per *Bat* and *Rob* in Experiment 2.1.

2.3 Results

Experiment 1: Root angle measurements

Although not as precise as the original hemispherical basket design, which could identify root angle within a couple of degrees (Oyanagi 1994), the plastic basket pots used in this trial allowed root angle data to be collected with sufficient resolution

(within approximately 7°) to distinguish differences in seedling root angle between *Bat* and *Rob* (Figure 2.8).

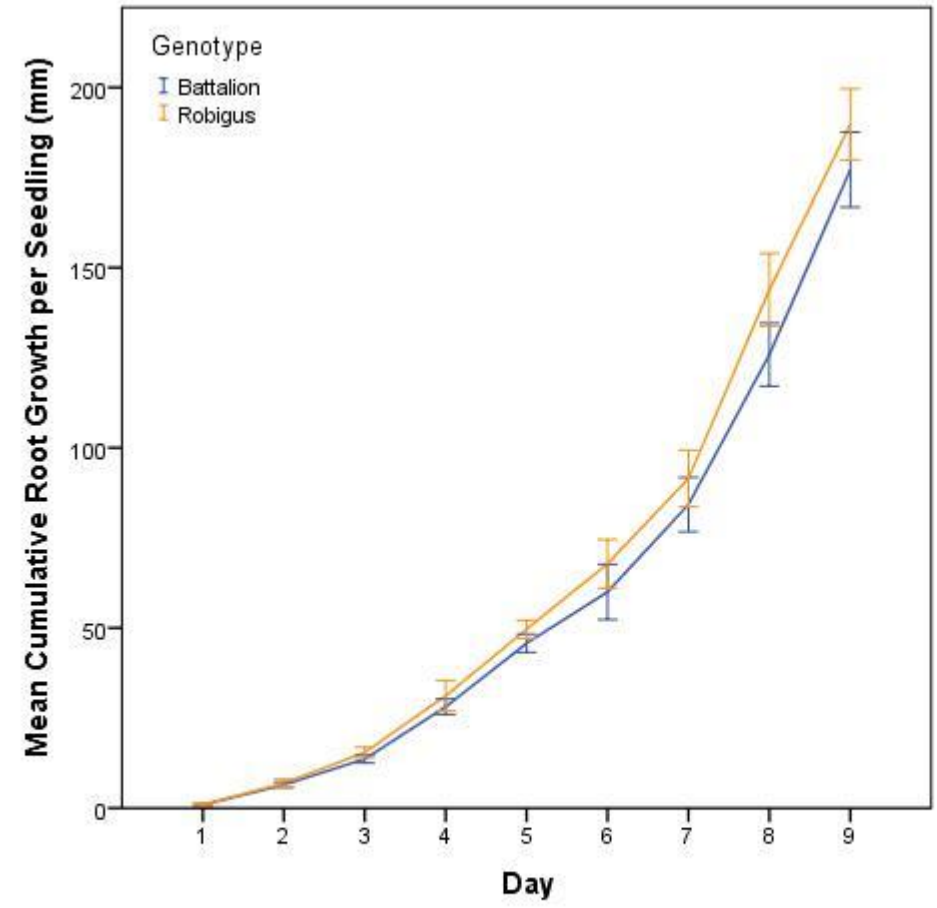


Figure 2.7: Cumulative root length of genotypes *Bat* and *Rob* during the course of Experiment 1. There were no significant differences in root elongation between the two genotypes.

The first three pairs of columns in Figure 2.8 illustrate the three experimental replicates of this first trial to check for consistency of results, and the fourth pair of columns pools data from these three replicates. With the exception of the third repeat, which was nearly significant (p-value = 0.072), these results show that *Rob* has a significantly (p-value $\leq$ 0.023) wider seedling root angle spread than *Bat*.

Alternatively, this could be phrased as *Rob* displaying a shallower root phenotype than *Bat*, as its roots tended to be located closer to the soil surface that those of *Bat*.

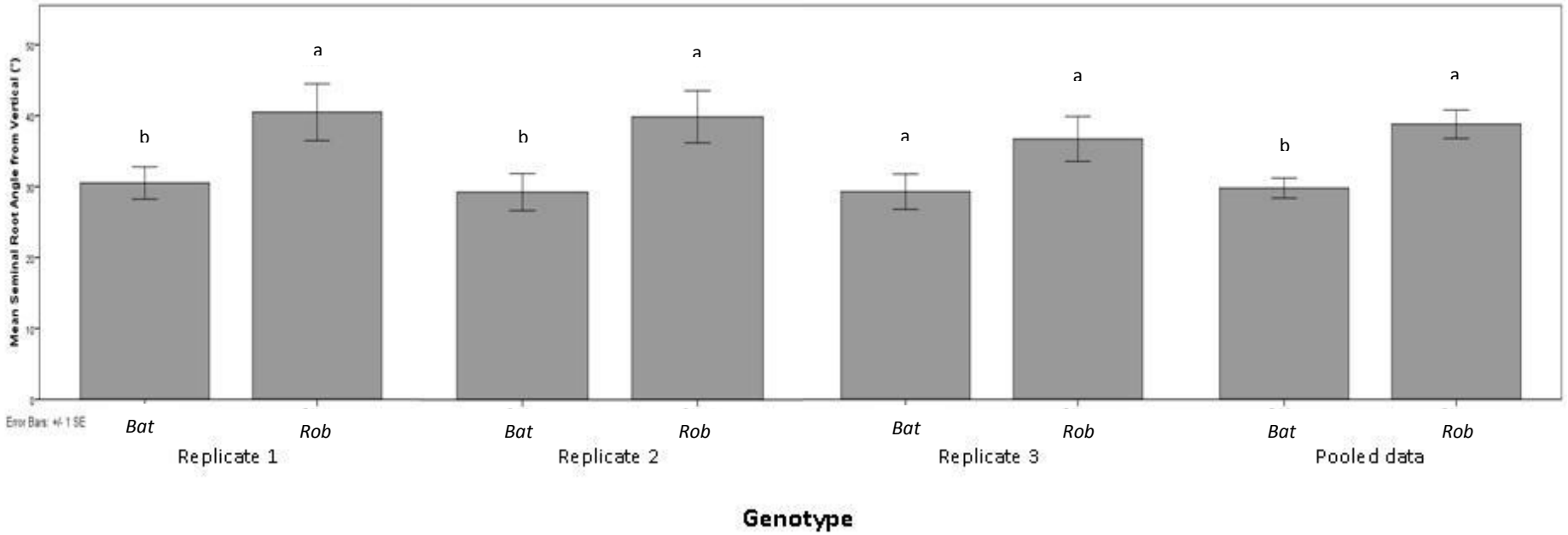


Figure 2.8: Seminal root angles of wheat seedlings when harvested, at approximately second leaf stage of growth. The angles to vertical made by both roots from the first pair of seminal roots are taken as individual data points, with the mean calculated as the average of all data points. N is the number of roots measured for each treatment: for individual replicates, N = 20 – 29, for pooled data N = 66 (*Rob*) and 82 (*Bat*). The pooled data was calculated as the mean of all individual data points across the three replicates. Columns are means ± SE, letters above columns are letters of mean discrimination between genotypes within replicates.

These roots grew in a relatively unrestricted environment, and they had grown to a sufficient length 7 - 8 days after planting to emerge from the basket pot and into the soil of the outer pot, thus being ready to harvest. This was confirmed upon harvesting the first trial of Experiment 1, 9 days after planting the pre-germinated seeds.

Experiment 2.1: Changes in water availability

In Experiment 2, two inter-related variables of interest were added to the simple methodology of Experiment 1 by changing the water status of half of the pots – by reducing the amount of water added to the pots at the start of the experiment by 75% to create water-limited conditions. In the well-watered pots, the gravimetric water content was approximately 0.4 g g^{-1}, and in water-limited pots it was approximately 0.13 g g^{-1}. The soil water matric potential was calculated using the curve in Figure 2.3. In the water-limited pots, soil matric potential was significantly lower (p-value < 0.001) than in the well-watered pots (Figure 2.9). Soil matric potential did not vary significantly within watering treatments during the experiment.

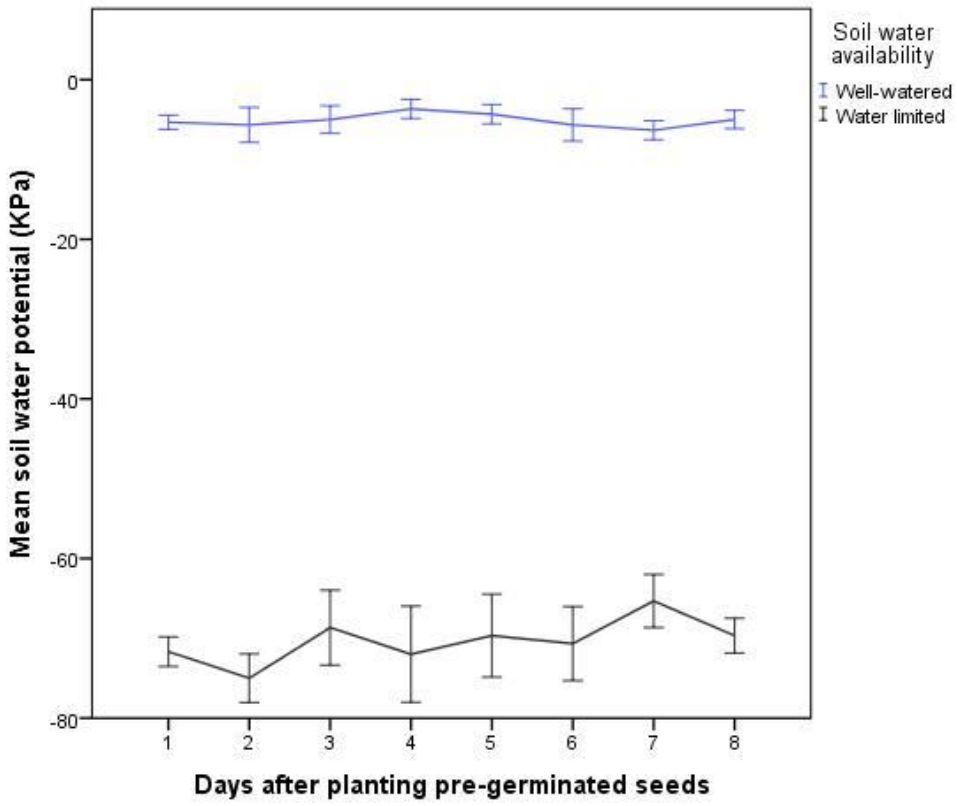


Figure 2.9: Time course of soil water potential in Experiment 2. Lines are means ± SE of 12 replicates.

Experiment 2.2: Changes in soil strength

The two different water treatments resulted in significant (p-value < 0.001, Student's T-test) differences in the average soil strength between well-watered (approximately 2.5 MPa) and water-limited (approximately 2.2 MPa) pots, with soil being about 11% stronger in the water-limited treatments, although the highest values of soil strength recorded per pot did not vary between treatments. There were no differences in soil strength between cultivars.

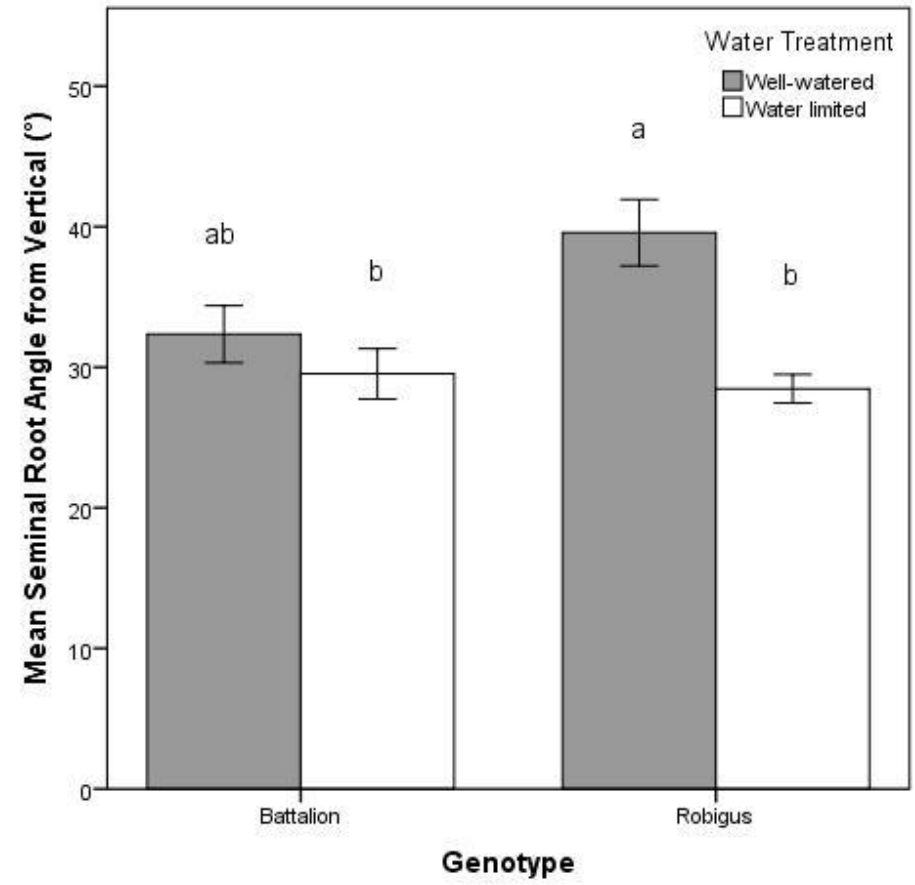


Figure 2.10: Mean root angles of individual seminal roots, from 10 plants of each treatment: well-watered conditions (as in Experiment 1) or water-limited conditions. Columns are means ± SE of 10 replicates. Letters above columns indicate whether or not there are significant differences between all treatment combinations (ANOVA, p-value < 0.05, Bonferroni post-hoc). ANOVA also allowed comparison between treatments within genotypes, *Bat*: p-value = 0.302, *Rob*: p-value = 0.022. *Bat* has a consistent root angle under both treatments, but *Rob* root angle is significantly deeper under water-limited conditions.

Although soil water availability did not alter the root angle of *Bat*, under water-limited conditions *Rob* root angles decreased by around 10° and created a deeper seedling root angle than under well-watered conditions (Figure 2.10). This was not entirely unexpected due to the necessary plasticity of roots in a highly variable environment, however the reaction of *Rob* to either the differences in water content or soil strength

is extreme enough to be considered two significantly different root phenotypes (Figure 2.10, p-value = 0.022).

Experiment 3: Effects of temperature

Cold temperatures did not significantly change the seminal root angle of either *Bat* or *Rob* (Figure 2.11 and Table 2.4); however, the rate of root elongation was significantly inhibited (Figure 2.12) by 24%, 45% and 89% at 10 °C, 6 °C and 2 °C respectively. Root growth was so constrained in the 2 °C treatment that it was not possible to collect root angle data from these pots, as the roots did not emerge from the inner basket pots by the end of the experiment. Pooling the three temperature treatments that did produce root angle data, there is still a significant difference between genotypes (Table 2.4, p-value = 0.044), however this difference is lost if the data from the 22 °C treatment is excluded (p-value = 0.525, Independent Samples Mann-Whitney U-test). Again, *Rob* was more responsive to environmental (temperature) variability than *Bat* (as indicated by the large SEs in Figure 2.12).

Table 2.4: Table of p-values from experiment analysis, comparing root angles. Genotype tested by independent samples Mann-Whitney U test. Temperature and Genotype x Temperature tested by independent samples Kruskal-Wallis test.

Variable	**Significance**
Genotype	0.044
Temperature	0.368
Genotype x Temperature	0.136

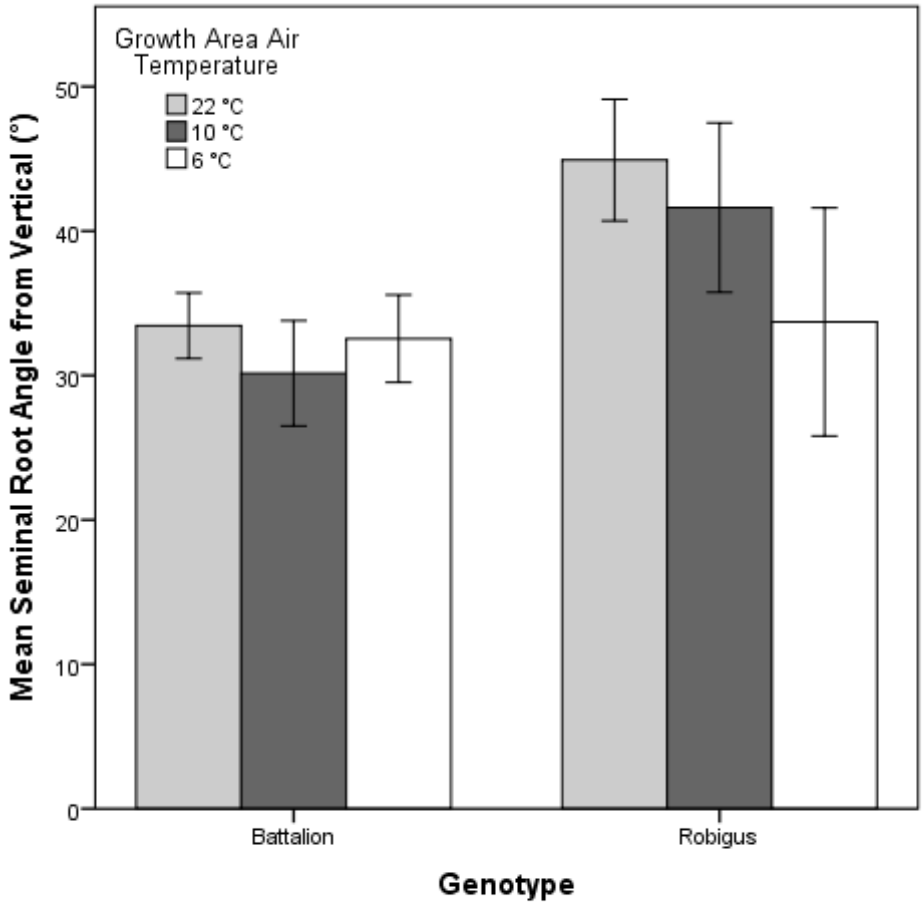


Figure 2.11: Effects of air temperature on root angle. The 2 °C treatment is not included in this figure because root growth was so restricted that the roots did not penetrate through the basket pot, thus no root angle data was available. There were no significant differences between specific genotype-temperature treatments (see table 2.4).

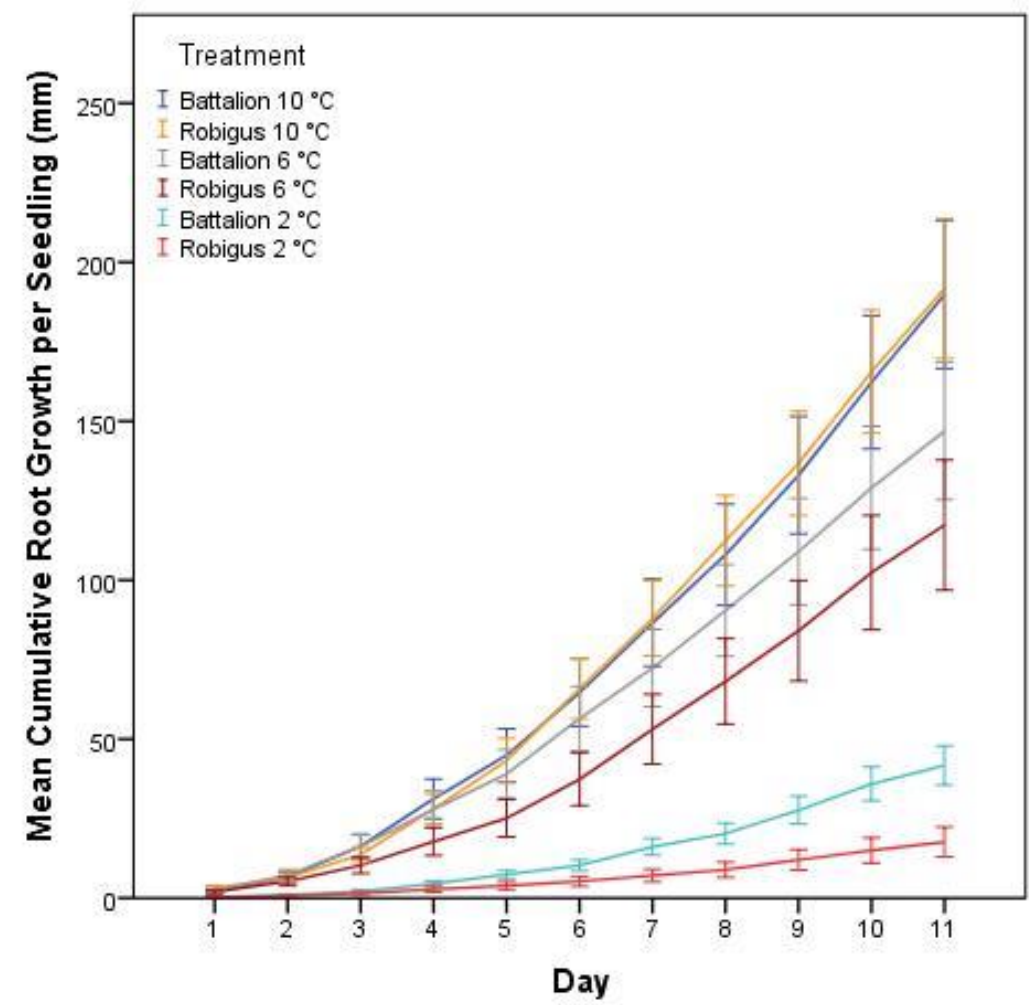


Figure 2.12: Mean cumulative root growth as a function of local environmental air temperature. Univariate analysis of variance with time as a co-variate, treatment is highly significant, p-value < 0.001.

Experiment 4: Phenotyping of multiple cultivars

Repeating Experiment 2.1 on a larger set of genotypes (Table 2.1) identified significant differences between some, although not all, genotypes (Figure 2.13). *Rob* continued to show a larger amount of variation in seminal root angle than *Bat*, and indeed any other genotype. *Bat* was the most consistent deep phenotype, and *Ist* was identified as a significantly shallow phenotype. Overall though, many genotypes

produced average seminal root angles in the range of 35° to 40°, and were not statistically different to one another.

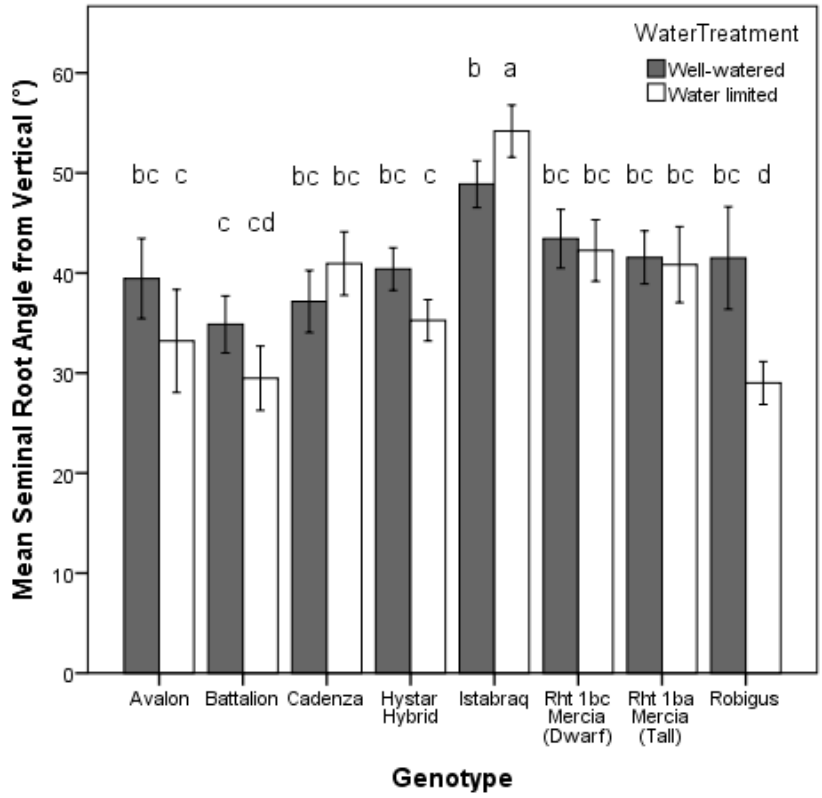


Figure 2.13: Seminal root angles of wheat seedlings grown under well-watered (grey columns) and water-limited (white columns) as recorded at point of harvest, approximately second leaf stage of growth. Columns are means ± SE of 18 – 28 replicate roots. Means calculated from the individual angles to the vertical made by the first paired seminal roots of each plant. Letters above columns indicate significant differences (One-way ANOVA, Bonferroni post-hoc, p-value < 0.05).

Some interesting differences become apparent under water-limited conditions. *Rob* again shows a highly plastic response, consistent with that displayed in experiment 2.1. *Ist* was also phenotypically plastic, as its seminal root angle is also signifcantly different (p-value < 0.05) under water-limited conditions. Interestingly, it shows an opposite reaction to *Rob*; rather than showing a deep rooting phenotype under drier

conditions, *Ist*'s roots grow at a shallower angle into the surrounding soil. This response stands out as unusual, and in contrast to what would be expected, given that deep roots are associated with water scavenging in dry environments.

2.4 Discussion

2.4.1 Suitability of basket pot method

The basket pot phenotyping system developed in this chapter detected significant differences in seminal root angle between UK wheat cultivars (Figures 2.7, 2.11 and 2.14). Scanning and imaging root zones using X-ray computed tomography (which can achieve a spatial resolution of < 500 nm, and indicate the angle of root growth to a single degree (Tracy *et al*. 2010)) would be a more accurate method than using the basket pots, as resolution of root angle in the latter can only be done categorically rather than quantitatively. Nevertheless, the basket pot method does produce 3D data on root angle in soil media, with the advantages of being both cheaper and quicker than CT imaging.

Although it would provide 3D data that could be repeatedly measured over time, using gel media in pots or cylinders is not a suitable method for wheat root phenotyping. Previous studies using this method (e.g. Iyer-Pascuzzi *et al*. 2010) have been conducted on rice (*Oryza* sp.), which is better adapted to anoxic conditions than other cereals, including wheat (Waters *et al*. 1991). Even when pre-germinated, wheat seeds struggle to grow and establish roots in gel media (Hodgkinson, *unpublished data*).

One of the major disadvantages of the basket method over a 2D transparent surface method, such as gel plates (Bengough et *al*. 2004) or transparent pots (Richard *et al*. 2015) is that it provides only a snapshot measurement of root angle, and cannot be used to trace root growth over time. This weakness is shared by the transparent soils method of measuring root angle (Downie *et al*. 2012); the growth media for this method is not transparent until a liquid is added at the point of imaging, which shares the same refractive index and thus causes the media to become transparent. However, as this method is less destructive than the basket pot method and allows imaging of the complete root system at the end of the experiment.

Currently the only method which allows repeated 3D measurements of the wheat root zone is X-ray computed tomography, which was deemed unsuitable for this research due to the time constraints, the sample size constraints, and the costs associated with it, compared with the basket method.

Some of the results observed in this chapter are consistent with previously published root angle observations for the featured genotypes: *Bat* showed a steep rooting angle and narrow root system spread in a wax disc root penetration study (Whalley *et al*. 2013). Meanwhile *Rob* had a wider spread of roots, and was less able to penetrate strong layers than *Bat* and other steeper rooting genotypes. These findings lead Whalley *et al*. (2013) to hypothesise that roots with steeper rooting angle are also more likely to penetrate hard layers than roots with shallower rooting angles. Interestingly, Jin *et al*. (2015) found that under increased mechanical impedance, wheat roots grew at a significantly steeper angle than under low soil resistance, which

further supports the hypothesis that steep rooting angle in young wheat plants aids soil penetration.

2.4.2 Soil water content and soil strength in the basket pots

Given the results of experiments 2.1 and 2.2, it was not possible to determine whether changes in root angle under different watering schedules were due to soil water content, soil strength, or a combination of both. However, these two traits are also closely related under field conditions (Whalley *et al*. 2005), so while it is impossible to isolate the effects of these two factors, it is still possible to screen for seedling traits that would be potentially also be observed in the field. The average soil strength across all pots was higher than expected (approximately 2.3 MPa), and would be expected to inhibit root growth in some plants; higher values of soil strength are associated with severe reductions in root elongation, although the precise effects are species and soil specific. Soils with root penetration resistances of approximately 0.5 MPa decreased elongation by 90% in maize and 44% in pea, compared to loosely packed control soils (Bengough and Mullins 1990). Wheat, however, has been shown to grow with only minimal impediment at least 2.0 MPa (Merotto and Mundstock 1999). In the experiments in this chapter, clearly root growth was not limited, so it is possible that the penetration resistance readings were increased artificially, perhaps by hitting the side or base of the plastic basket pot. It is also possible that these high readings came from using a fixed-needle penetrometer, rather than rotating-needle. Fixed-needle penetrometers can increase soil strength readings by up to a factor of three (Whalley *et al.* 2005).

2.4.3 Phenotypic plasticity in root angle

The root system is highly plastic in its development to allow it to exploit the environment in which it grows (Malamy 2005). As such, when considering if a genotype can show phenotypic plasticity in root angle, it can be argued that the actual question is one of whether the genotype is showing a significant degree of plasticity, greater than the stochastic variation that would be expected in plant development (Pigliucci 2005). If it is assumed that root angle changes in response to the environment are an example of continuous variation (and some previous studies, such as Onderdonk and Ketcheson (1973) and Jin *et al*. (2015) support this assumption), then the relative plasticity of rooting angle within the eight genotypes featured in this chapter can be established by comparing their reaction norms.

A reaction norm is the relationship between the environmental factor (for example from this study, decreased soil water content) and the change in the organism's phenotype (in this case root angle) (Stearns 1989). The way in which the phenotyping screen in Experiment 4 was carried out does not allow the reaction norms of the eight genotypes of interest to be plotted. However, genotypic differences in response could be assessed by comparing percentage change in root angle under the two different water regimes, or by using a statistical test to compare the well-watered and water-limited root angles to test for statistically significant differences between the two phenotypes. In this case, statistical differences between well-watered and water-limited phenotypes occurred in two cultivars: *Ist* and *Rob*.

Table 2.5: Comparison of changes in root angle with decreasing soil moisture content, * indicates significant differences in root angle (p-value < 0.05).

Cultivar	**Change in seedling root angle from well-watered (%)**
Ava	- 11.0
Bat	- 10.2
Cad	+ 7.5
Hys	- 7.7
Ist	+ 19.0 *
Tall	- 1.7
Dwarf	- 2.7
Rob	- 30.1 *

Only *Ist* and *Rob* showed significant plasticity in phenotype (Table 2.5), but interestingly the two cultivars showed different directions of change. In drying soil, *Rob* showed a deeper angle of growth, which seems adaptive given the theory that deeper roots allow better access to water (Oyanagi 1994); however, *Ist* displays a significantly shallower seedling root angle in drier conditions. While *Cad* also shows a small change in the same manner as *Ist*, it is not significantly different to the well-watered phenotype.

The phenotype is typically defined as the physical outcome of a genotype in a particular environment, although Forde (2009) identified developmental instability (also referred to as stochasticity) as potentially playing a third, underappreciated role in the expression of the phenotype. This developmental instability is random deviation from the expected phenotype of a particular combination of genotype and environment. Phenotypic plasticity has a purely genetic component, in that the genotype controls how responsive an organism is to its environment. There is also an environmental component, wherein the local environment provides external stimuli to

shape the phenotype. Developmental instability may act both as part of the genotype and a non-genetic component of phenotypic plasticity, alongside the environment, by causing stochastic variation in the development of cells and organs within the plant; it may also influence how responsive cells, organs and individuals are to environmental stimuli (Forde 2009). Applying this principle to the results of this chapter may potentially explain some of the variation in root angle of wheat. It could be hypothesised that *Ist* and *Rob* have more developmental instability, which causes them to show greater degree of change to different environments than other genotypes.

The mechanism behind the phenotypic plasticity of seedling root angle (and adult root system distributions) remains unknown, but possible hypotheses may involve the movement towards water (Oyanagi 1994), avoidance of soil hypoxia (Passioura 2006), improved nutrient uptake (Lynch 1995), or changes in hormone ratios in the developing roots in response to soil drying (Band *et al*. 2012, Sato *et al*. 2015). It should be noted as a limitation on the temperature study that it was air temperature, and not soil temperature, that was measured in the Snijder Cabinets. Furthermore, the soil temperature in the pots would have been more responsive to low air temperature than a field soil, which would be better insulated as the temperatures drop in winter, and slower to warm up again in spring as air temperature rises (Brown Beckel 1957). Therefore, it cannot be said that there is no possibility of temperature affecting root angle in the field, just from the results of this chapter.

2.4.4 Implications of diversity of seminal root angle in UK wheats

The genetic diversity of seminal root angle in UK wheats suggests that there are either physiological or yield advantages associated with specific rooting angles. While the UK climate does not generally cause environmental extremes that threaten plant survival in the same way as in Australia or India (Wasson *et al*. 2012), there may still be advantages of having roots in specific parts of the soil profile, depending on the location of the field.

2.5 Conclusions

This chapter aimed to identify a suitable method for measuring root angle and identifying differences in root angle between genotypes. Although the resolution of root angle was not as sensitive as a fine mesh net, these basket pots were still a valid foundation for an experimental system that measures seedling root angle. The basket pot system identified genotypes with atypical rooting angles and reactions to environmental differences, most importantly:

- Genotypic differences in root angle identified in this these trials were generally consistent with previous trials by Whalley *et al*. (2013), with *Bat* showing with a narrow spread of deep roots.

- Unexpected rooting behaviour within these genotypes was also revealed, with *Rob* showing a previously unreported ability to modify morphological response under different conditions.

- The consistent shallow root phenotype of *Ist* will be useful for future trials investigating how RSA impacts on wheat growth, with *Bat* as a consistent deep rooting phenotype for comparison (as described in Chapters 3 and 4).

Chapter 3: An investigation of early vegetative growth and physiology of shallow and deep rooting wheat seedling phenotypes

3.1 Introduction

3.1.1 Importance of the root system during early growth

As evidenced in Chapter 2, UK wheats show genetic variation in seminal root angle and root responsiveness to local soil conditions (Figure 2.13). This suggests that there may be some benefit bestowed by the angle at which roots grow, even in the relatively benign UK climate. However, it is not known *whether* and *how* this variation in root angle may benefit wheat crops grown in the temperate UK, either in the early growth stages or later in the plant's life cycle. In Australian wheats, narrow seedling root angle correlates well with increased rooting depth and root length density within deep layers of the soil profile, improving water uptake during grain filling and increasing yields (Kirkegaard *et al*. 2007, Manschadi *et al*. 2008). The average UK soil profile will generally stay much wetter throughout the growing season than a severely droughted, arid Australian soil profile (cf. Lilley and Kirkegaard 2011, Dodd *et al*. 2011). In the south east of the UK, where wheat is commonly grown, the average annual rainfall varies from approximately 550 mm – 950 mm (Met Office 2016). The Australian wheatbelt covers a far larger area with greater climatic variation, but the majority of the wheatbelt receives approximately 300 mm - 600 mm per annum on average (Land Commodities 2014). The advantages of growing deep roots to access any available stored water are readily apparent, and although much of the current research has been undertaken in Australia, other hot and water-stressed regions may

also benefit from deep-root phenotyping, such as the tropics and the Mediterranean. Whether deep rooting phenotypes are necessary or perform better than shallow rooting phenotypes in temperate soils in the UK (or elsewhere across northern Europe or America), remains to be determined.

Benefits of root positioning in the soil profile in the early stages of growth (approximately 21-22 in Zadoks code (Zadoks *et al*. 1974)) are rarely considered in the literature, although there is evidence that early root vigour benefits later stages of wheat development (Richards *et al*. 2010, Rebetzke *et al*. 2014), especially in areas prone to water limitations. Early seedling RSA traits, such as total root length and maximum spread of roots, were associated with number of grains, extended grain-filling period, and yield in mature, field-grown recombinant inbred lines (RILs) of wheat-spelt (*T. aestivum* x *T. spelta*) hybrids. QTLs for early seedling vigour and late plant maturation (in this study defined as the point at which 50% of the main shoots of a plot displayed yellowing of peduncles) were co-located, resulting in a higher grain yield (Xie *et al*. 2017). Similarly, cultivars with a steep seedling rooting angle had greater relative root density at depth and higher yields in semi-arid regions prone to summer droughts, due to their improved access to stored water deep in the soil profile (Manschadi *et al*. 2010).

Conditions when winter and spring wheats are sown in the UK are typically wet, although sustained periods without rainfall are not unusual and these may become even more frequent during the summer months (Dodd *et al*. 2011). These periods of limited rainfall can be severe enough to decrease yield. Also, rainfall patterns vary significantly within the UK, with the north and west typically receiving far greater

annual rainfall than the south and east of the country (Fowler *et al*. 2005). Similar rainfall patterns are predicted in the future, even with the expected effects of climate change. As such, seedling access to water in the early stages of growth may be significantly affected by RSA. Following rainfall, just-germinated seedlings may show no differences in water uptake regardless of whether they display shallow or deep rooting systems. However, if the seeds germinate in drier conditions in well-draining soils, a deeper root system may allow better access to water stored below the surface layer.

In saturated soils, a deep root system may expose young plants to waterlogging and potentially hypoxia (Trought and Drew 1980, Malik *et al*. 2002), and in extreme cases the seminal roots can die (Thomson *et al*. 1992). Since seminal roots make the greatest contribution to water uptake of wheat crops (Wasson *et al*. 2012) even after development of the crown roots, this could profoundly affect plant development and yield. Outdoor pot trials found that waterlogging decreased yields of winter wheat (cv. Maris Huntsman) by 2 – 19 %, depending on frequency of events and length of time for which the root system was waterlogged (Belford 1981). If mildly waterlogged (water table at 5 cm below soil surface) at the seedling stage, then most plants recovered well and yields were only slightly (≈ 2%) reduced, but frequent severe waterlogging (water table at the soil surface) at the seedling, tillering and booting phases greatly diminished yields (≈ 19%).

Early formation of a vigorous root system aids crop establishment by facilitating water uptake and nutrient scavenging, and providing a secure anchor for the developing plant, protecting it from being uprooted by, for example, strong winds or grazing

animals (Fitter 2002). In wheat, root system anchorage is important in limiting yield losses due to root lodging (Berry *et al*. 2007). In wheat seedlings, seminal roots growing at angles nearer to the vertical offer protection against being uprooted by vertical forces, whereas roots nearer to the horizontal provide better protection against forces acting in the horizontal direction (Ennos 1991). UK wheats are not commonly grazed, but are still at risk of lodging through a combination of heavy rains, poor anchorage in saturated soils and strong winds (Berry *et al.* 2004). The other roles of the root system such as water and nutrient uptake, and their ability to convey signals about the root zone environment from the roots to the shoot, make early root system development vital to healthy crop development.

A shallow root system in drying soils, or soils prone to rapid cycles of wetting and drying, may cause ABA synthesis and accumulation in the roots (Zhang and Davies 1989). ABA synthesised in the roots may be transported to the plant shoot and to the leaves, where it triggers stomatal closure as a response to low soil moisture availability (Loss and Siddique 1994). Alternatively, foliar ABA accumulation may be stimulated by loss of leaf cell turgor (Pierce and Raschke 1980). However, as leaf cell turgor is lost when water uptake is less than that needed to match transpiration, there is still a close relationship between root water uptake and leaf ABA concentration. Root positioning in a drying soil profile can significantly affect leaf ABA concentration; with plants with relatively more roots near the soil surface accumulating more ABA than plants with deeper growing roots (Saradadevi *et al*. 2016).

In light sandy soils, or in areas with adequate early season rainfall, early plant vigour is associated with deeper rooting in wheat (Ludwig and Asseng 2009), even by late stages of growth. As such, a variety that quickly grows a deep root system soon after germination might be expected to have a higher yield potential (Richards *et al*. 2002, Liao *et al*. 2004). Similarly, having roots with access to a continuous water source maintains plant water status, thereby preventing drought responses such as root and shoot ABA accumulation (Dhanda *et al*. 2004, Saradadevi *et al*. 2016). Preventing significant ABA accumulation is desirable (when growing conditions are favourable), as ABA can inhibit growth both by reducing stomatal conductance (and thus photosynthetic carbon gain) and directly limiting cell expansion and division (Peleg and Blumwald 2011). Later in the life cycle, accumulation of ABA in the female reproductive parts (gynoecia) of wheat flowers before anthesis is correlated with reduced grain setting (Westgate *et al*. 1996).

3.1.2 Aims of this chapter

This chapter will investigate whether variation in seminal root angle under different soil moisture gradients affects early shoot growth and the ability to cope with periods of restricted water availability in two cultivars of wheat. Growing both cultivars in large pots with vertical moisture gradients (created by watering either the soil surface or saucers at the base of the pot) tested the first hypothesis: matching seedling root angle with the direction of gradient will improve early seedling leaf extension rates. Later in development, withholding water from half the pots of each treatment tested the second hypothesis: seedling root angle and local soil moisture gradients affected

plant responses (stomatal conductance, leaf extension rate, shoot and root ABA concentrations, and ultimately plant biomass) to soil drying.

3.2 Materials and Methods

The cultivars *Bat* and *Ist* were selected for this trial because they showed contrasting root phenotypes under both well-watered and water-limited conditions (deep and shallow seminal root angles, respectively – Figure 2.13). The experiment was a 2 (genotypes – *Bat* and *Ist*) x 2 (watering location – to the top and base of the soil profile) x 2 (water availability – well-watered or drying soil) factorial design (Figure 3.1). Due to the limitation of available bench space a maximum of 60 pots could be used per experiment; therefore, in each experiment there were 7 pots of each treatment, with 4 spare pots, in the event of failed germinations. In addition to the main pot trial, two racks of blue roll (Kimberly-Clark Professional™ WYPALL™ L20) tubes were set up to monitor root extension rates of the two cultivars, as described in Chapter 2.2.6, Experiment 1 (Figure 2.5), to establish whether cultivars differed in root extension rate.

3.2.1 Plant growth

For each trial approximately 100 unsterilised wheat seeds of both *Bat* and *Ist* were geminated on damp filter paper in separate Petri dishes, and left in the dark at room temperature. After 24 hours, 60 seeds of each genotype were selected based on similar size and similar progression of radicula growth. The selected seeds were then planted out in a walk-in CE room at Lancaster Environment Centre with average day/night

temperature of 24/19 °C, 12 hours of artificial lighting per day/night cycle, daytime light intensity at plant height of approximately 600 µmol m^{-2} s^{-1} PPFD, and relative humidity averaging approximately 60%.

A further 10 pre-germinated seeds from each cultivar were wrapped in blue roll and placed in water filled plastic racks, and left to grow in the same CE room.

3.2.2 Pot system

To measure seedling root angle in this trial, basket pots (Figures 2.2 and 2.3) were placed in larger pots (before planting out the pre-germinated seeds), as described in Chapter 2.2.2. These outer pots (10 L volume, 22 cm high, 28 cm in diameter) were filled with equal weights of roughly sieved John Innes no. 2 (mesh size 20 mm), and placed in saucers. The basket pots were positioned within the outer pots such that the top of the basket pot was barely concealed under the soil surface. Each pot was then weighed, and then received a further 2 L of water at the soil surface, to bring the pot to drip point. The pots were then arranged in a random block design on two parallel benches in the CE room, and allowed to drain. After 48 hours, the saucers beneath the pots were emptied, if necessary. Two pre-germinated wheat seeds of the same cultivar were then planted in each pot (one in the basket pot, and one as a spare in the soil outside of the basket pot), and then the entire pot (including the saucer), was weighed again. This weight was considered as the starting weight of the pot, and pots were returned to this weight when re-watering. When a seed within a basket pot failed to germinate, the second (spare) seedling was not transplanted into the basket pot, so root angle data was not collected for that pot. Non-appearance rates differed between

genotypes: 3% for *Bat* and 40% for *Ist*. Using spare plants meant that even if root angle data was unavailable, a plant was still available for leaf extension, stomatal conductance, leaf water potential, ABA, and biomass measurements.

To establish the first four treatments (before the additional water-limited treatments described below), half the pots of each cultivar were watered from the surface, and the other half from the saucer below the pot (see Figure 3.1).

3.2.3 Daily measurements

The 20 blue roll tubes were unwrapped daily and all roots measured using a transparent 30 cm ruler, for the first 9 days of growth. The total root growth in the 24 hour interval for each plant was used for analysis.

Throughout the experiments (28 days for Experiment 1 and 30 days for Experiment 2, described below), the soil water content of the pots was measured daily at the surface of the pot and at the base of the pot by an ML2x theta probe, inserted directly in the soil surface for surface water content readings, or through the side of the pot approximately 50 mm above the base of the pot for deeper soil profile water content. All theta probe and pot weight measurements were taken immediately before re-watering. Moisture release characteristics for the substrate were previously determined (Figure 2.3).

4 days after planting, the shoots of the wheat plants were clearly visible above the soil surface, and large enough to allow daily leaf length measurements with a transparent

plastic 30 cm ruler, recorded to the nearest mm. Leaf elongation measurements were taken until the first destructive harvest. After 14 days, the first expanded leaves were large enough to allow stomatal conductance readings using a transient-time porometer (Model AP4, Delta-T Devices, Cambridge, UK). Porometer readings were taken daily, halfway through the photoperiod (12:00 – 14:00), until the end of the experiment, using the most recently fully expanded leaf available on each plant. The sensor head was positioned on the abaxial surface of the leaf, approximately two thirds of the way up from the stem.

The first destructive harvests began after 18 days of growth. At this stage, the two trials differed from each other in the method of harvesting and further data collection, to provide complementary data sets.

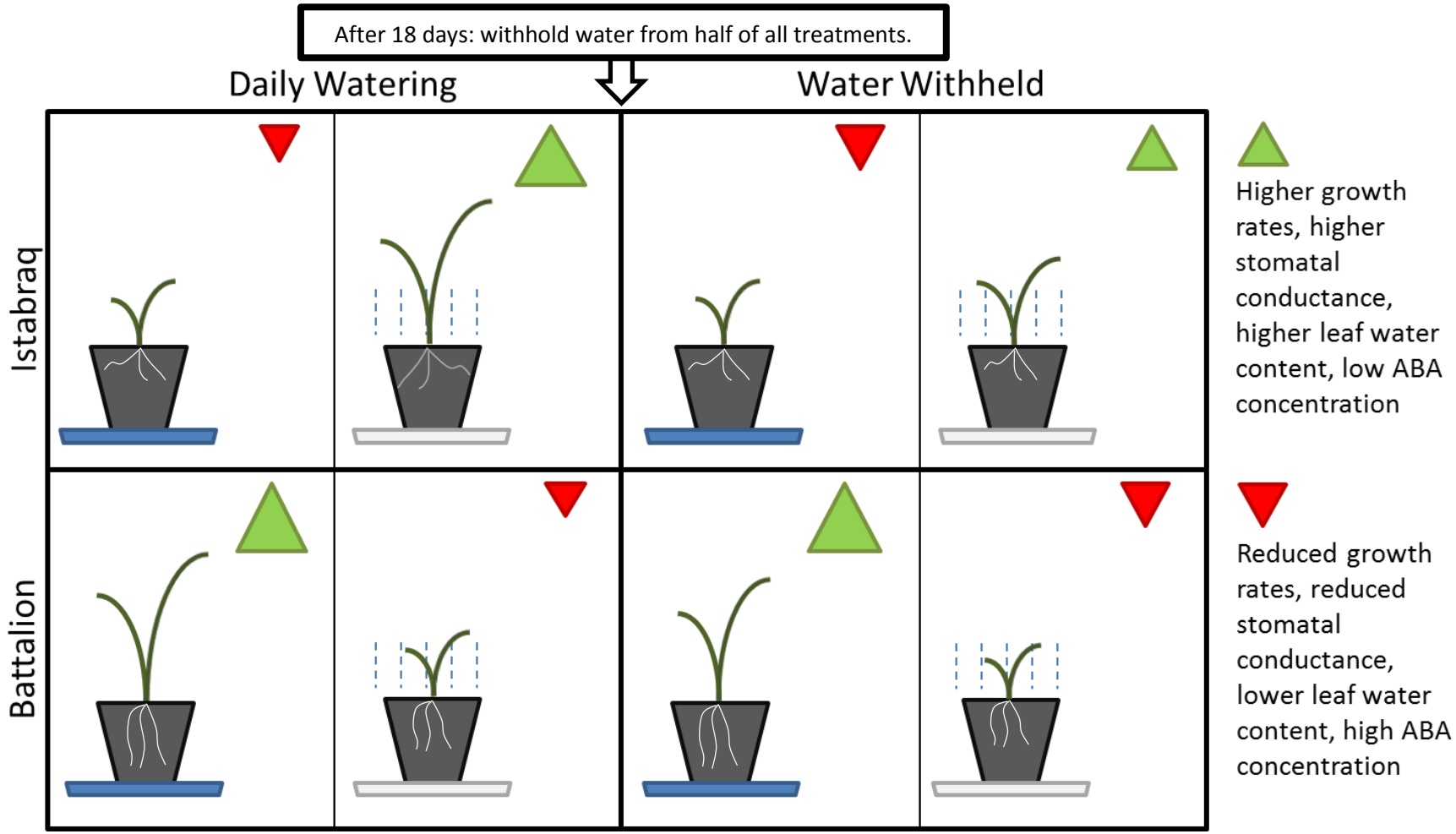


Figure 3.1: Diagram of experiment system, showing the eight distinct treatments and hypothesised outcomes.

Experiment 1

After 18 days of growth, when all plants had reached at least the third leaf stage, water was withheld from half of all the previous four treatments (formed by all the possible combinations of the two cultivars treatments and two watering location treatments). Seedling growth rate measurements stopped, and leaf water potential (Ψ_{leaf}) and leaf tissue ABA concentration determined for the oldest leaf on Days 18, 21, 25 and 28. To measure Ψ_{leaf}, 5 mm leaf discs were punched from the oldest leaf from two randomly selected plants of each treatment. The excised disc was immediately placed in a clean sample holder and wrapped in foil to minimise possible water loss. After collecting all discs (taking approximately 20 minutes in total), sample holders were unwrapped and placed into C52 chambers (Wescor Inc., Utah, USA). Samples were then left to equilibrate within the chambers for three hours before readings were taken with a HR-33T Dew Point Microvolt meter (Wescor Inc., Utah, USA). Psychrometer chambers were calibrated using salt solutions of known osmotic potentials, and leaf water potential (MPa) measurements were converted from voltage readings based on the calibration.

For ABA sampling, the leaves which were sampled for Ψ_{leaf} were cut from the plant stem with scissors as soon as the discs had been wrapped in foil, folded into individual 1.5 ml microtubes (Sarstedt, Nümbrecht, Germany), and then immediately submerged in liquid nitrogen. The first leaves from the plants not sampled for Ψ_{leaf} were then also sampled for leaf tissue ABA analysis and frozen in liquid nitrogen. All samples were kept below -20 °C before being freeze-dried for 48 hours. The freeze-dried samples were then ground to a fine powder and extracted with distilled, deionised water at 1:30

mg:µl in a 1.5 ml microtube, before being placed on a shaker plate overnight at 4 °C. The following morning, ABA concentrations (ng/g dry weight) were determined by competitive radioimmunoassay (RIA) (based on the method described in Quarrie *et al*. (1988)). Samples were centrifuged for four minutes to remove plant material held in suspension, and then the RIA was conducted according to the protocol described by Rothwell (2014), using radiolabelled ABA (DL-cis/trans [3H] ABA) and the antibody MAC 252. Radioactive material is bound as a precipitate and quantified by the addition of a scintillation fluid, allowing bound radioactivity to be measured by fluorescence using a scintillation counter (Packard TriCARB 1600TR Light Scintillation Analyser, Canberra, CT, USA). A standard curve was produced using eight ABA solutions of known ABA concentration ((±)-ABA (A1049, Sigma-Aldrich)), which were analysed at the same time as the wheat leaf samples.

On day 28, the final ABA and Ψ_{leaf} tissue samples were taken. The remaining aboveground biomass was harvested and each individual plant weighed before being dried in a paper bag for three days at 60 °C, and then weighed again for dry biomass. Basket pots were recovered from the bulk soil and root angles recorded.

Experiment 2

After 18 days of growth, when all plants had again reached third leaf stage, water was withheld from half of the pots, as per Experiment 1. In this trial, samples for Ψ_{leaf} and ABA leaf tissue analysis were taken from just one plant of each treatment on Days 18, 20, 22, 24, 26, 28 and 30 of the trial. After placing sample holders in the psychrometer chambers and freezing the ABA samples, the remaining aboveground biomass of the

eight sampled plants was harvested and weighed, before being dried in a drying oven for 72 hours and weighed again. The basket pots were extracted and root angles recorded. Any visible root tissue from both within and outside of the basket pots was quickly harvested and washed, then placed into 1.5 ml microtubes and freeze-dried for ABA analysis. The amount of root available was very small compared to the volume of soil, as such roots were often difficult to extract. For this reason, any root tissue found within five minutes of removing the shoot was collected and frozen; however, this meant that some of the roots were usually left in the soil, making root biomass comparisons impossible. Root ABA analysis was conducted in the same way as leaf tissue analysis, except for being extracted at a ratio of 1:40 mg:µl rather than 1:30 mg:µl.

3.2.4 Soil water content at end of experiment

After the final harvest of a pot, a sample of soil was taken from the top 3 cm of the pot, and from the bottom 3 cm of the pot, weighed on a 0.0001g balance, then dried at 60 °C for approximately 120 hours, and weighed again on the same balance, to measure gravimetric water content.

Using theta probes to monitor soil water content worked reasonably well at the surface of the pots, but pushing the probe through pre-made holes in the sides of the pot did not produce reliable data for soil water content at the bottom of the pot (Table 3.1). While the methods generally correlated significantly, the theta probe readings at the base of the pot were not correlated with the gravimetric soil water content measurements for soil taken from the base of the pots.

The pots were made of thin plastic and they would warp and bend as the probe was inserted, creating air gaps around the probe's spikes, and causing disruption to the soil environment in the area around the holes. Theta probe readings suggested that soil water content was much lower at the base than the surface in all treatments, but the gravimetric soil water content data displayed in Figure 3.3 suggest otherwise. Due to this discrepancy, the theta probe data is not presented.

Table 3.1: Pearson's coefficients of correlation between methods of measuring soil water content, * denotes correlation is significant at p-value = 0.05, ** denotes correlation is significant at p-value = 0.01.

	θ probe reading at surface	θ probe reading at base	Surface soil gravimetric water content (g/g)	Base soil gravimetric water content (g/g)
θ probe reading at surface				
θ probe reading at base	0.419 **			
Surface soil gravimetric water content (g/g)	0.689 **	0.441 *		
Base soil gravimetric water content (g/g)	0.654 **	0.312	0.566 **	

Table 3.2: A side-by-side comparison experiment schedule for the first two methods described in Chapter 3.2.3. Note that the table is not scaled, and the days after planting columns do not match up between rows. Ψleaf = leaf water potential, gs = stomatal conductance, LER = leaf extension rate.

Days after planting	4	14	18	21	25	28			
Exp. 1	Start daily LER measurements.	Continue LER measurements. Start measuring gs daily.	Begin withholding water treatments. Stop LER measurements. Continue gs readings. Harvest oldest leaf of every plant for ψ_{leaf} and ABA.	Continue gs readings. Harvest oldest leaf of every plant for ψ_{leaf} and ABA.	Continue gs readings. Harvest oldest leaf of every plant for ψ_{leaf} and ABA.	Last gs readings. Harvest oldest leaf of every plant for ψ_{leaf} and ABA. Harvest and weigh shoot biomass. Record root angles.			
Days after planting	4	14	18	20	22	24	26	28	30
Exp. 2	Start daily LER measurements.	Continue LER measurements. Start measuring gs daily.	Begin withholding water treatments. Stop LER measurements. Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass.	Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.	Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.	Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.	Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.	Continue gs readings. Harvest one plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.	Final gs readings. Harvest last plant from each treatment for ψ_{leaf}, leaf & root ABA, shoot biomass. Record root angles.

Experiment 3

Ten plants of each cultivar were grown in the CE room under well-watered conditions. Before planting, the seeds were pre-germinated on damp filter paper as described in Chapter 2.2.2, but left for 48 hours, to allow development of the primary root. One L pots were filled with 550 g of John Innes no. 2, and watered with untreated water until drip point (approximately 300 ml) per pot. The top layer of soil was carefully excavated from each pot and a cone with an internal apex angle of 110° (in keeping with the average seedling root spread of *Ist*) made of laminated paper was positioned and partially covered using some of the excavated soil. There was a small hole at the top of the cone made using the point of a pin. The primary root of a germinated seed was very gently positioned in the hole, such that this root could continue to grow towards vertical, but subsequent seminal roots of both cultivars would be forced to grow in a shallow formation. After the seed was positioned, the rest of the cone and the seed were fully buried using the remaining excavated soil (see Figure 3.2).

The plants were kept well-watered for 12 days until they reached Zadoks stages 13/14 (Zadoks *et al*. 1974), by which point the leaves were sufficiently large to allow stomatal conductance readings using an AP4 Porometer. Readings were taken daily during the middle of the photoperiod (13:00 – 14:00), from the most recently extended leaf on each plant. The sensor head was positioned on the abaxial surface of the leaf approximately one third of the leaf length from the tip, for consistency. The pots were then weighed and re-watered. After two days of stomatal conductance readings, water was withheld for the next six days, to compare genotype responses to drying soil. Stomatal conductance readings continued daily.

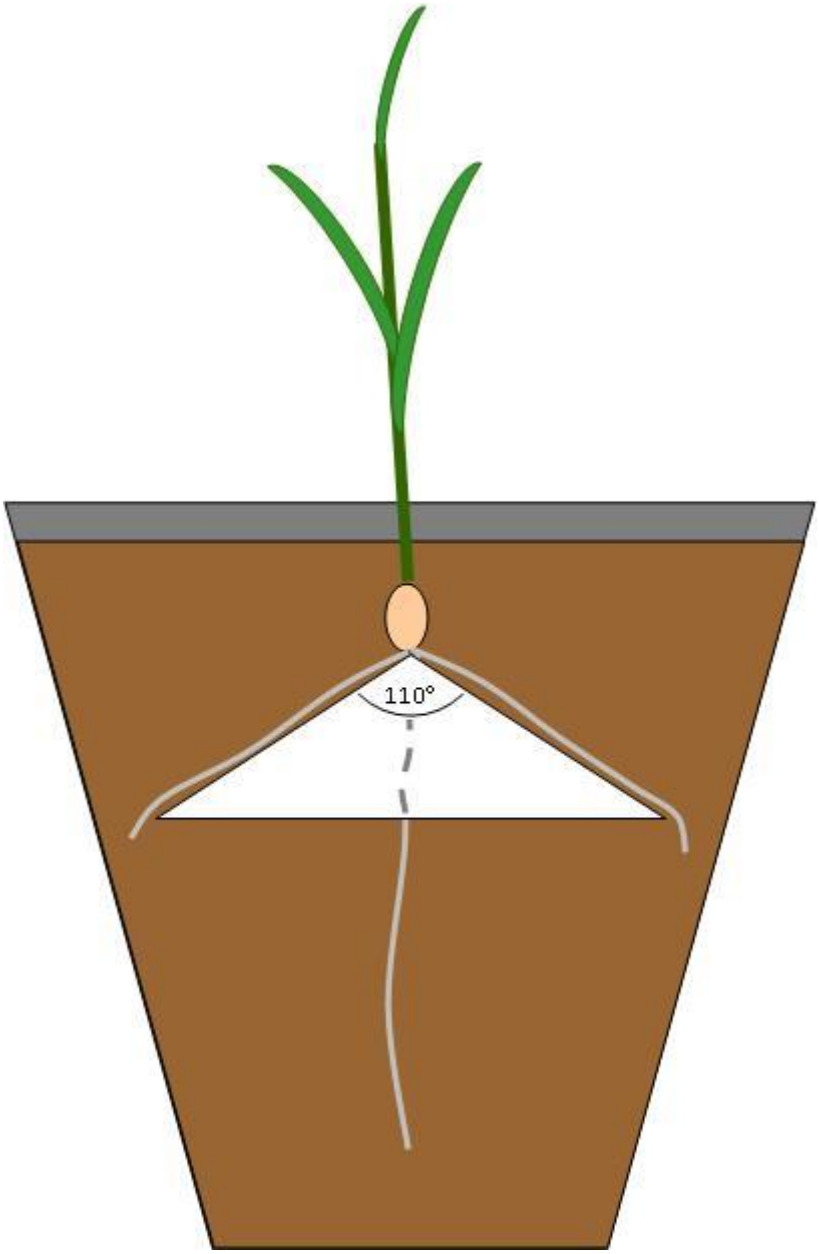


Figure 3.2: Cut away view into a pot where root angle has been constrained by a cone with internal apex angle 110°. The primary root is allowed to grow vertically through a hole in the top of the cone; the subsequent seminal roots grow down the outside of the cone to force a shallow seedling root angle.

After one week of porometry, the leaf that was used for the stomatal conductance readings from plant was excised to prepare microscope slides. Light microscopy was used to image the epidermal cells and calculate stomatal density index. The abaxial surface of the leaf was then coated with a thick layer of clear nail lacquer. After approximately eight minutes, the lacquer was peeled from the leaf, and mounted on a

microscope slide. Lacquer impressions were taken from the base, middle, and apex of the leaf. Stomatal conductance readings were taken nearest the apex of the leaf, but impressions were taken from along the length of the leaf to account for differences in stomata distribution along the leaf.

These slides were then imaged using a SPOT Insight 3.2.0 Color microscope camera and Spot Basic Image Capture Software (SPOT Imaging, Diagnostic Instruments, Inc., Sterling Heights, Michigan, US) through a Leitz Biomed 020-507.010 microscope, at 238x magnification. Three images were taken per leaf (base, middle and apex) to give 30 images per genotype. Stomatal density was calculated as the number of whole stomata per 1200 x 837.5 μm area and then scaled to a count per mm^2. Stomata at each separate leaf section were counted and compared individually, at the base, middle and apex of the leaf, as well as the sum of all sections per leaf to compare between genotypes.

3.2.6 Statistics

Statistical analyses were conducted in SPSS version 22 (IBM Corp, NY, USA). Means were compared using ANOVA or, when the variance of the data were not homogeneous as determined with use of Levene's test, then a relevant non-parametric test such as the Mann-Whitney U-test or the Kruskal-Wallis test was used instead. Temporal effects of treatments were analysed by treating time as a variable, and incorporating it as a factor in multi-way ANOVAs along with the main factors under investigation: genotype, watering location (pot surface or pot base), and watering

treatment (continuously watered daily or water withheld after first leaf harvests). Pearson's correlation coefficient was used to explore relationships between variables.

For comparison of stomatal density, the numbers of stomata per unit area at each leaf section (base, middle, and tip) of the two cultivars were compared against each other by Student's T-test. Leaf section was also compared as a factor by ANOVA.

3.3 Results

Experiments 1 & 2

3.3.1 Root and shoot growth

Plant root systems were grown through basket pots in this experiment to check that genotypic differences in seminal root angle were consistent with previous results (Figure 2.13, see also Whalley *et al*. 2013). Again, *Bat* had a significantly (p-value = 0.003) deeper rooting phenotype than *Ist* (Figure 3.3). Overall, watering location did not significantly (p-value = 0.135) affect root angle, although the seminal roots of *Ist* grew at a shallower angle under surface watering than when basally-watered (consistent with Figure 2.13), thus the genotype x watering location interaction was significant (p-value = 0.016). Water was not withheld until after the majority of the seminal roots had penetrated through the basket pot, so this factor was not included in root angle analysis.

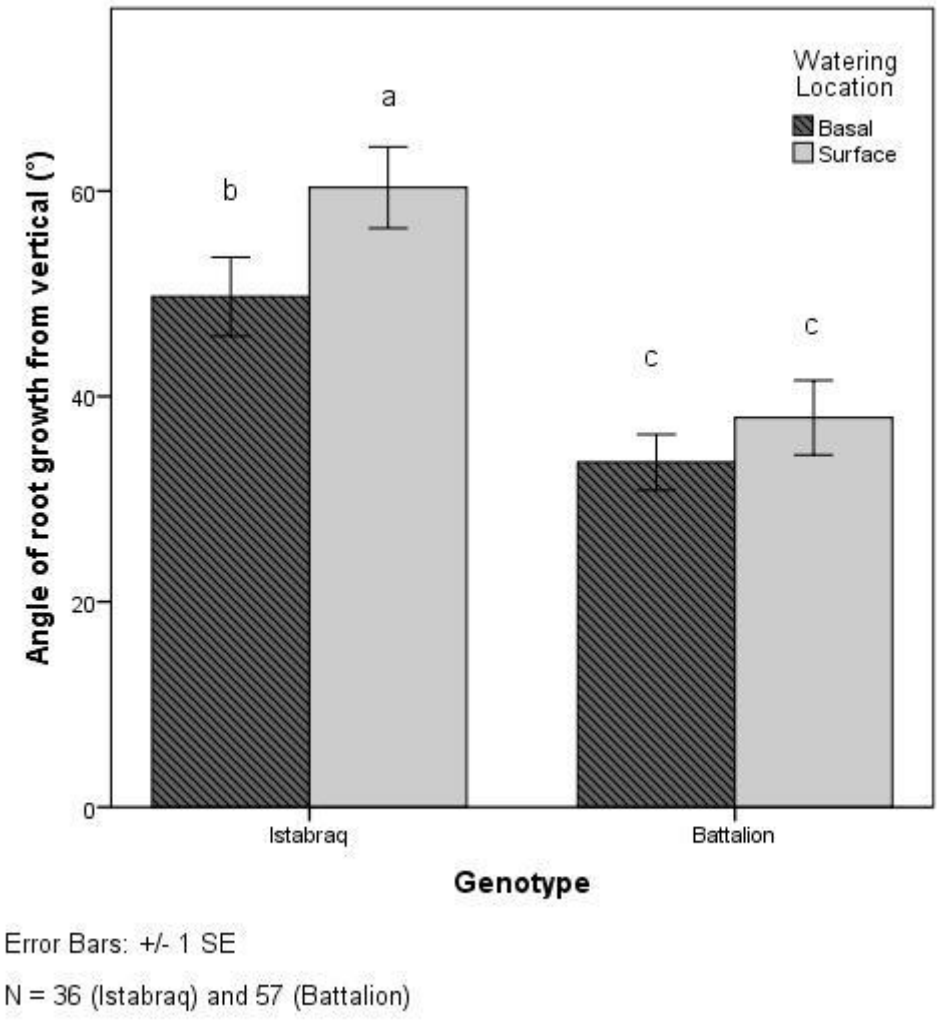


Figure 3.3: Seedling root angles, separated by genotype and watering location. *Ist* grew roots at significantly shallower angles than *Bat*, consistent with previous data (Figure 2.13). Data were combined across Experiments 1 and 2.

In the blue roll experiment, root elongation (RE) did not significantly differ between the two genotypes (Figure 3.4, p-value = 0.897). Although roots grew slower than previously (Figure 2.7), the average RE was still adequate to ensure penetration through the sides of the basket pot.

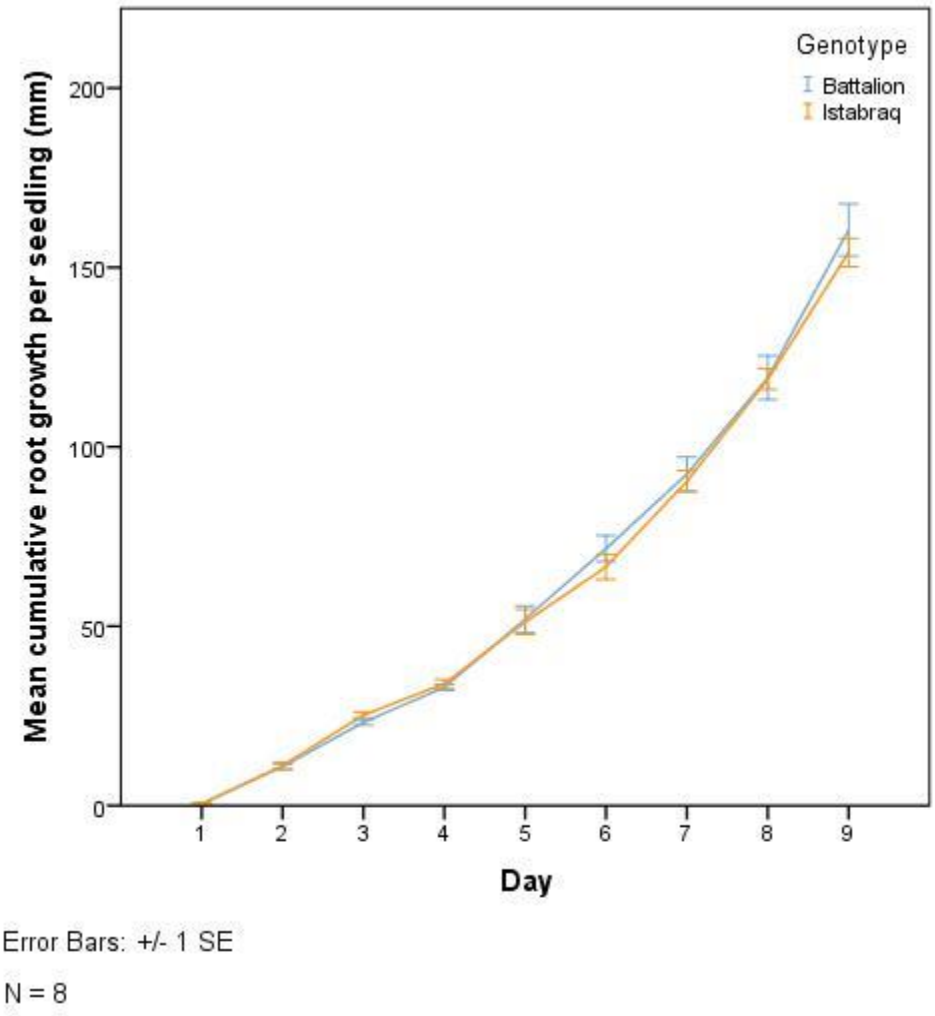


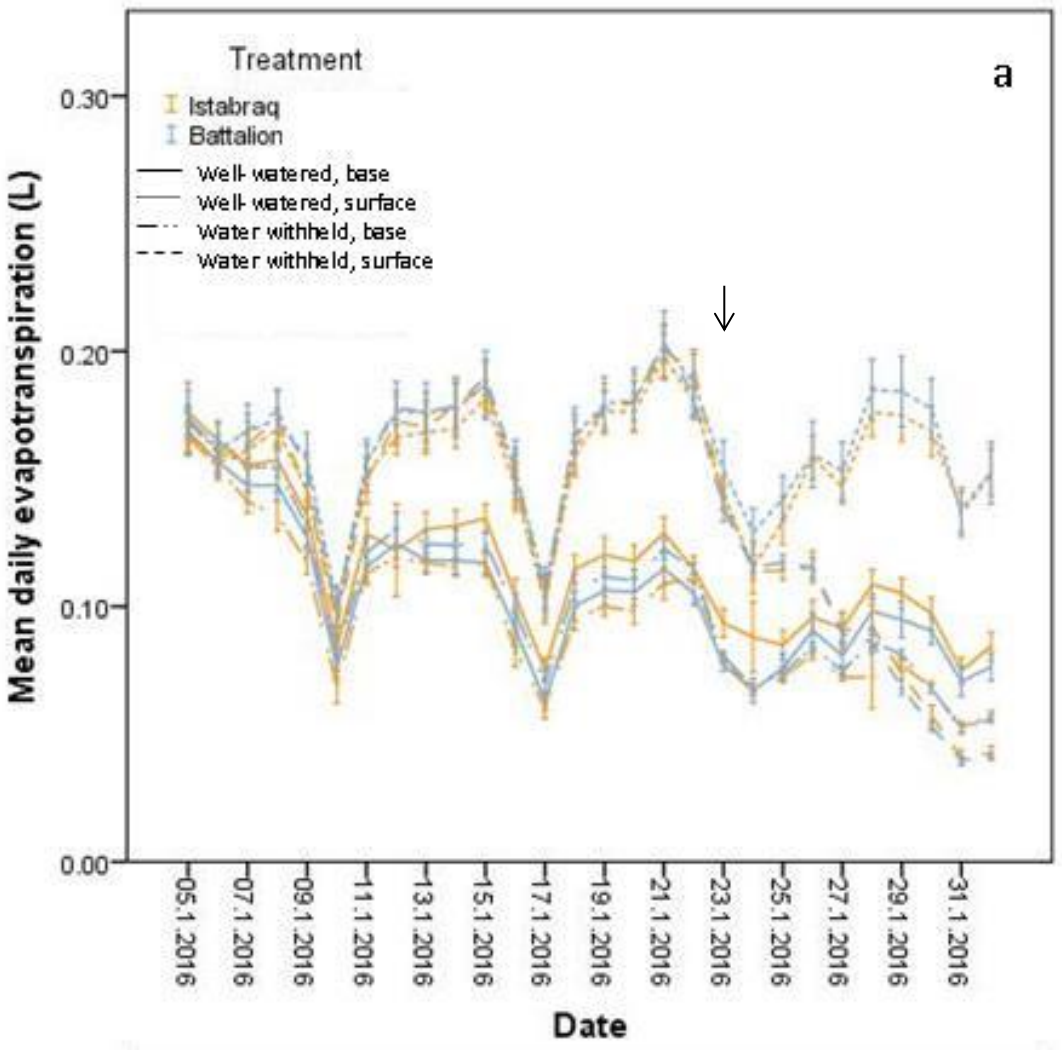
Figure 3.4: Cumulative root length of genotypes *Bat* and *Ist*. There were no significant differences in elongation between the two genotypes (p-value = 0.897, One-way ANOVA).

Leaf extension rate (LER) was not affected by genotype (p-value = 0.070) or watering location (p-value = 0.880), nor was there any interaction between these variables (p-value = 0.862, Table 3.3). Aboveground biomass at harvest was not significantly affected by any of the individual factors, or by any interaction effects.

3.3.2 Soil water content

Evapotranspiration was monitored by weighing the pots every day (Figure 3.5) before re-watering, and measuring gravimetric soil water content at harvest (Figure 3.6).

Soil moisture gradients were better established in the basally-watered pots than the surface watered pots (Figure 3.6), probably since surface evaporative losses were greater than drainage from the base of the pot (Figure 3.5). Withholding water resulted in more water being lost from the surface-watered treatments than the basal-watered treatments over the same time period (Figure 3.5).



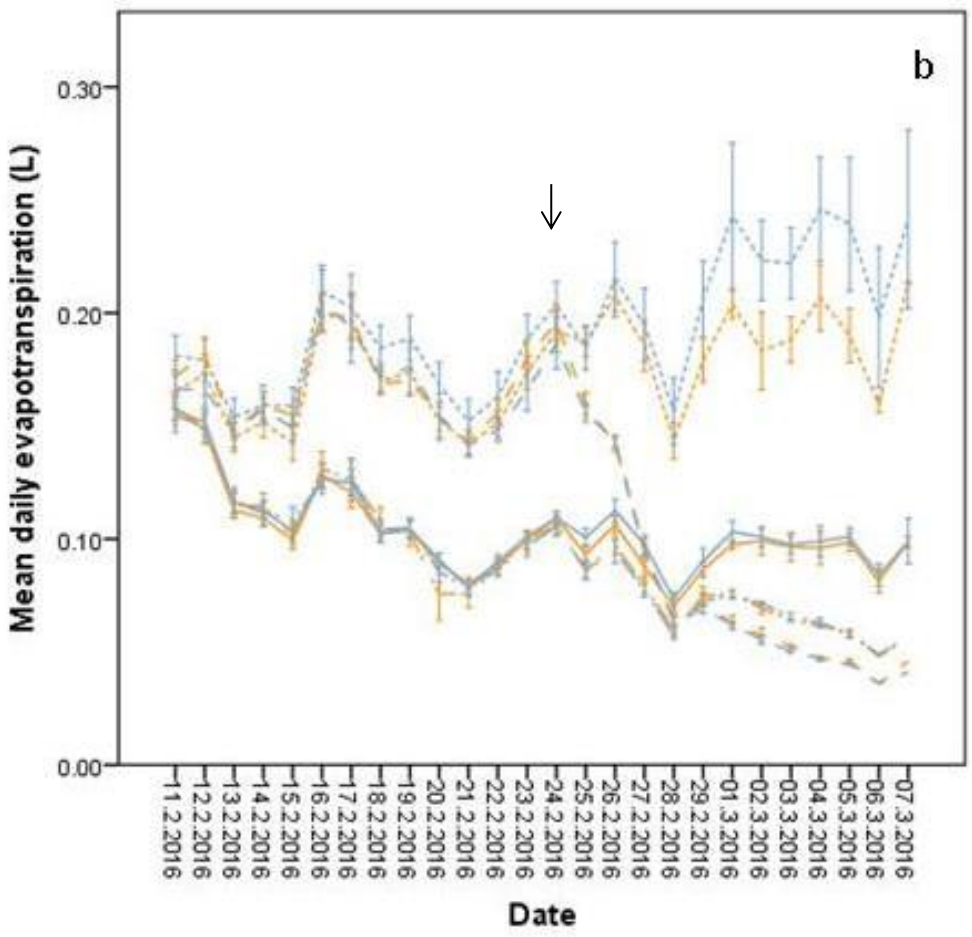


Figure 3.5a, b: Water use over time from a) Experiment 1 and b) Experiment 2. Arrows mark the dates at which water was withheld from half of the treatments. For these graphs, water use incorporates all water lost through evapotranspiration in the 24-hour period between measurements.

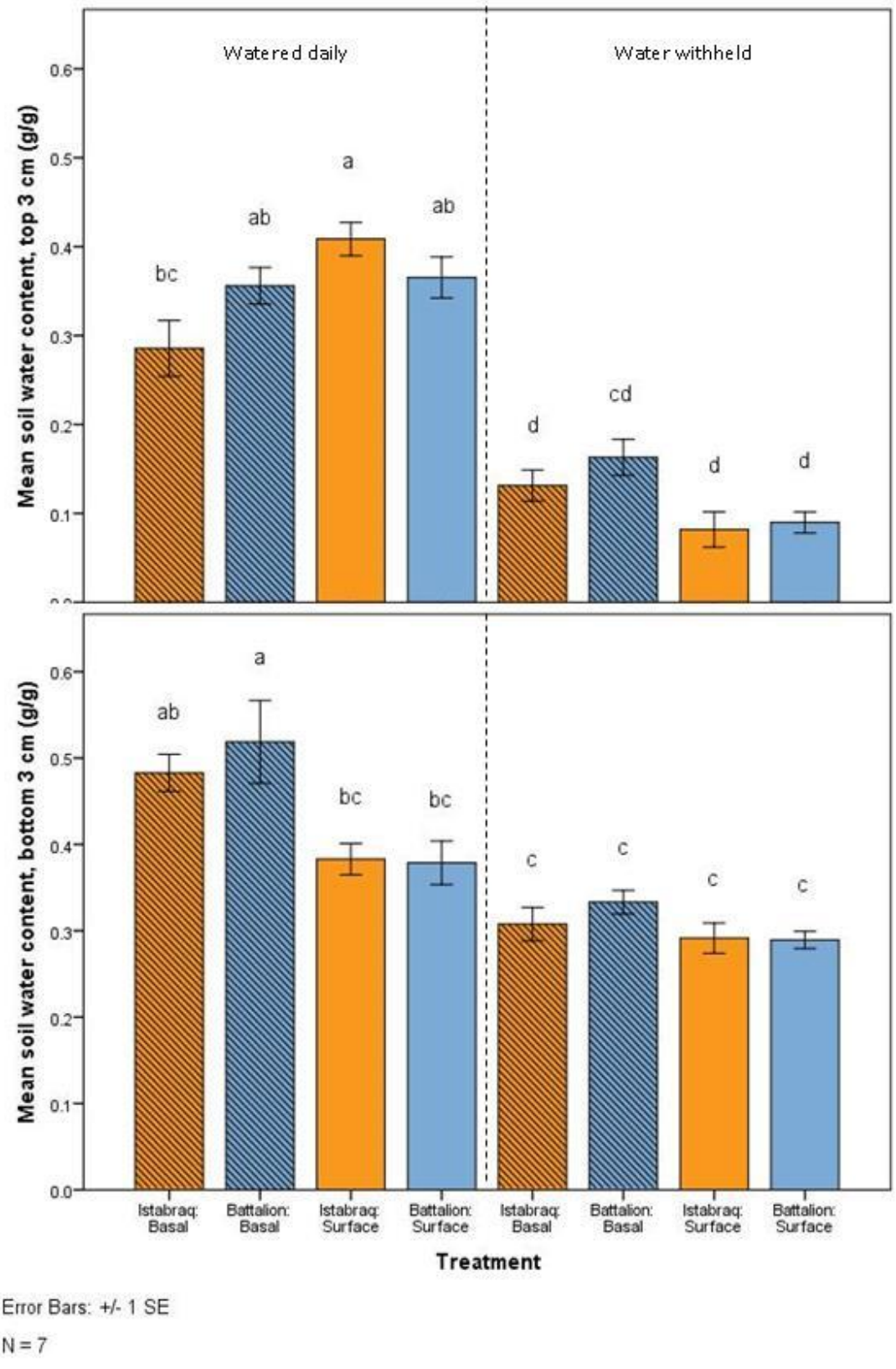


Figure 3.6: Gravimetric soil water content of soil taken from a) upper and b) lower 3 cm of all pots at point of harvest in Experiment 2. Letters of mean discrimination distinguish significant differences between the eight treatments (separately for top and bottom of pot), calculated by ANOVA with a Bonferroni post-hoc test.

Table 3.3: ANOVA table with p-values and, where significant, F-values in italics to establish significant main effects and interactions on plant growth and physiology variables. Data are pooled from both experiments, except for RE (Experiment 1 only) and root ABA (Experiment 2 only). Where necessary (Levene's test significant) a relevant non-parametric test was used instead. **Bold** indicates a significant result, * denotes significance at p-value = 0.05, ** denotes significance at p-value = 0.01. † indicates a non-parametric test was used instead of ANOVA.

Factor(s)	**Root angle**	**RE**	**LER**	**Stomatal conductance**	**Leaf ABA concentration**	**Root ABA concentration**	**Ψ_{leaf}**	**Aboveground biomass (DW)**
Genotype (G)	**0.003**** *9.373*	0.897	0.070	**0.018***†	0.762	0.661	0.091	0.957
Watering Location (WL)	0.135	N/A	0.880	**<0.001****†	**0.018*** *6.425*	0.188	0.055	0.121
Water Availability (WA)	N/A	N/A	N/A	**<0.001****†	**0.012*** *6.076*	0.288	**<0.001****†	0.336
G x WL	**0.016*** *4.737*	N/A	0.862	0.976	0.392	0.518	0.060	0.863
G x WA	N/A	N/A	N/A	**0.025*** *5.059*	0.051	0.250	0.514	0.904
WL x WA	N/A	N/A	N/A	0.764	**0.011*** *6.554*	**0.037*** *4.383*	0.620	0.658
G x WL x WA	N/A	N/A	N/A	0.941	0.438	0.618	0.554	0.607

3.3.3 Main effects by individual factors

Genotype effects

Other than root angle, the only physiological variable affected by genotype was stomatal conductance (Table 3.3). Stomatal conductance of *Ist* was significantly (p-value = 0.018, Mann-Whitney U-test) higher than that of *Bat* (by 9%). None of the other physiological variables measured in the two experimental replicates (LER, Ψ_{leaf}, leaf ABA concentration, root ABA concentration, and aboveground biomass) differed significantly when genotype was examined as the sole factor (Table 3.3).

Watering location effects

Watering plants from the base significantly (p-value < 0.001, Mann-Whitney U-test) increased stomatal conductance by 38% compared to surface watering of plants. Leaf ABA concentration of surface-watered plants was significantly (p-value = 0.018) higher than in basally-watered plants (by 15%). Whether pots were surface or basal-watered had no significant effects on root ABA concentration, LER, Ψ_{leaf}, or aboveground biomass. Due to the different drying rates between the surface and basal watered treatments (Figure 3.5), total pot weight was used to check if the whole pot soil water content was responsible for the observed differences in stomatal conductance and leaf ABA concentration. Shoot weight was negligible compared to the combined weight of water, soil and the pots (< 1%), and so it was ignored for this analysis. One-way ANOVAs suggest that pot weight *alone* was not responsible for the changes in stomatal conductance (p-value = 0.059) or foliar ABA (p-value = 0.084).

Soil drying effects

Withholding water significantly (p-value < 0.001, Mann-Whitney U-test) decreased stomatal conductance by about 15%, significantly (p-value < 0.001, Mann-Whitney U-test) decreased Ψ_{leaf} by 30% and significantly (p-value = 0.012, one-way ANOVA) increased foliar ABA concentration by 14% (Table 3.3). As discussed above, pot weight alone does not account for these changes. However, pot weight was significantly positively correlated with stomatal conductance (Pearson's correlation coefficient = 0.233, p-value < 0.01), and significantly negatively correlated with leaf ABA concentration (Pearson's correlation coefficient = -0.214, p-value < 0.01). Pot weight did not correlate significantly with Ψ_{leaf}.

3.3.4 Interaction effects of genotype, direction of soil water gradient, and soil drying

No single factor significantly affected root ABA concentrations, but watering location and soil water availability in combination significantly (p-value = 0.037) affected root ABA levels (Table 3.3, Figure 3.7). Withholding water from plants that were previously surface-watered increased root ABA concentrations 66% more than in the other treatments.

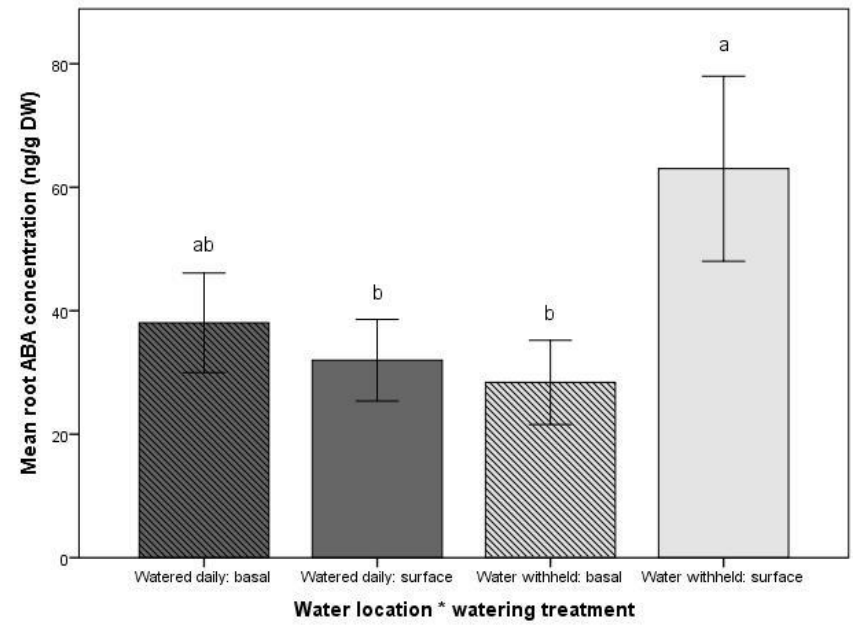


Figure 3.7: Root ABA concentration (from Experiment 2 only). Data are means ± SE of 14 replicates. Letters of mean discrimination denote significant differences between combined treatments. Two-way ANOVA, p-value = 0.037.

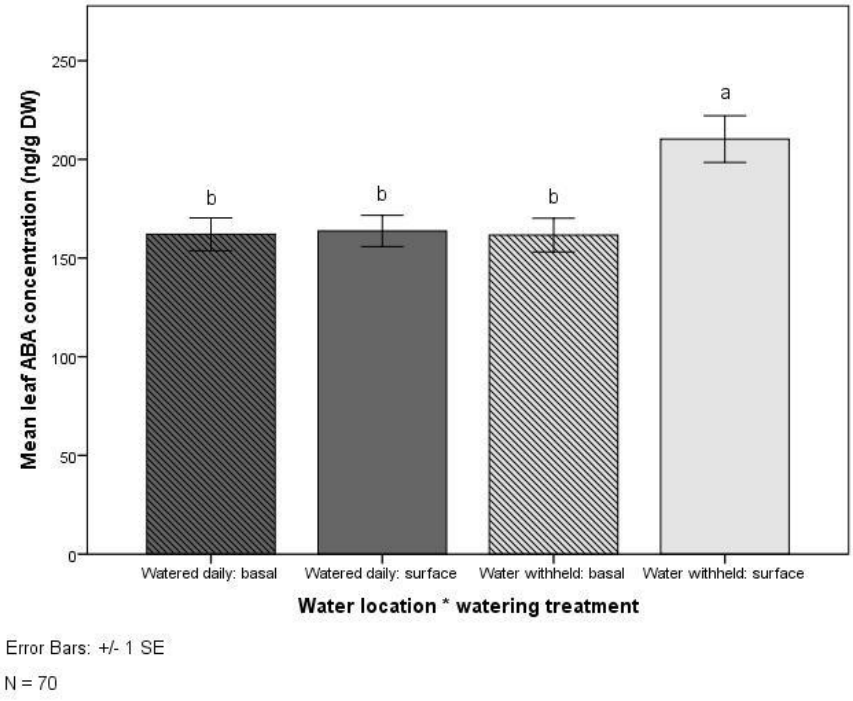


Figure 3.8: Leaf ABA concentrations from both experimental replicates. Letters of mean discrimination denote significant differences between combined treatments. Two-way ANOVA, p-value = 0.011.

Withholding water from previously surface-watered plants increased both root (Figure 3.7) and leaf (Figure 3.8) ABA levels; although the increase was not as extreme for foliar ABA (29%) as for root ABA (66%).

Genotype and water availability had a significant interaction effect on stomatal conductance (p-value = 0.025, Figure 3.9), and near-significant effects on leaf ABA concentration (p-value = 0.051, Figure 3.10); meanwhile genotype and watering location had a near-significant effect on Ψ_{leaf} (p-value = 0.06, Figure 3.11) (Table 3.3). Withholding water decreased stomatal conductance in both genotypes, but to a greater extent in *Ist* (18%) than *Bat* (10%). In contrast, withholding water had no effect on leaf ABA concentration of *Ist* but increased leaf ABA concentrations of *Bat* by 27%. Ψ_{leaf} follows a similar to pattern to stomatal conductance, although in this case there is no change in *Bat*; watering *Ist* from the surface decreases Ψ_{leaf} by 24% compared to basal watering.

To summarise, withholding water increases leaf ABA concentration and decreases stomatal conductance of *Bat*, while Ψ_{leaf} is maintained. In contrast, although stomatal conductance of *Ist* decreases when water is withheld, there is no corresponding decrease in ABA, and Ψ_{leaf} falls.

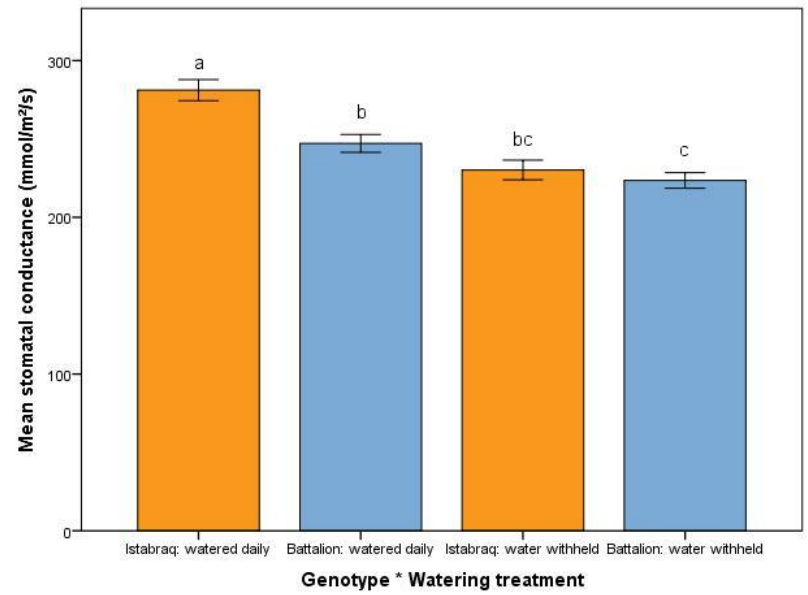


Figure 3.9: Stomatal conductance readings from both experiments demonstrating interaction by genotype and watering treatment (well-watered *versus* droughted). Letters of mean discrimination denote significant differences between combined treatments. Two-way ANOVA, p-value = 0.025.

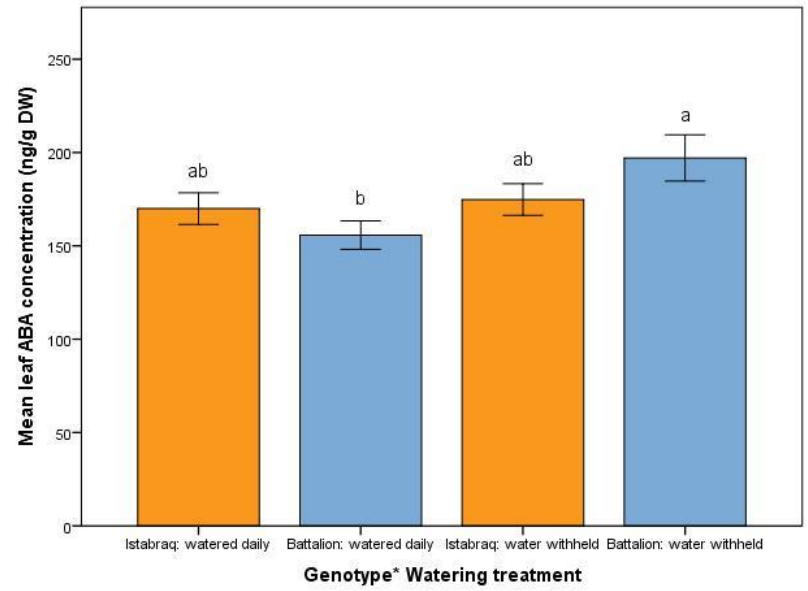


Figure 3.10: Leaf ABA levels from Experiments 1 and 2, demonstrating interaction by genotype and watering treatment (well-watered versus droughted). Letters of mean discrimination indicate differences between combined treatments, although differences in this case tended towards significance. Two-way ANOVA, p-value = 0.051.

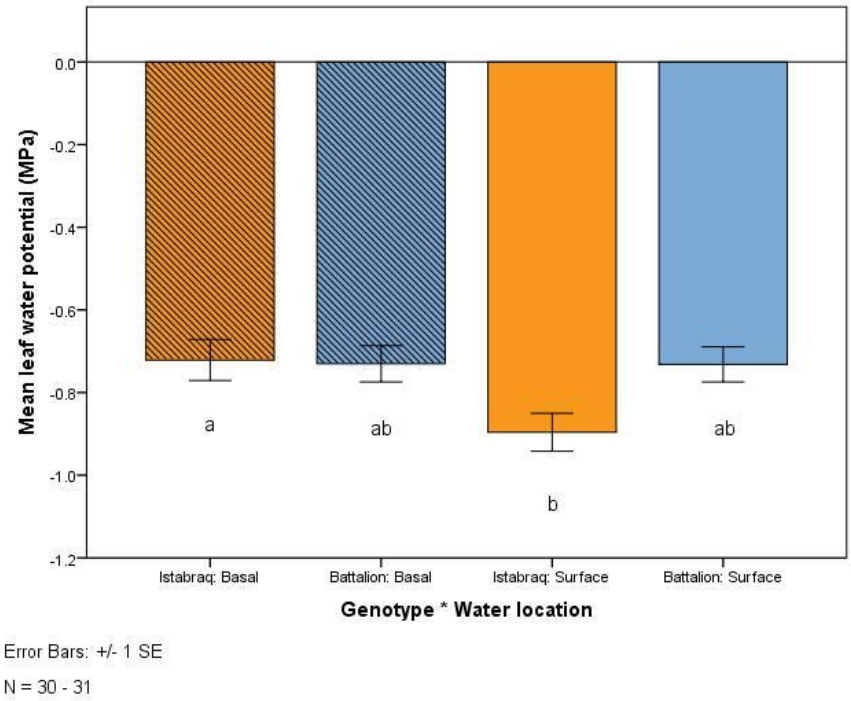


Figure 3.11: Ψ_{leaf} readings from Experiments 1 and 2, demonstrating interaction by genotype and watering location (basal irrigation *versus* surface irrigation). Letters of mean discrimination indicate differences between combined treatments, although differences in this case tended towards significance. Two-way ANOVA, p-value = 0.06.

3.3.5 Changes in variables over time

The number of days after imposing a water withheld treatment significantly (p-value < 0.05) affected many of the variables of interest (Table 3.4).

Table 3.4: Table of variables significantly affected by time, or by the interaction of time and another variable.

Factor(s)	**Dependent variable**	**p-value**
Time	Ψ_{leaf}	< 0.001
Time	Leaf ABA concentration	< 0.001
Time	Stomatal conductance	< 0.001
Time x water location	Stomatal conductance	< 0.001
Time x water treatment	Stomatal conductance	< 0.001

Figure 3.5 indicates that soil drying increased over time, and stomatal conductance plotted over time (Figure 3.12) are consistent with this result, and the data presented in Table 3.4. In Figure 3.12, re-watering the base of the pot daily significantly increased stomatal conductance compared to when water was first withheld. Meanwhile, in plants that were originally surface watered, but then received no further water, stomatal conductance appeared to decrease over time from their starting point.

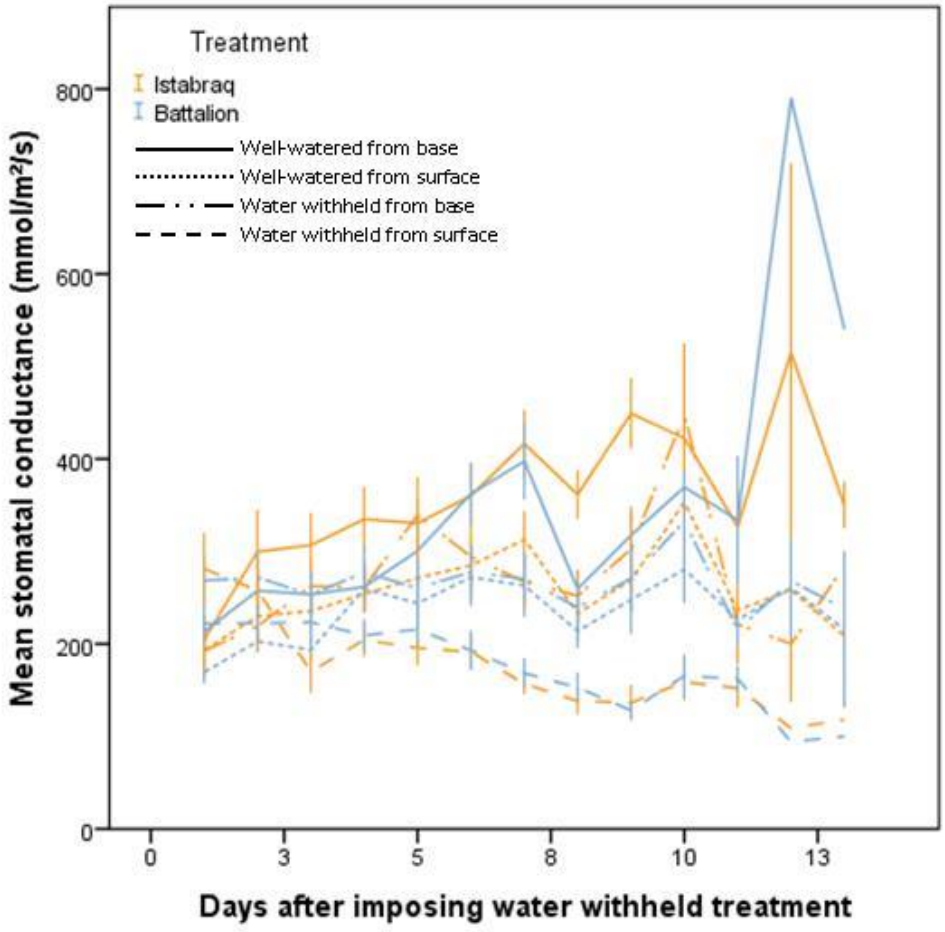


Figure 3.12: Average stomatal conductance over both experiments, measured daily. Day 0 marks the last day on which the water withheld treatments received water. One-way ANOVA, p-value < 0.001 for both factors.

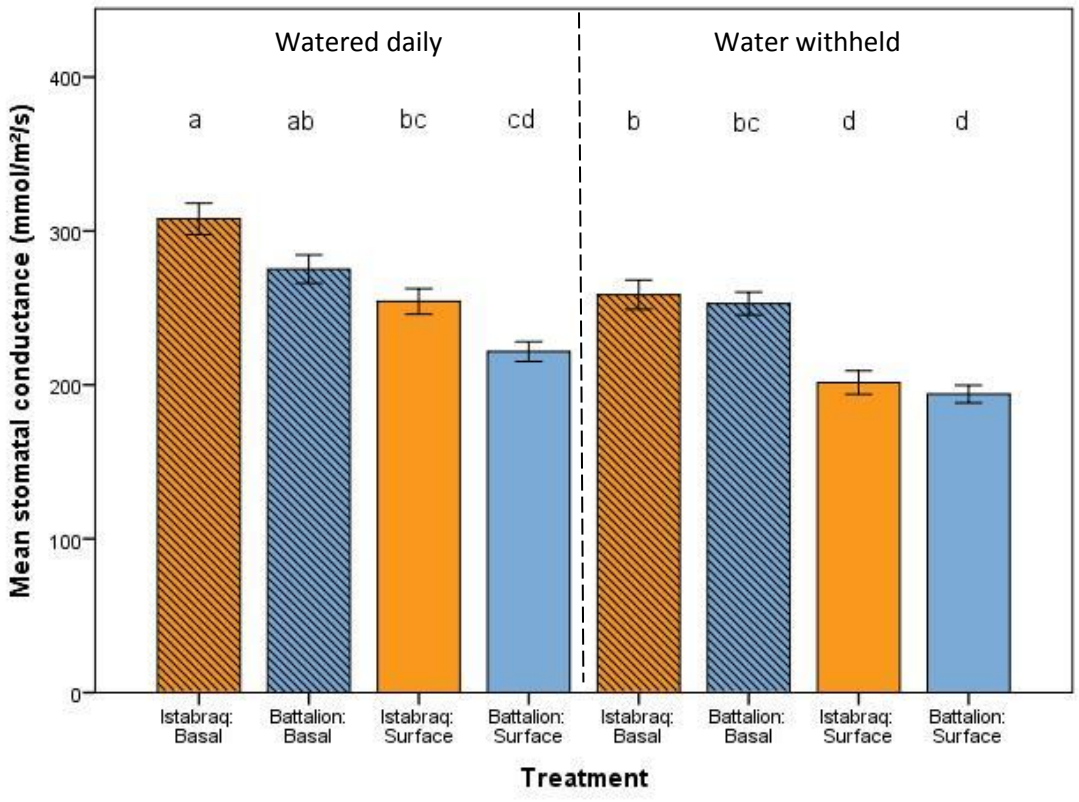


Figure 3.13: Pooled mean stomatal conductance over the course of both experiments, measured from the most recently expanded leaf. Letters above columns are letters of mean discrimination, as calculated by ANOVA with Bonferroni post-hoc test (p-values < 0.05).

When pooling all trials and possible treatments, *Ist* had a significantly (p-value = 0.018) higher stomatal conductance (by 8%) than *Bat*. Soil drying decreased stomatal conductance of *Ist* to a greater extent, as indicated by the letters of mean discrimination in Figure 3.13. When looking at differences in stomatal conductance between all eight possible treatments there are some interesting overlaps between treatments. Watering both genotypes from the base resulted in a significantly higher stomatal conductance than almost all other treatments. Average stomatal conductance is statistically the same between daily, basally watered *Bat*, daily surface-watered *Ist*, and both genotypes when their basal-watered pots receive no further water. However,

the surface watered treatments of both genotypes show significant decreases in stomatal conductance when water is withheld.

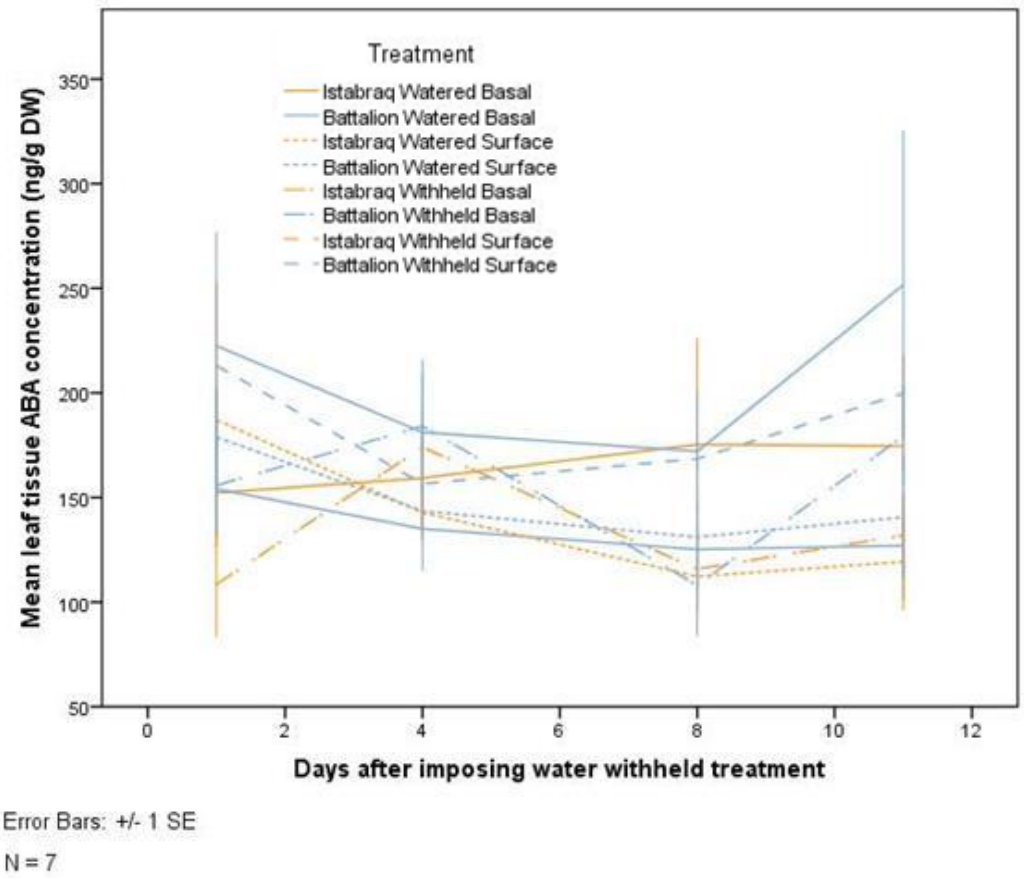


Figure 3.14: Leaf ABA concentrations over time (Experiment 1 only, due to large SE from Experiment 2). Day 0 marks the last day on which the water withheld treatments received water. One-way ANOVA, p-value < 0.001.

There were no clear patterns of leaf ABA concentration by treatment over time (Figure 3.14), although significant differences between treatments over time were detected by ANOVA (Table 3.4). Figure 3.15 suggests that these differences lie in the accumulation of ABA in the previously basally-watered *Bat* plants, and previously surface-watered treatments for both *Bat* and *Ist*. In Figure 3.14, the surface well-watered treatments for both genotypes appear to decrease in ABA concentration over time, which would also have influenced the ANOVA in Table 3.4.

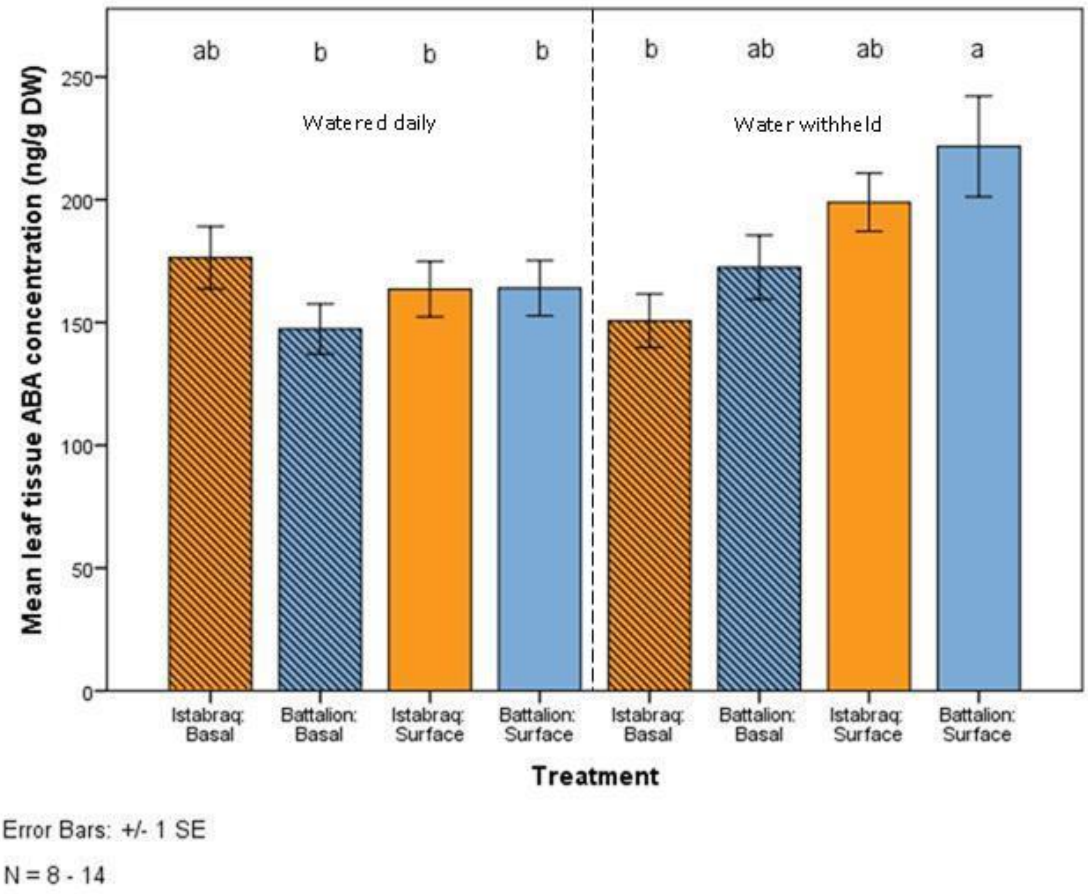


Figure 3.15: Leaf ABA concentrations. Letters of mean discrimination indicate significant differences, p-value ≤ 0.05 (One-way ANOVA, Bonferroni post-hoc). One-way ANOVA finds treatments significantly different, p-value = 0.001.

When data from both experiments and all treatments are pooled, there are few significant differences between treatments (Figure 3.15). Withholding water from previously surface-watered *Bat* plants increased leaf ABA accumulation to a greater extent than from previously basally-watered *Ist* plants and all of the plants that were watered daily, except for daily basally-watered *Ist*. Figure 3.15 suggests that *Bat* increases leaf ABA concentration under soil drying, when *Ist* does not, as indicated by a near-significant (p-value = 0.051) genotype x watering location interaction.

A one-way ANOVA for root ABA concentration over time showed that the number of days after first withholding water was significant (p-value = 0.016), however, none of

the eight specific treatment combinations differed significantly (p-value = 0.225) in mean root ABA concentration. A two-way ANOVA found no interaction effect of number of days post water withdrawal and treatment combination on root ABA concentration (p-value = 0.337). Thus, it appears that all root ABA concentrations rose over time, without significant differences between treatments in rate of concentration increase.

3.3.6 Relationships between dependent variables

Table 3.5: Pearson's correlation coefficient values for key traits of interest, where * denotes significance at p-value ≤ 0.05 and ** denotes significance at p ≤ 0.001 (2-tailed). The data are pooled from both experimental trials.

	Stomatal conductance	Ψ_{leaf}	Leaf ABA concentration	Root ABA concentration	Aboveground dry biomass
Stomatal conductance					
Ψ_{leaf}	.027				
Leaf ABA concentration	-.198 **	.126			
Root ABA concentration	.061	-.071	.098		
Aboveground dry biomass	.512 **	.205	-.131	-.169	

Stomatal conductance significantly decreased as leaf ABA concentration increased (Table 3.5). Increased biomass was highly significantly correlated with increased stomatal conductance, perhaps indicating increased rates of photosynthesis in larger plants. There are no significant relationships between Ψ_{leaf} or root ABA concentration and any of the other dependent variables in Experiments 1 and 2 (Table 3.5).

3.3.7 Experiment 3

Root angle, stomatal conductance and stomatal density

Stomatal conductance remained significantly (p-value = 0.002, Student's T-test) higher in *Ist* compared to *Bat* when root systems were grown on top of cones buried in soil to force the roots to grow with a minimum angle of spread of 110°, equivalent to the shallowest root system shown by *Ist* in (Figure 2.13).

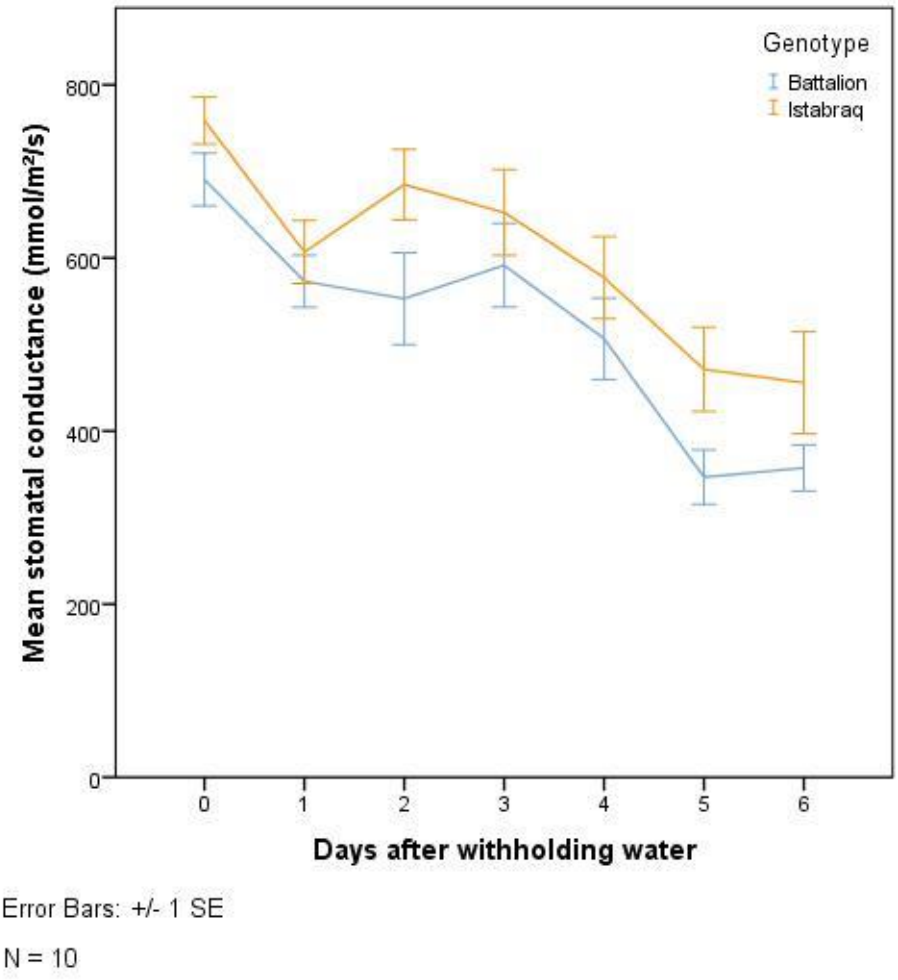


Figure 3.16: Stomatal conductance over time during the soil drying phase in Experiment 3. *Ist* has significantly (p-value = 0.002, Student's T-test) higher stomatal conductance than *Bat* almost throughout this phase.

Figure 3.16 confirms significant genotypic differences in stomatal conductance, as previously observed in Experiments 1 and 2. Using images captured through a light microscope, stomatal density on the abaxial leaf epidermis was higher for *Ist* than *Bat* (p-value = 0.005). On average, this amounted to 19.5% more stomata per unit area (Figure 3.17). This was especially apparent at the tips of the leaves, where stomatal density was significantly higher in *Ist* than *Bat* (p-value = 0.002). The higher stomatal density of *Ist* is consistent with, and may at least partially explain, the twice-observed result of this same genotype having higher stomatal conductance than *Bat*.

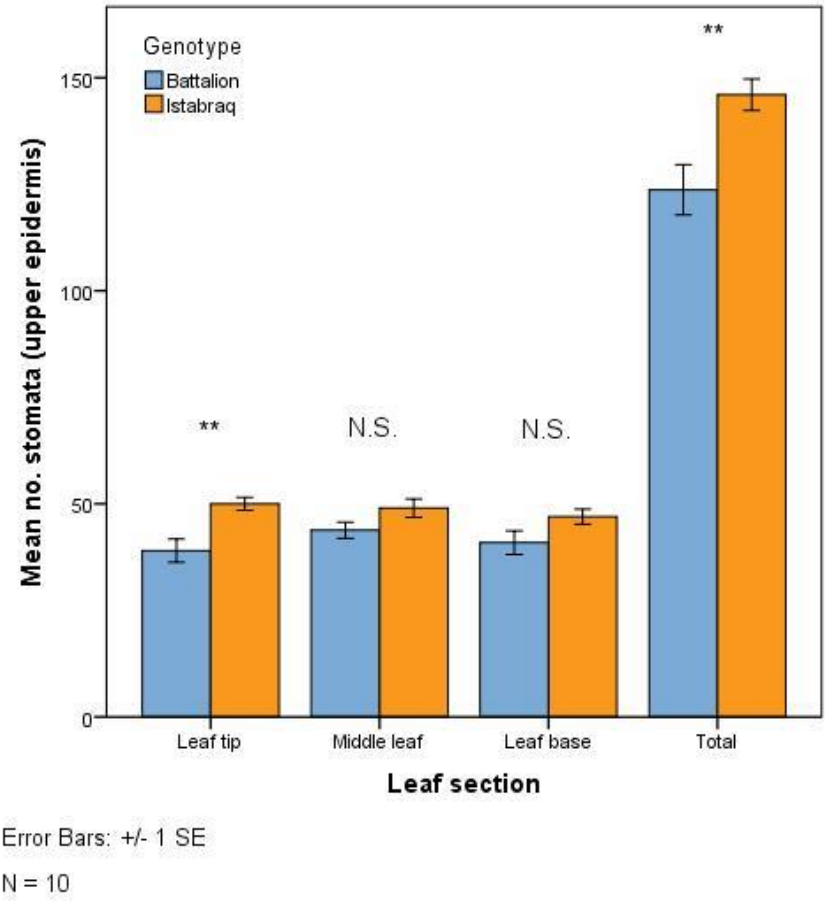


Figure 3.17: Counts of stomata per mm^2 of abaxial leaf epidermis at the leaf tip, mid-section, and base, and the mean averages of the sum of these three sections per leaf, compared across genotypes. N.S. denotes no significant difference in number of stomata between genotypes, ** denotes significant differences with p-value $\leq$ 0.005 (as calculated by Student's T-test).

There were no significant ($p = 0.598$, one-way ANOVA) differences in stomatal density between the three leaf sections (top, mid-section and base), nor any significant ($p = 0.370$, two-way ANOVA) interaction effects of leaf section and genotype.

3.4 Discussion

While *Ist* and *Bat* show distinct differences in seedling root angle (Figures 2.15 and 3.2), root angle phenotype appears to have little effect on the developing plant in the early stages of growth, which can be inferred from the lack of genotypic differences for most variables (Table 3.3). Only stomatal conductance significantly differed between cultivars, with the lower stomatal conductance of *Bat* (Figures 3.5 and 3.17) apparently explained by its lower stomatal density (Figure 3.17). However, both watering location (base/ surface) and water treatment (watered daily/ water withheld), had significant effects on multiple variables (Table 3.3), and sometimes interacted with genotype.

3.4.1 Early vegetative growth

There were no differences in RE between cultivars when grown in blue roll tubes (Figure 3.4). Slower root extension in this experiment, compared to the experiments of Chapter 2, was likely because the blue roll racks were not placed directly below the light and heat sources (as they were for the experiments in Chapter 2), thus lower air temperatures could explain the slower rate of root growth.

During early seedling growth, there were no significant genotype and watering location effects on LER (Table 3.3). Similarly, no differences were observed in dry biomass at harvest, other than the number of days before harvest, i.e. the longer a plant had to grow, the bigger it was when harvested. Although water deficits limit dry matter accumulation by inhibiting leaf elongation (Boyer 1968, Munns *et al*. 2000) and stomatal conductance (Turner 1986, Sperry *et al*. 2002), which in turn limits photosynthesis (Parry *et al*. 2011), neither genotype experienced significant limitation on dry matter accumulation, suggesting a relatively mild stress. Biomass accumulation is critical for wheat yields as this will increase the plant's leaf area, and thus their effectiveness in capturing light, and in high-input systems this trait has essentially already been optimised (Parry *et al*. 2011). Even with a relatively large leaf area, stomatal closure in response to soil water deficits and high evaporative demand can still limit photosynthesis, by restricting CO_2 intake.

Rapid growth and accumulation of root biomass has been associated with increased LER and shoot biomass in young wheat plants (Rebetzke *et al*. 2014). In this chapter, neither cultivar grew more vigorously than the other in terms of shoot or root growth. Even under differently oriented soil moisture gradients, which hypothetically could have influenced how much water a deep or shallow root system could access, the cultivars did not differ in water usage (Figure 3.4), LER (Table 3.3) or aboveground biomass at harvest (Table 3.3). Seedling vigour has been identified as a key trait in conserving soil water in Mediterranean-type environments with in season rainfall, such as those found in areas of southern Europe and western Australia. Vigorous young wheat plants produce aboveground biomass more rapidly than less vigorous varieties, thus the leaf canopy covers a greater area, shading the soil surface and

reducing water loss through evaporation (Duan *et al*. 2016). Vigorous plants may also have improved photosynthetic capacity through higher leaf area (Parry *et al*. 2011). Since *Bat* and *Ist* had similar RE and LER, root angle does not appear to be important in determining vigour.

3.4.2 Stomatal conductance

Although stomatal conductance readings were consistently significantly (p-value = 0.018) higher for *Ist* than for *Bat* (by 9%), and stomatal conductance and photosynthesis are curvilinearly related (Ye and Yu 2008), there were no differences in shoot dry weight (Table 3.3) between genotypes, despite a highly significant positive correlation between stomatal conductance and shoot dry weight. Thus, this could be another example of the effects of time having a more significant effect on shoot mass than either genotype or water treatment. Or it could be related to decreased leaf water potential in some of the *Ist* treatments under soil drying, as leaf cell turgor (not measured) is also associated with leaf growth (Bouchabké *et al*. 2006).

None of the other physiological variables measured in Experiments 1 and 2 (leaf extension rate, leaf ABA concentration, root ABA concentration, Ψ_{leaf}, and aboveground biomass) differed significantly with genotype as the sole factor. As genotypic differences in stomatal conductance were not associated with consistent changes in ABA and Ψ_{leaf}, an alternate hypothesis of genotypic differences in stomatal density was tested. Indeed, *Ist* had nearly 20% more stomata per unit area than *Bat*, which apparently explains genotypic differences in stomatal conductance. Although

stomatal density may vary along the leaf (Mott and Buckley 1998), genotypic differences were consistent along the leaf (Figure 3.17).

3.4.3 ABA concentrations

Although genotype had no effect on leaf or root ABA levels, *Bat* leaf ABA concentrations increased significantly after watering ceased, while leaf ABA concentrations of *Ist* were more stable, thus there was a significant genotype x watering treatment interaction (Fig. 3.10). The same reaction in leaf ABA concentration was observed in the interaction effect of watering treatment and watering location (Figure 3.8), and root ABA concentration (Figure 3.7). No other singular factor, or factors in combination, had a significant effect on root ABA levels. There is still considerable debate over whether ABA synthesised and accumulated in the roots is transported to the leaves to close stomata (Wilkinson and Davies 2002), or if another signal associated with reduced water uptake causes ABA to be synthesised in the leaf, and thus close the stomata (McAdam *et al*. 2016). Using the methods described in Chapter 3.2, there is no way to establish the origin of the ABA detected in the leaf and roots. The ABA concentrations reported in this chapter cannot be claimed to support conclusively either of the two ABA synthesis models discussed in Chapter 1. Superficially, the greater responsiveness of shoot ABA concentrations in contrast to the root ABA concentrations is more consistent with the shoot-synthesised ABA hypothesis (McAdam *et al*. 2016), which proposes that ABA is synthesised in the shoots under soil drying, and then transported to the roots. An alternative hypothetical argument is that there was simply a greater change in Ψ_{leaf} than root water potential (Ψ_{root}), but Ψ_{root} was not measured in these experiments. The leaf-

synthesised ABA theory does not fully fit with the data presented in this chapter, as there was only one example of increased root ABA concentration in any treatment where an overall increase of leaf ABA is reported: the coinciding peaks of Figures 3.7 and 3.8. In all other cases, there is no visual or statistical pattern in foliar and root ABA concentrations.

Both watering from the surface (as opposed to the base) and withholding water significantly decreased stomatal conductance (Figure 3.13), but these patterns are not so clearly defined in foliar ABA concentrations (Figure 3.15), which might have been expected to increase inversely to stomatal conductance (Dodd 2013). However, Figure 3.15 does show the same trend observed in Figures 3.10 of *Bat* increasing leaf ABA concentration under soil drying, when *Ist* does not.

Leaf ABA concentrations were significantly higher in plants grown in pots watered from the surface than from those watered from the base. Even when watered daily, pots watered from the surface experienced greater soil drying than pots watered basally (Figure 3.4). Having seminal roots in drying soil significantly increases shoot ABA levels (Ali *et al*. 1998, Martin-Vertedor and Dodd 2011, Saradadevi *et al*. 2016). In the pots that were re-watered daily, the surface layers would go through a cycle of wetting and drying every 24 hours, which can produce an ABA signal if maximum soil water deficit is sufficiently low. In the pots which were not watered, the surface dried out more than the base, regardless of where they had originally been watered (Figure 3.6), resulting in the water withheld from surface treatments being the driest of all pots (Figure 3.5).

The data from these experiments demonstrate the expected negative relationship between stomatal conductance and leaf ABA concentration (Wilkinson and Davies 2002) (although not root ABA concentration (Jacobsen *et al*. 2009), where increased ABA correlates with decreased stomatal conductance (Pearson's correlation coefficient = -.198, $p < 0.001$, Table 3.5). For example, in the surface-watered plants leaf ABA concentration was significantly (p-value = 0.018) higher than for basally-watered plants; by contrast stomatal conductance was significantly decreased in surface-watered plants (p-value < 0.001). The most reasonable explanation for these relationships is that they are from the response to soil drying, which is already well documented (Martin-Vertedor and Dodd 2011, Saradadevi *et al*. 2016). This relationship is maintained under contrasting water availability treatments (Table 3.5).

3.4.4 Leaf water potential (Ψ_{leaf})

Withholding water decreased Ψ_{leaf} (Table 3.3, Figure 3.11). There was also a near-significant interaction of genotype with watering location, with surface-watered *Ist* having a (nearly) significantly lower Ψ_{leaf} than basally-watered *Ist*, whereas *Bat* had a similar Ψ_{leaf} to both *Ist* treatments. Not only does this suggest that Ψ_{leaf} in *Ist* is more sensitive to a soil moisture gradient than *Bat*, but it is contrary to the hypothesis that Ψ_{leaf} will be greater when the rooting phenotype matches the soil moisture gradient. Thus, shallow rooting *Ist* had higher Ψ_{leaf} when grown in pots watered from the base, compared to those watered from the surface.

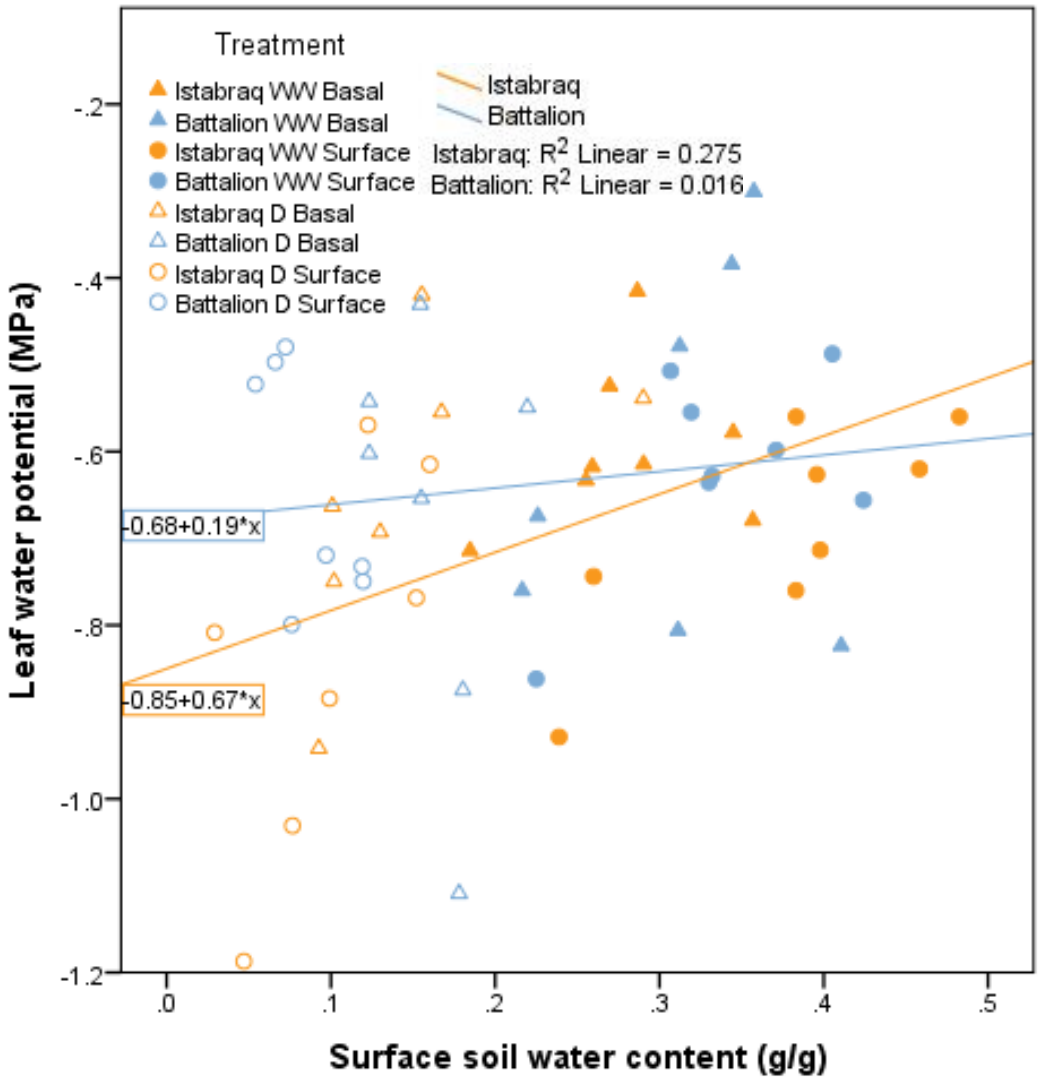


Figure 3.18: Ψ_{leaf} plotted against soil water content, by treatment. There is a significant effect of soil water content on Ψ_{leaf} (as established by Kruskal-Wallis test, Table 3.3), however the steeper slope of *Ist* compared to *Bat* indicates that Ψ_{leaf} in *Ist* is more sensitive to soil drying. ANCOVA for effect of genotype with soil water content as a covariate reported a significant genotype x soil water content interaction effect on Ψ_{leaf} (p-value = 0.042). Each data point is an individual plant.

An alternative interpretation of Figure 3.15 is that *Ist* seedlings growing in surface irrigated pots, with more roots near the surface of the pot, are more sensitive to soil drying than *Bat* seedlings. In Figure 3.11, only one column of four showed a considerable change (between the two *Ist* treatments, where the surface-watered

treatment decreased compared to the basally-watered treatment). This could be explained by differences between genotypes in Ψ_{leaf} response to soil drying. To test this hypothesis, leaf water potential was plotted against soil water content (Figure 3.18), which revealed that *Bat* was better at maintaining Ψ_{leaf} as the soil dried.

Being able to phenotype for drought tolerance in cultivars is important in field-based breeding trials, but the techniques used in these experiments are not generally practical to apply to large scale field studies. Measurements of stomatal conductance and tissue samples for ABA and Ψ_{leaf} analysis are valuable methods for investigating genotypic differences in water status in small scale, controlled environment settings. However, these techniques cannot be easily applied to larger scale phenotyping and/or breeding trials, they would simply be too time consuming and labour intensive. In field settings, alternative water status measurements would be more practical. An alternative would be to measure canopy temperature: well-hydrated plants generally have cooler canopies (Blum *et al*. 1989, Munns *et al*. 2010), and lower canopy temperature can also be used as selection criteria for breeding drought resistant wheat (Fischer *et al*. 1998). This method is already being incorporated into large scale wheat phenotyping platforms in the field, such as the field 'Scanalyzer' at Rothamsted Research, Harpenden (Andrade-Sanchez *et al*. 2014, Virlet *et al*. 2017).

3.4.5 Contrasting strategies for coping with soil drying

Although both genotypes responded to soil drying by reducing stomatal conductance (Figures 3.9 and 3.13), they also differed in response because *Bat* significantly increased foliar ABA levels in response to soil drying (Figure 3.10), whereas *Ist*

decreased Ψ_{leaf} when watered from the surface compared to the base of the pot (Figure 3.18). Considered together, these results suggest that the two genotypes may have contrasting strategies for coping with soil drying. Different behaviours between species or cultivars in response to soil water deficits and atmospheric vapour pressure deficits (VPD) can be described as isohydric or anisohydric behaviours (Tardieu and Simonneau 1998). Isohydric plants maintain almost constant Ψ_{leaf} independent of changes in soil water status, whereas anisohydric plants show a marked decrease in Ψ_{leaf} with increased evaporative demand and drought conditions. Increased ABA levels and decreased stomatal conductance in some treatments in *Bat* appear to be an isohydric response, with tighter regulation of stomatal opening to conserve plant water status. Meanwhile, *Ist* does not show significant increases in ABA even under soil drying, but does show significantly reduced Ψ_{leaf} under soil drying, which is more typical of an anisohydric response (Gallé *et al*. 2013) (summarised in Figure 3.19).

It is possible that if the experiment had run for longer, *Bat* would have begun to behave more like *Ist*, as some wheat (and barley) cultivars seem to change from isohydric to anisohydric behaviour under increasing water deficit (Munns *et al*. 2010). No clear mechanism for this change has yet been proposed, however grape vines (*Vitis vinifera* L.) exposed to sustained PRD altered their responses from isohydric to anisohydric (opposite response to wheat) due to increased sensitivity of the stomata to transpiration and VPD (Collins *et al*. 2010). With this proposed mechanism in mind, it may be that sustained exposure to drying soil reduces the sensitivity of stomata in wheat, explaining Munns *et al*.'s (2010) observation of changing behaviour in wheat and barley. However, this is only speculative, and further investigation of these

cultivars (perhaps in combination with other an/isohydric cultivars) is required, to explain the mechanism behind this response.

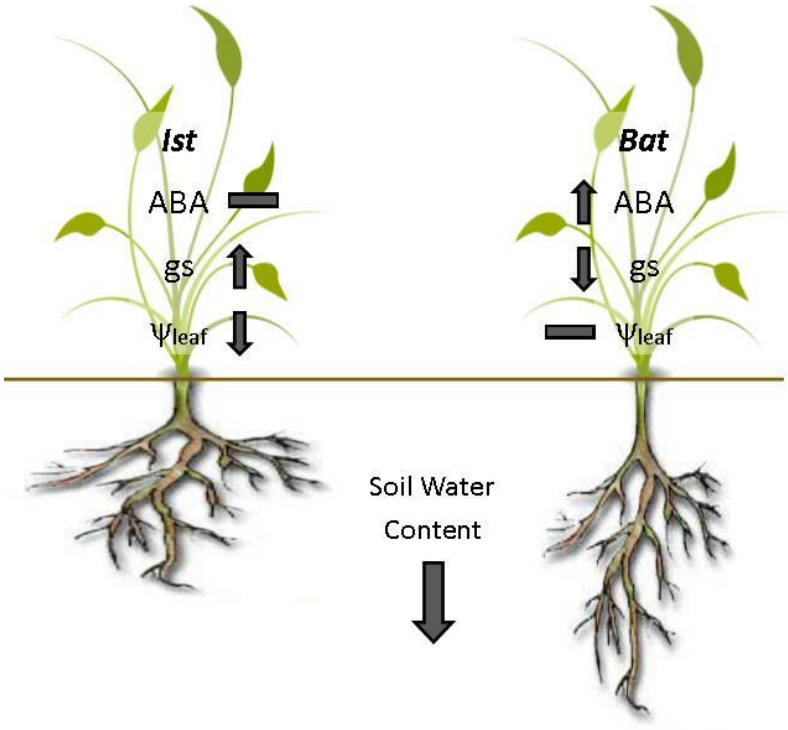


Figure 3.19: Summary of differences in response to soil drying between the two genotypes. The maintaining of Ψ_{leaf} in *Bat* is suggestive of an isohydric response, whereas the loss of Ψ_{leaf} in *Ist* suggests an anisohydric response.

3.4.6 Soil water gradients and pot trials

Establishing soil water gradients in pots, in an attempt to replicate patterns of wetting and drying in field soils, was partially successful in these experiments. Daily watering from the either surface or the base of the pot created a soil moisture gradient, but this was abolished by withholding water due to the combined effects of plant water uptake and surface evaporation. Regardless of whether the pots were originally basal or surface watered, as the pots dried out more water was present in the lower half of the pots than the top (Figure 3.3). Surface-watered pots lost more water over each 24-hour

period than pots that were watered at the base (Figure 3.4), making it difficult to compare plant water use between treatments, as opposed to evapotranspiration.

In hindsight, one way in which this methodology could have been improved would have been to cover both the soil surface in the pots, and the water surface in the saucers beneath the pots, to prevent unequal evaporative losses between different treatments. On the other hand, covering the pots and saucers would have slowed the rate of water loss, reducing or delaying a plant response. This would be compounded by the continued root growth, and the increasing homogeneity of root distributions within the pots between genotypes. Over time the shallow root phenotype of *Ist* would be lost in a pot environment, as the roots may hit the sides of the pot and grow downwards. As the root systems become pot bound, phenotypic differences in root systems between genotypes will be eliminated.

3.4.7 Other methodological remarks

Large 10 L pots, used for the experiments described in this chapter, allowed the roots more space to grow before being constrained by the sides of the pot, with the aim of maintaining the deep/shallow phenotypes. Wheat phenotyping trials for response to elevated atmospheric CO_2 and higher temperatures were repeated in 1.4 L pots and 7.5 L (10 cm x 100 cm) plastic columns. Genotypic x environment interactions and their resultant phenotypes were inconsistent between the two container sizes under the same CO_2 and temperature conditions (Bourgault, *et al*. 2017). While phenotypic differences in seedling root angle were maintained between in these studies (Figures 2.15 and 3.2), we cannot know if the choice of container is obscuring other phenotypic

responses because no other physiological measurements were made in Chapter 2. A meta-analysis on previous studies on the effect of pot size found that it generally reduces growth by limiting photosynthesis per unit leaf area (Poorter *et al*. 2012); although there have also been contrasting reports (see Ray and Sinclair 1998) of decreasing pot size causing increased photosynthetic rate in bean (Carmi *et al*. 1983) and no change in photosynthetic rate in soybean (Krizek *et al*. 1985). To prevent pot size from imposing any limitations, it is suggested that plant biomass should not exceed 1 g L^{-1} of soil (Poorter *et al*. 2012). The maximum aboveground plant mass at harvest was approximately 1.8 g and root mass, while not measured due to the difficulty in extracting the whole root system from the soil in the large pots (as well as needing to freeze dry what was collected immediately), could reasonably be assumed to be even less. Therefore, it is unlikely that the root systems were constrained by pot size during this experiment, or that root constraint significantly affected plant responses.

3.5 Conclusions

Genotypic differences in root angle did not significantly affect the early growth and physiology of young wheat plants. However, some interactions between genotype and irrigation treatment suggest that:

- The two cultivars differ in their response to soil drying, with *Ist* showing no changes in ABA concentration despite stomatal closure, whereas *Bat* leaf tissue ABA concentration increases under soil drying as stomatal conductance decreases.

- Soil drying decreases Ψ_{leaf} of *Ist*, but *Bat* maintains Ψ_{leaf} as soil dries. These contrasting results suggest that *Bat* favours a conservative water use strategy, closing the stomata during soil drying to maintain Ψ_{leaf}, whereas *Ist* is less conservative, at the expense of Ψ_{leaf}. These could respectively be considered examples of isohydric and anisohydric plant behaviours.

- The only consistent difference between cultivars across all three experiments (increased stomatal conductance in *Ist*) was best explained by increased stomatal density. There is no evidence to suggest that root angle phenotype directly influenced stomatal conductance.

While genotypic differences in seedling root angle did not explain the difference in stomatal conductance (as plants responded as they had in Experiment 1, when root angle was constrained in Experiment 3), the physiological effects of soil moisture gradients are likely more pronounced in the field environment, where narrow root angle may enhance water capture from deeper soil layers. Thus, *Ist* and *Bat*, along with other cultivars expected to show differences in root phenotype, were grown in the field (Chapter 4) to further explore the relationship between root and soil moisture distribution, and plant response.

Chapter 4: Field study of root distribution within the soil profile of a UK wheat crop

This work presented in this chapter is the result of collaboration with researchers at Rothamsted Research. It is acknowledged that core samples were taken from pre-existing field trials, and so the field design was developed before this study commenced. The penetrometer and neutron probe data were collected by Rhys Ashton, the plant height data by Marion Dorlanne, and the statistical methods were selected through discussion with Richard Whalley and Rodger White. Rhys Ashton, Colin Webster, and Marion Dorlanne all assisted in the collection of the soil cores, and the root washing of those cores.

4.1 Introduction

4.1.1 Roots and field soils

Chapters 2 and 3 have established the presence of genetic variation in seedling root angle between UK wheat cultivars, although the effects of these differences on the developing seedlings appear to be minimal. Previous studies have highlighted the importance of seedling root angle as a predictor of rooting depth in the mature plant (Manschadi *et al*. 2008, Wasson *et al*. 2012). Therefore, this chapter will focus on the root systems of the genotypes phenotyped in Chapter 2, when grown in the field, and any relationships with water use efficiency (WUE) and yield.

Between 2012 and 2015, annual UK wheat yields averaged 14.6 million tonnes (DEFRA 2016), and leading up to 2011, the average yield was around 7.9 t ha^{-1} (Curtis and Halford 2014). This is considerably higher than both the global average yield of 3.03 t ha^{-1} and the EU average yield of 5.19 t ha^{-1} (Curtis and Halford 2014). The relatively high yields in the UK can mostly be attributed to a mild climate where rainfall is distributed broadly evenly through the year. Even in the UK, yields of winter wheat can be restricted by water availability (Dodd *et al*. 2011). However, even in dry summers, water is available at depths as shallow as 60 cm at relatively high matric potentials (Whalley *et al*. 2007, 2008), which could be accessed by roots. Since water use (transpiration) is linearly related to crop yield (Passioura 1977), this represents an untapped resource that might be usefully exploited to further increase wheat yields.

The inability of roots to access water is commonly attributed to a low root length density at depth (Gregory *et al*. 1978a, b). For this reason, rooting depth of wheat in the UK has been of considerable interest (e.g. Lupton *et al*. 1974; Gregory *et al*. 1978a; Barraclough and Leigh 1984; White *et al*. 2015). Lupton *et al*. (1974) found little difference between the rooting depths of tall wheats in comparison with semi-dwarf wheats which had recently been introduced to the UK. However, within a selection of 21 wheats that are currently grown commercially in the UK, there is recent evidence that some lines are more effective at accessing deep water than others, with positive correlations between deep water extraction and yields in drought, although differences in water uptake at depth were not sufficiently large or consistent to identify extreme performers with any certainty (Ober *et al*. 2014). This could be caused by genotypic differences in root length density, where even if the roots of all

genotypes grow to the same depth, the proportion of root material in different layers of the soil profile may vary between genotypes, as was found to be the case in potato (Puértolas *et al*. 2014). Alternatively (or possibly in combination with different patterns of root density), this may be partly due to the impact of management on rooting depth. For example, sowing depth can have a large impact on both the amount and depth of the root, while total root mass was closely correlated with the accumulation of (aerial) thermal time (Barraclough and Leigh, 1984). Early sowing led to deeper roots, especially until early spring (March) although the rooting depth was similar between early and late sown wheat thereafter. Taken together, these results indicate limited genetic differences in wheat root distribution with depth in the soil profile under UK conditions. Similarly, a comparison of 40 different wheat genotypes at two different field sites in Australia found little effect of genotype in determining rooting depth, the number of shallow roots or the number of deeper roots (Wasson *et al*. 2014). The field sites (i.e. soil type) had the greatest effect on the distribution of roots with depth, with one of the sites encouraging a much greater root length density at depths shallower than approximately 1 m in all the genotypes. In contrast, the soil at the shallow rooting site was denser towards the soil surface than the deep rooting site, and trapped more water in the upper soil layers.

Soil properties can influence rooting depth and distribution in many ways. In denser soils, roots are forced to exploit cracks and pores, forcing them to grow in a particular direction and reducing the randomness of their distribution in the soil profile (Wasson *et al*. 2014). Increased soil density also increases root impedance, which can increase the steepness of the angular spread of the root system in wheat (Jin *et al*. 2015). Both of these factors could help to explain the greater root length density depth in Wasson

et al.'s (2014) paper. Meanwhile, high soil density at the soil surface may have prevented deep rooting due to the difficulty with which roots penetrate hard compacted layers (Kubo *et al*. 2004, Botwright Acuña and Wade 2012).

In the field, deep roots are almost exclusively found in pre-existing pores (White and Kirkegaard 2010), thus deep rooting is likely to be largely determined by the quantity of deep pores. Gao *et al*. (2016) suggested that increases in soil strength with depth may confine roots to pre-existing pores, especially when penetrometer resistances in the bulk soil are much greater than 2.5 MPa (see also Busscher *et al*. 1986). An interesting feature of root length distribution with depth in field studies is that root length density decreases exponentially with depth (e.g. Gerwitz and Page 1974; Fan *et al*. 2016) which contrasts to many laboratory experiments with re-packed soils (e.g. Manschadi *et al* 2008; Jin *et al*. 2015; Gao *et al*. 2016), where there is relatively high root density at depth and a less noticeable exponential decrease of root length density with depth. Thus, differences in pore distribution with depth may explain the limited genetic differences in wheat root distribution with depth, but this has received little attention under UK conditions, especially with respect to deep roots.

4.1.2 Aims of this chapter

This chapter has two main goals. First, root length density was compared with vertical changes in soil structure in winter wheat grown in the UK. While root length distributions of field-grown wheat have been reported (e.g. Gregory *et al*. 1978a; Barraclough and Leigh, 1984; White *et al*. 2015) and they conform to the empirical root length density distribution of Gerwitz and Page (1974), they have not been

compared with soil structure and physical characteristics. Indeed, Rich and Watt (2013) note that few field studies report both root and soil conditions; this study will provide new evidence to address this deficiency. Second, the possibility that root length distributions with depth are in part determined by genetics was evaluated by comparing tall and dwarf near isogenic lines (NILs) (Rht-B1a Mercia (*Tall*), and Rht-B1c Mercia (*Dwarf*)) as well as four wheat cultivars commercially grown in the UK. Measurements were made in two successive seasons on adjacent fields with similar soil profiles, with the effects of soil structure, genotype and season on the distribution of roots with depth considered. Although direct measurement of root angle is not possible under field conditions, drawing on data from Chapter 2 these field trials will test the hypothesis that seedling root angle can be used to predict rooting depth and proportional distribution of roots of mature plants grown in field conditions.

4.2 Materials and Methods

4.2.1 Experimental sites

Experiments were conducted on neighbouring Broadmead (2014) and Warren Field (2015) sites at Woburn Experimental Farm, Bedfordshire, UK (52°01'11.2"N 0°35'30.4"W). The soil at both sites is a deep silt-clay loam. The vertical gradient in texture is negligible on Warren Field, but on Broadmead some parts of the trial site had a greater sand content at depth. On both sites, the surface layer (approximately 30 cm) has a higher organic matter content and is less dense. Soil properties are summarized in Table 4.1. The soil profiles of both Warren Field and Broadmead are consistent with description of a soil profile by Weir *et al*. (1984) that should be

expected to produce high yields of winter wheat. The soils are broadly consistent with good, grain-producing soils in the UK.

Table 4.1: Description of site and soil properties of Woburn Experimental Field Station, Bedfordshire, UK.

Property		**Units**	
Location		Latitude	5201’06’’N
		Longitude	0035’30’’W
Soil type		SSEW group	Typical alluvial gley soil
		SSEW series	Eversley
		FAO	Fluvisol
Sand (2000 – 65 μm)	Surface soil	g g^{-1} dry soil	0.538
Silt (63 – 2 μm)		g g^{-1} dry soil	0.203
Clay (< 2 μm)		g g^{-1} dry soil	0.260
Texture		SSEW class	Sandy clay loam
Particle density		g cm^{-3}	2.587
Organic matter		g g^{-1} dry soil	0.038

4.2.2 Field management

The field sites were sown in the same manner in both years: 96 separate 9 m x 1.8 m plots, divided into four fully randomised blocks, with each block containing 23 plots of different wheat cultivars and one fallow plot. The experiment is also described by Whalley *et al*. (2017). Cultivars and fallow plots were randomly arranged within each block. The plots were sown on 10/10/2013 in 2013/14 and 26/09/2014 in 2014/15. The field sites were rain fed with no additional irrigation. Soil moisture measurements were taken, and soil cores were collected approximately 1 m from the end of each specific plot.

4.2.3 Wheat genotypes

Of the 23 available genotypes, five were selected for soil coring trial in 2014, and six in 2015, based on previous laboratory phenotyping experiments (Whalley et al. 2013, Chapter 2). The 2014 genotypes were Battalion (*Bat*), Hystar Hybrid (*Hys*), Rht-B1c Dwarf Mercia (*Dwarf*), Rht-B1a Mercia (*Tall*), and Robigus (*Rob*). *Dwarf* and *Tall* were NILs. The 2015 genotypes were the same as for the previous year, with the addition of Istabraq (*Ist*), which was chosen due to its observed shallow rooting habits under laboratory conditions.

4.2.4 Field measurements

Neutron probe (CPI HydroProbe model 503TDR) readings were taken in the field at approximately monthly intervals. Neutron probes detect soil moisture by releasing fast neutrons that collide with hydrogen atoms in water molecules in the soil. Upon collision, the fast neutrons lose energy; the detection of slow neutrons provides an estimate of water content (Schmugge *et al*. 1980). Aluminium access tubes were installed approximately 100 cm from the end of selected plots and measurements were made at depths of 10, 25, 50, 75, 100, 125 and 145 cm. Soil strength was measured by taking readings using a soil penetrometer in both years (Whalley *et al*. 2008, 2017). Where possible, penetrometer strength profiles were taken to a depth of 52.5 cm. Atmospheric conditions and rainfall were measured and recorded by a weather station on the experimental farm.

4.2.5 Soil cores to estimate rooting

Cylindrical soil cores were taken from the Broadmead plots between 03/06/2014 and 13/06/2014 and from the Warren Field plots between 25/06/2015 and 03/07/2015 using a soil column cylinder auger (Van Walt Ltd, Surrey, UK). The cores were 100 cm long and 9 cm in diameter. They were extracted approximately 1 m in from the end of the wheat plots at the end opposite to the one with the neutron probe access tube. In 2014 one core was taken from three of the blocks for each genotype of interest, resulting in three replicates per genotype. In 2015 four cores were taken for each genotype, one from each block, and thus four replicates per genotype. Once extracted the cores were stored at 4 °C (at Rothamsted Research, Harpenden, UK) inside two 105 cm lengths of polyethylene guttering and wrapped in a black polyethylene bag, until analysis.

Cores were divided into five sections, each approximately 20 cm in length. These sub-cores were then broken approximately 5 cm from both ends to reveal fresh faces exactly as described by White and Kirkegaard (2010), which were 10 cm apart in the original core. The core faces were viewed at 3.95x magnification and imaged using a Leica M205 FA stereomicroscope (Leica Microsystems), and Leica Application Suite Advanced Fluorescence (LAF AF) software (version 2.6.0, Leica Microsystems). Each face was photographed six times to ensure that the entire face was recorded. The images were 1.4 MP in size with a resolution of 37.8 pixels per cm. When a whole face was not recovered (the cores were sometimes stony and crumbly, particularly at depths below 60 cm), then fewer images were recorded, but the entirety of the

available face was photographed. All photographs were exported as TIF files to Adobe Photoshop CS5.1.

To estimate root penetration through each face, 10 sections 1 cm^2 in size were selected by overlaying 2 mm gridlines on the images from that face using Adobe Photoshop CS5.1., and using randomly generated coordinates to identify the 10 squares for analysis. The coordinates were generated using the RANDBETWEEN function in MS Excel 2010. The images were manually compared where the coordinates generated may have caused possible overlap, and when overlaps were identified, the second image was discarded and another 1 cm^2 section chosen through a newly generated pair of coordinates. The numbers of roots and pores visible within each individual 1 cm^2 were recorded in an Excel 2010 spreadsheet before being exported for statistical analysis. For this study, pores were defined as a visible airspace in and below the broken face of the soil core, with a diameter $\geq$ 0.7 mm. When roots were counted, it was also recorded whether the root was growing inside of a pore, or in the bulk soil. When roots were observed in pores, it was also recorded if they were sharing the pore with other roots, to look for possible evidence of root clustering in pores.

After the core break and photography procedures were complete, each 10 cm subsection of core was stored in a polythene bag and frozen at -23 °C. The subsections were then defrosted and the soil and debris washed out through a 0.5 mm sieve to retrieve as many root fragments as possible. These root fragments were then scanned on a flatbed scanner and analysed using WINRhizo (Regent Instruments Inc., Quebec, Canada).

4.2.6 Canopy measurements

Leaf area index (LAI) was measured with a ceptometer (Delta-T Devices, Burwell, Cambridge, UK) periodically during the season. Crop height was measured with a meter ruler. At harvest the grain yields were measured with a plot combine harvester.

4.2.7 Statistical analysis

All experimental data were analysed with GenStat v16 (www.vsni.co.uk). In each of the experimental years (2014 and 2015), 23 lines of wheat and a fallow plot were set out in a fully randomized complete block in four blocks, although root measurements were only made on a subset of these lines, as explained above. A different randomisation scheme was used in each year. The block structure, block/plots, was used for the statistical analyses with a treatment structure of "genotype" for yield measurements and block/plots/depth was used with the treatment structure "genotype x depth" for the penetrometer and root measurements.

Penetrometer data were analysed with REML (residual maximum likelihood), but these data required square root transformation to stabilize the variance with spline models, to account for the profile with depth. For ease of comparison with other published data, penetrometer data were plotted on the natural scale; it was not possible to plot the standard error of differences (SED) which was obtained from the transformed data. Similarly, the numbers of roots were transformed using square roots and the profiles modelled with regression (depth being treated as a variable) for a linear trend, and spline models to represent the non-linear departure from the linear

response. When fitting the spline function, a linear trend was used to explain the decrease in root numbers with depth and the spline curve was superimposed on the linear trend to account for the nonlinear nature of the root count with depth. Thus, the slopes of the linear trend were compared to determine if there were any significant differences in the interaction between genotype and depth were present. Given the low numbers of roots at depth, a variance determined from the surface layers was imposed on the deeper layers. The spline fits were compared using the Wald statistic from REML, and depth was treated as variable. Yield data was analysed with ANOVA. To compare pore occupancy, percentages of untransformed root counts were analysed by use of one-way and two-way ANOVA.

4.3 Results

4.3.1 Soil penetrometer resistance

Prior to any water uptake, the soil penetrometer resistance profiles are shown in Figure 4.1. These are similar for each year and both show that even in the absence of any soil drying (i.e. the soil profile was at field capacity) penetrometer resistance increases with depth. The effects of crop water uptake on soil penetrometer resistance are shown in Figure 4.2. REML analysis of the data in Figure 4.2 showed that genotype had a significant effect (p-value < 0.001) on the relationship between penetrometer resistance and depth. At 50 cm depth, *Bat* was the most effective at drying the soil (i.e. high penetrometer resistance compared with the fallow plot) while *Ist* and *Rob* had little effect on soil strength at this depth. In shallower layers, at approximately 20 cm, *Bat* was also the more effective wheat at drying the soil. In very

shallow soil, penetrometer measurements are poor at indicating soil drying (Whalley *et al*. 2017). Although penetrometer measurements were also made in 2014, the data set was more limited, because penetrometer resistance was not measured at the end of a drying period as in Figure 4.2; therefore, these data are not presented.

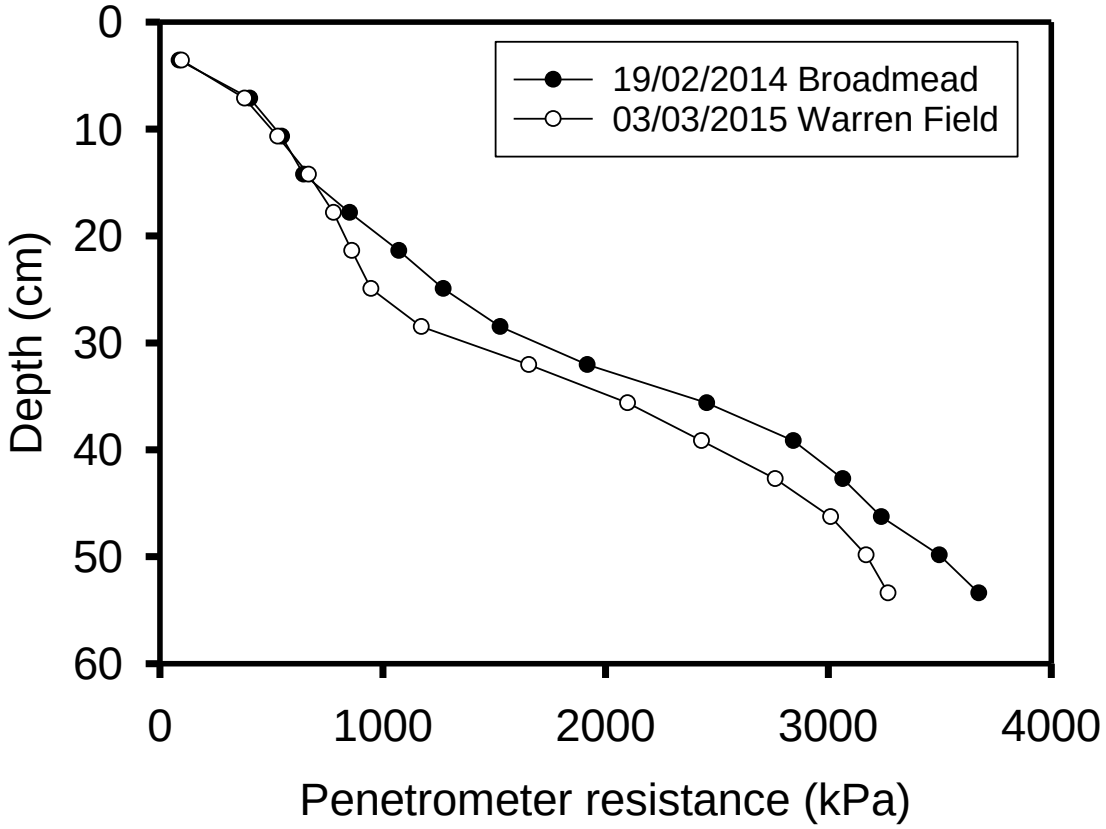


Figure 4.1: Soil strength with depth in the two field sites over the two years in early spring. These measurements were made before any soil drying had occurred. Data are the means of the four replicate plots per genotype, with five penetrometer measurements made in each plot.

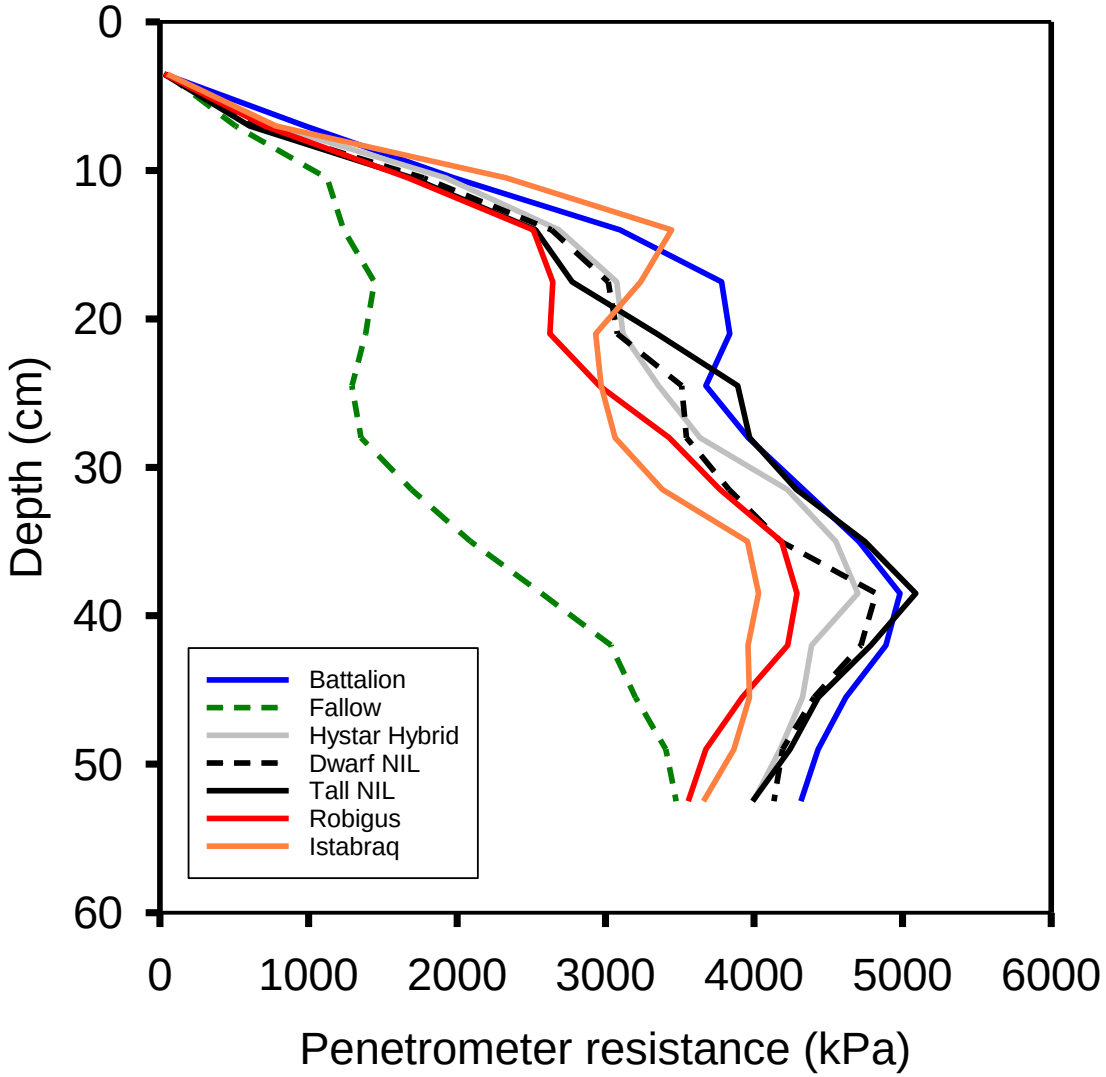


Figure 4.2: Soil penetrometer resistance profiles measured on 1 May 2015. Increases in penetrometer resistance are associated with increased soil drying and soil depth. These data can be used to compare soil drying by the different genotypes. On the fallow plot, there was no soil drying. REML analysis showed that the main effect of genotype was significant at p-value < 0.001. The effect of the interaction between genotype and depth was also significant at p-value < 0.001.

4.3.2 Soil water content

In Figure 4.3, neutron probe data are presented by showing the difference between water content at a reference time point when the soil prolife was at field capacity (at the beginning of March) and the water content at various times later in the season. Soil

moisture deficit and rainfall are also plotted in Figure 4.3 and are consistent with the pattern of soil water measured with the neutron probe.

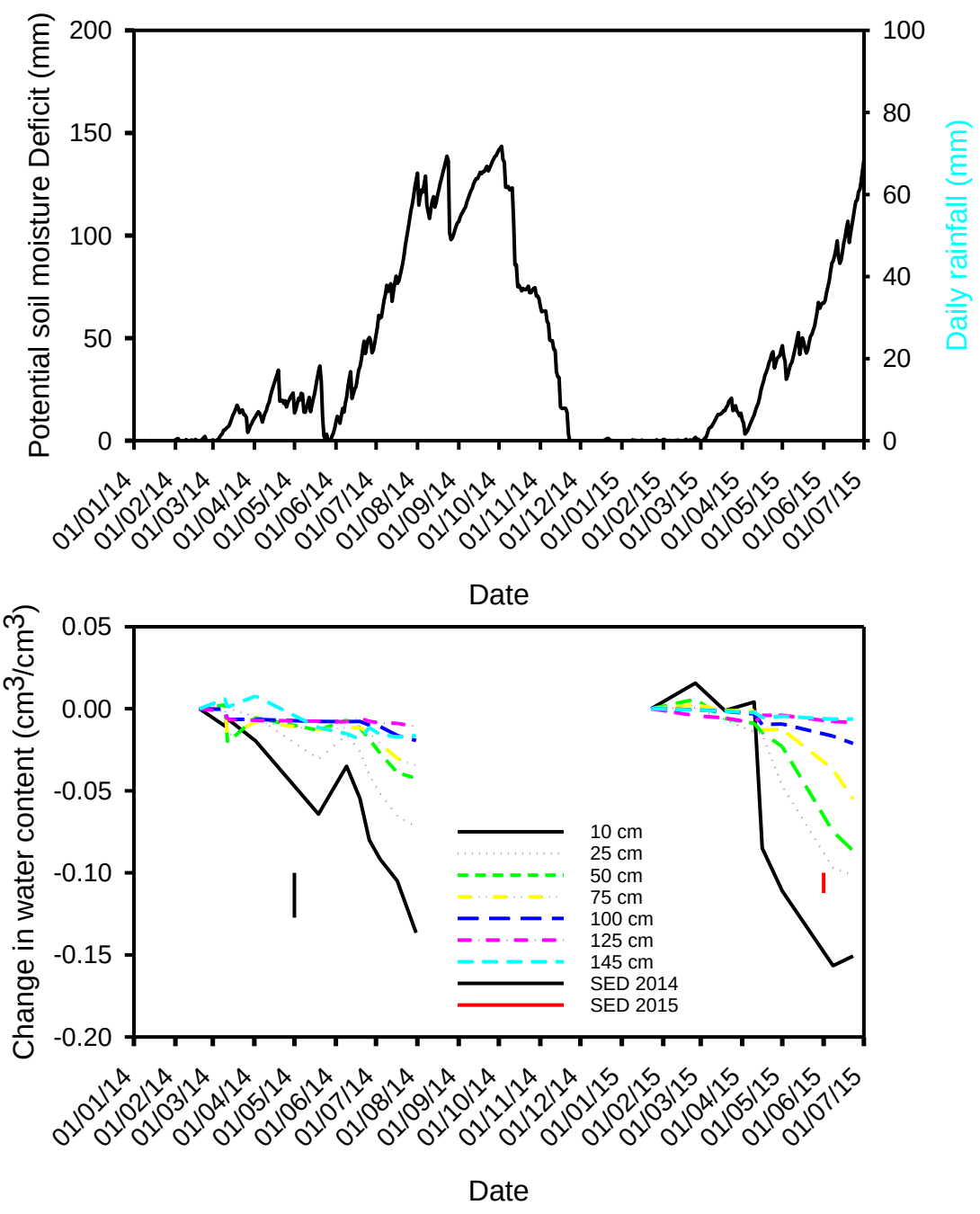


Figure 4.3: a) Potential soil moisture deficits for the duration of the experiment calculated from meteorological data with the Penman-Monteith method. Redrawn from Whalley et al. (2017) where it is presented as supplemental data; b) the change in water content at different depths in 2014 and 2015. These are the means taken across the different genotypes. In 2015 there was no effect of genotype on soil water content profile, but a significant effect was observed in 2014 (see Figure 4.4).

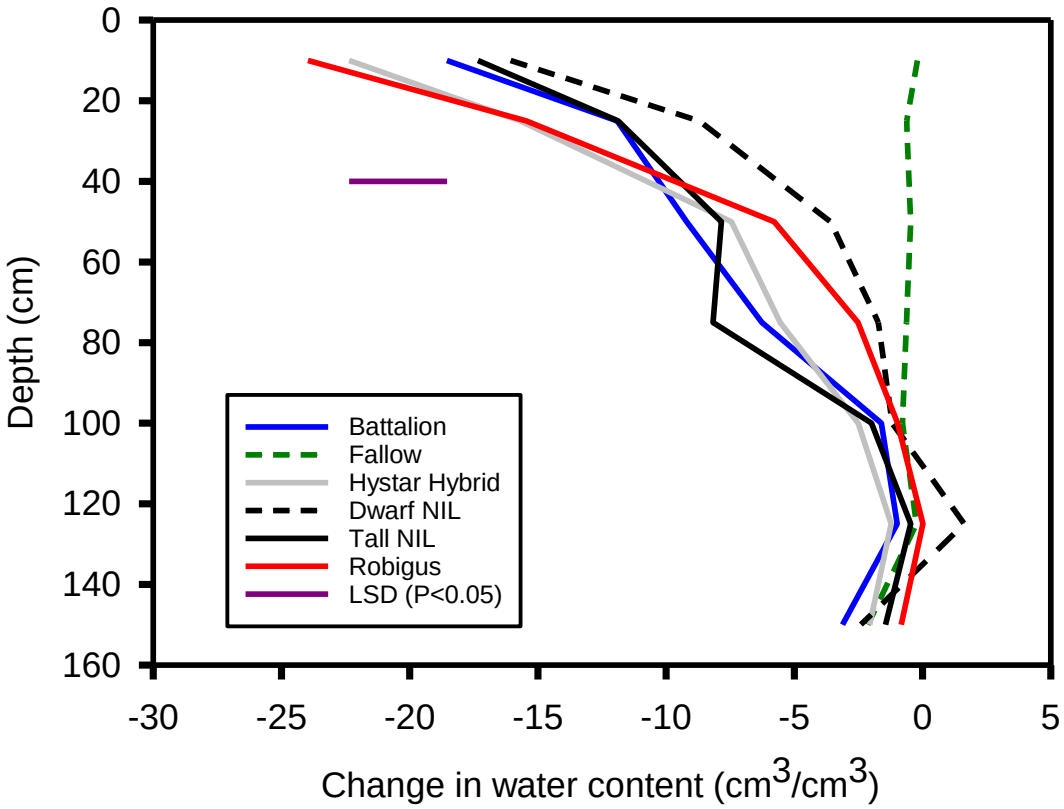


Figure 4.4: Change in water content with depth between 19/02/2014 and 17/07/2014 for the genotypes studied in 2014. Data for the fallow plot is also shown. In 2014, REML shows that there was a significant effect of genotype (p-value = 0.001). In 2015, there was no effect of genotype on soil water content profile and the main effect of depth on soil drying for both 2014 and 2015 is shown in Figure 4.5.

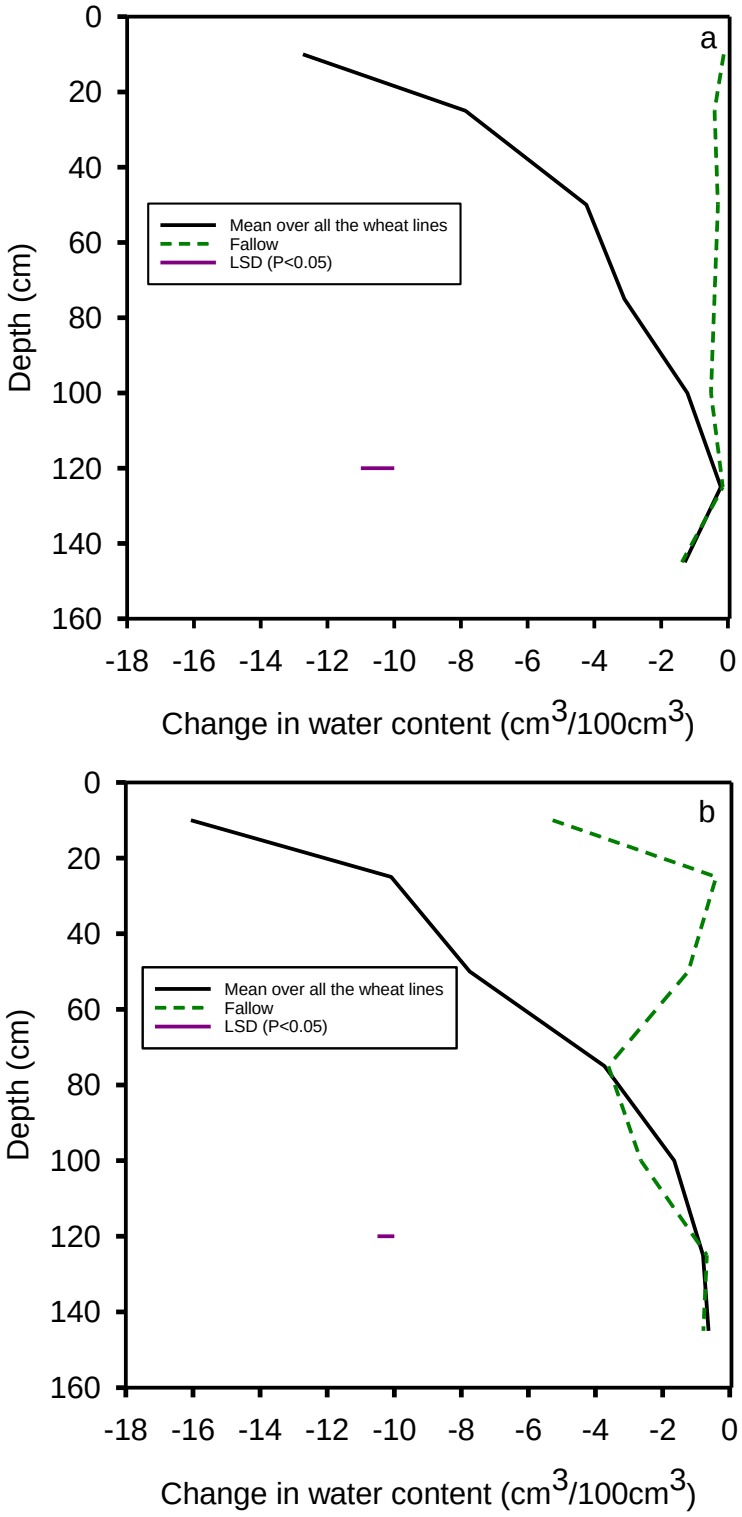


Figure 4.5a, b: Changes in soil water content between a) 19/02 and 17/07 in 2014 at Broadmead and b) 22/01 and 23/06 in 2015 at Warren Field, as a function of depth. For the sown plots these data are the means taken across the different lines. Data for the fallow plots are also shown. The main effect of wheat soil drying profile is shown in Figure 4.4 for 2014, but in 2015 only the main effects and interactions between time and depth were significant. In both years, the effect of depth was significant (p-value < 0.001).

These data show that there were very different temporal patterns of soil drying in 2014 and 2015. In 2015, the soil profile remained wet until mid-April when there was a period of intense soil drying, whereas in 2014 soil drying began earlier in the season. Genotype significantly affected the soil drying profiles in 2014 (Figure 4.3), with *Dwarf* least effective at drying the soil. At depths of approximately 50 to 80 cm, *Tall*, *Hys* and *Bat* dried the soil the most. In 2015, neutron probe measurements did not find any significant effects of genotype on soil drying. In 2014, the soil was dried to greater depth than in 2015 (Figure 4.5). However, estimated potential soil water deficits (Figure 4.3) showed that 2015 was drier than 2014. This was probably related to differences in field drainage between Warren Field and Broadmead.

4.3.3 Root depth profiles

Example photographs from depths of 5 cm and 95 cm are shown in Figure 4.6. Root numbers on ten 1 cm^2 areas were counted on the upper face of the cracked core and these data are plotted as a function of depth along with the counts of pores greater than 0.7 mm in size (Figure 4.7). As the cores were 10 cm in length, the total root count in 10 cm^2 is numerically equivalent to root length density in cm/cm^3, assuming that the roots are parallel. The interaction between genotype and depth had a significant effect on transformed root numbers in 2014 (p-value = 0.001) but not in 2015 (p-value = 0.869).

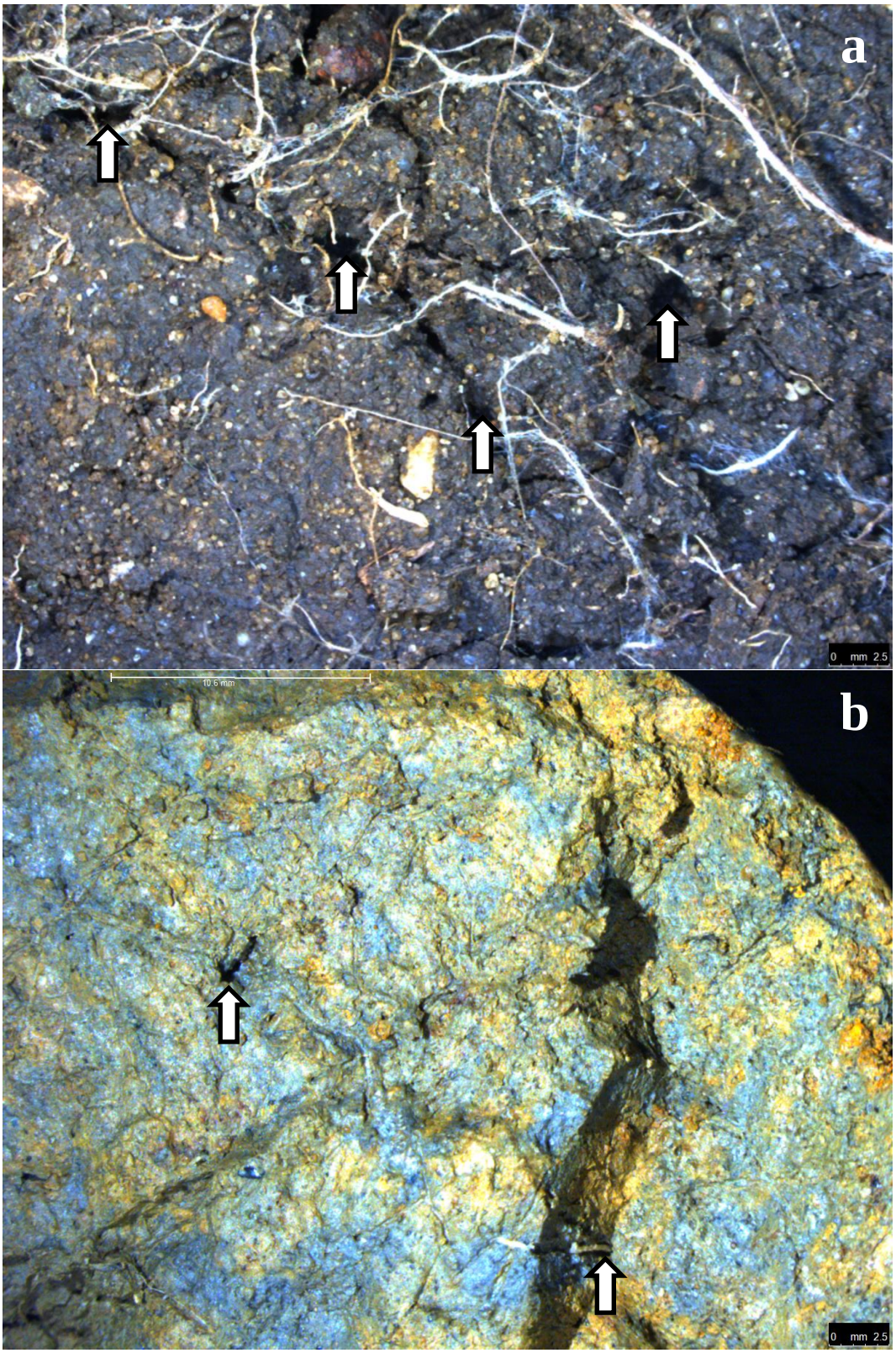


Figure 4.6a, b: Examples of photographs from which 1 cm squares were selected at random and used to count roots and pores following the core break procedure a) at 5 cm depth, and b) at 95 cm depth. White arrows indicate soil pores.

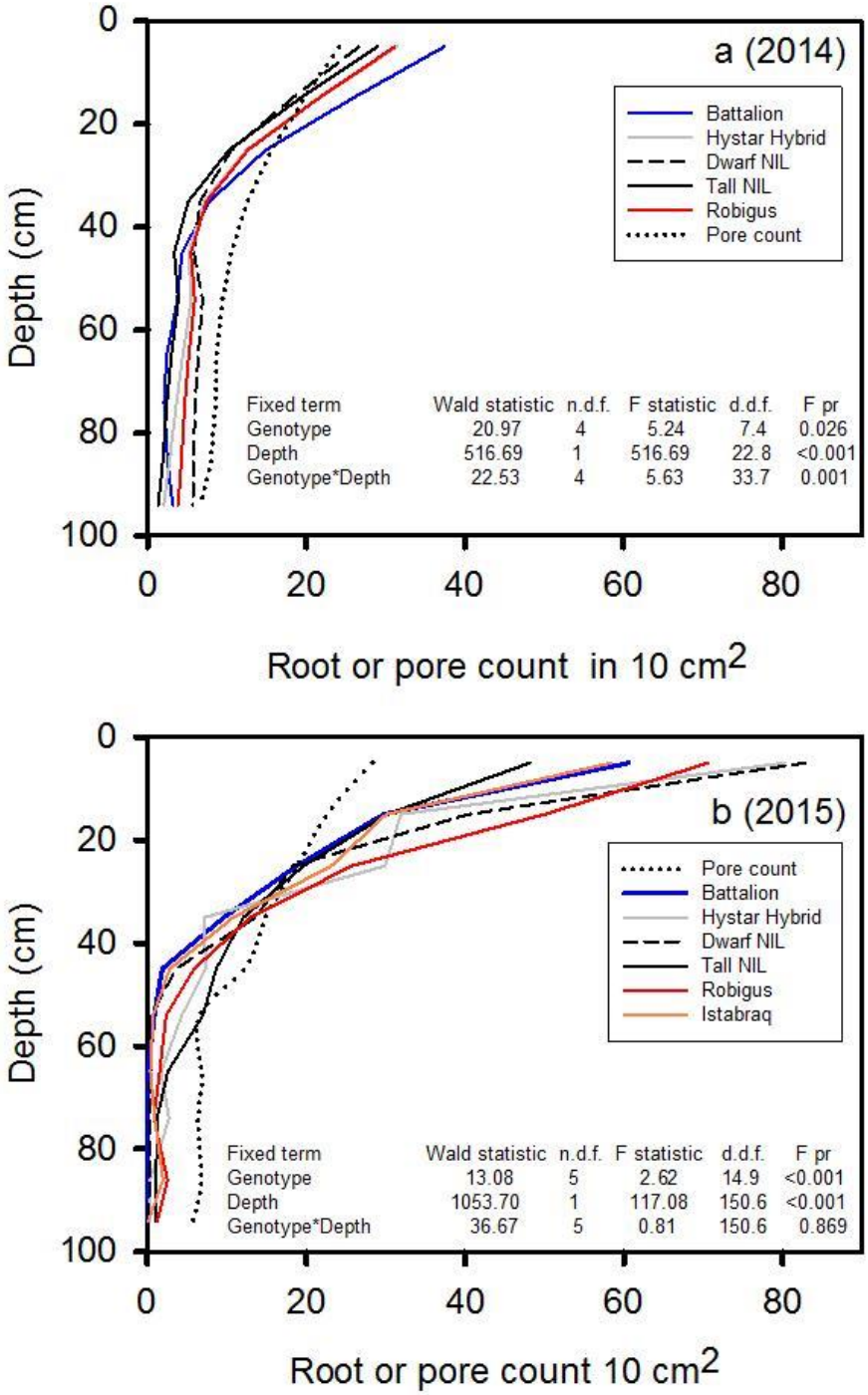


Figure 4.7a, b: The distribution of roots with depth in a) 2014 and b) 2015. These data were obtained with the core break method. A summary of the statistical analyses is also shown. The cores were 10 cm long and the number of roots counted on 10 areas in each 10 cm^2 is numerically equivalent to root length density in cm/cm^3. Output from REML analysis is shown and this applies only to the root data. The data were square root transformed before analysis, so LSDs cannot be presented. The only significant effect of pore count was that of depth in both 2014 and 2015.

In 2014, separate slopes plotting the linear trend of the decrease in root numbers with depth were needed for each cultivar, because in this year genotype had a significant effect on root length distribution with depth; in 2015 this significant effect was not observed and so the same slope across all cultivars was sufficient (see Table 4.2). Genotype had a significant main effect in 2014 (p-value = 0.026) and 2015 (p-value < 0.001) on the number of roots counted overall. *Bat* had many more roots in the surface layer than the other genotypes, while *Dwarf* had the greater number of roots at depth.

Table 4.2. Slopes for 2014 for each line and the common slope for 2015 on the square root scale (the rate of change per cm of depth). These data show that the root length distribution with depth in 2014 depended on genotype, but not in 2015. In 2014, the smallest negative slope for *Dwarf,* shows that it had the deeper rooting habit, while investing less in shallow roots (see also Figure 4.7). The more negative slope for 2015 in comparison with the slopes for 2014 reflects the shallower rooting in 2015.

Genotype	**Slope**	**Standard error**
Bat	-0.05314	
Hys	-0.04477	
Dwarf NIL	-0.02588	0.004450
Tall NIL	-0.04584	
Rob	-0.03709	
2015	-0.07888	0.003728

There were no significant effects of genotype on the number of soil pores counted (p-value = 0.072 in 2015 and p-value = 0.212 in 2014) nor was there any effect of the interaction between genotype and depth on pore count (p-value = 0.898 in 2015 and p-value = 0.098 in 2014). There was no reason to expect that genotype should affect pore count. However, these data suggested that the method used to break the cores did

not result in empty root channels due to roots being pulled out of the soil, to the extent that the inferences drawn on pore count data were affected. The distribution of pore counts with depth were similar on both Broadmead in 2014 and Warren Field in 2015.

The root washing method found no significant effects of genotype on root length density, determined by washing roots out of the 10 cm long cores in either 2014 or in 2015 (Figure 4.8). However, data from both root washing and core break methods (compare Figure 4.7 and Figure 4.8) showed similar differences between years (i.e. shallower rooting in 2015 and deeper rooting in 2014).

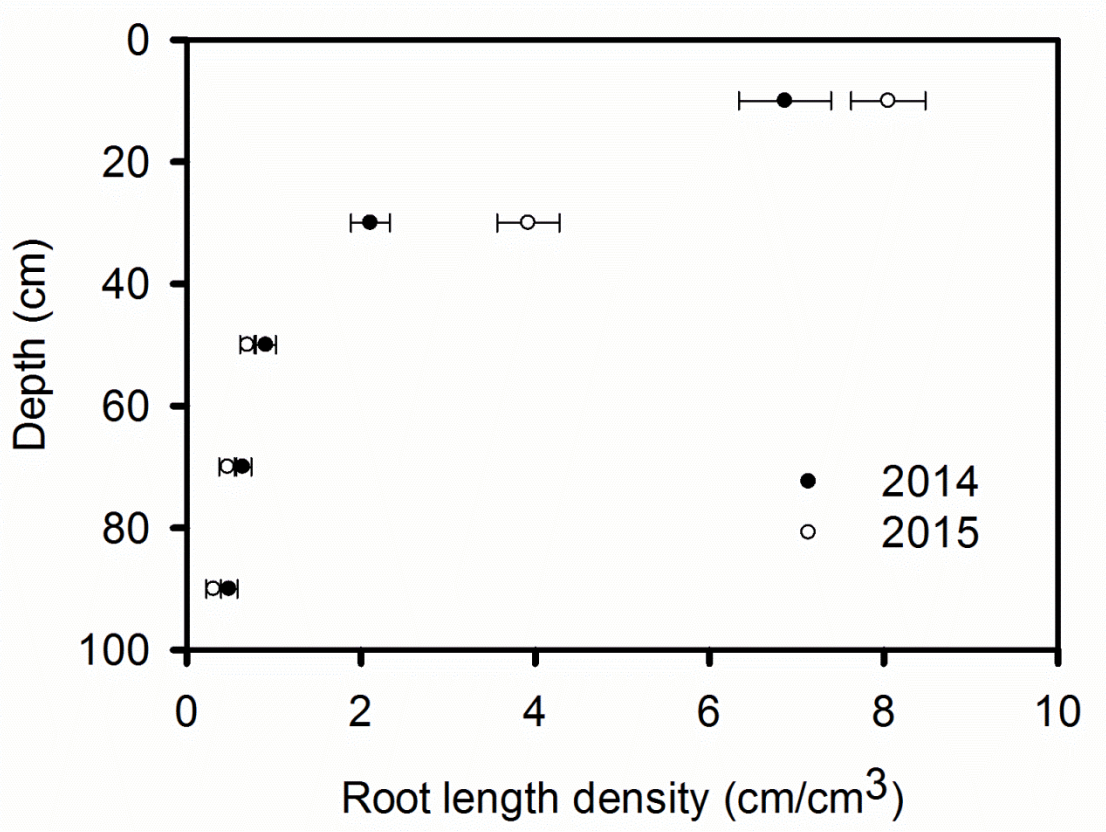


Figure 4.8: Root length density profiles in 2014 and 2015 determined from the root washing method. There was no significant effect of genotype in either 2014 or 2015. The SE of the means is shown for 3 replicates in 2014 and four replicates in 2015.

There were no significant genotypic effects on the proportion of roots found growing within pores compared to those growing through bulk soil (p-value = 0.131), nor did the proportion of roots growing within pores change significantly with depth (p-value = 0.778). A two-way ANOVA found no interaction between genotype and depth on percentage of roots found growing within soil pores (p-value = 0.183). Although there was a high level of variation between genotypes and depths (Figure 4.9), there are clearly many roots growing outside of pores in the bulk soil. Averaged across all genotypes and depths, 60% of roots were observed growing outside of pores.

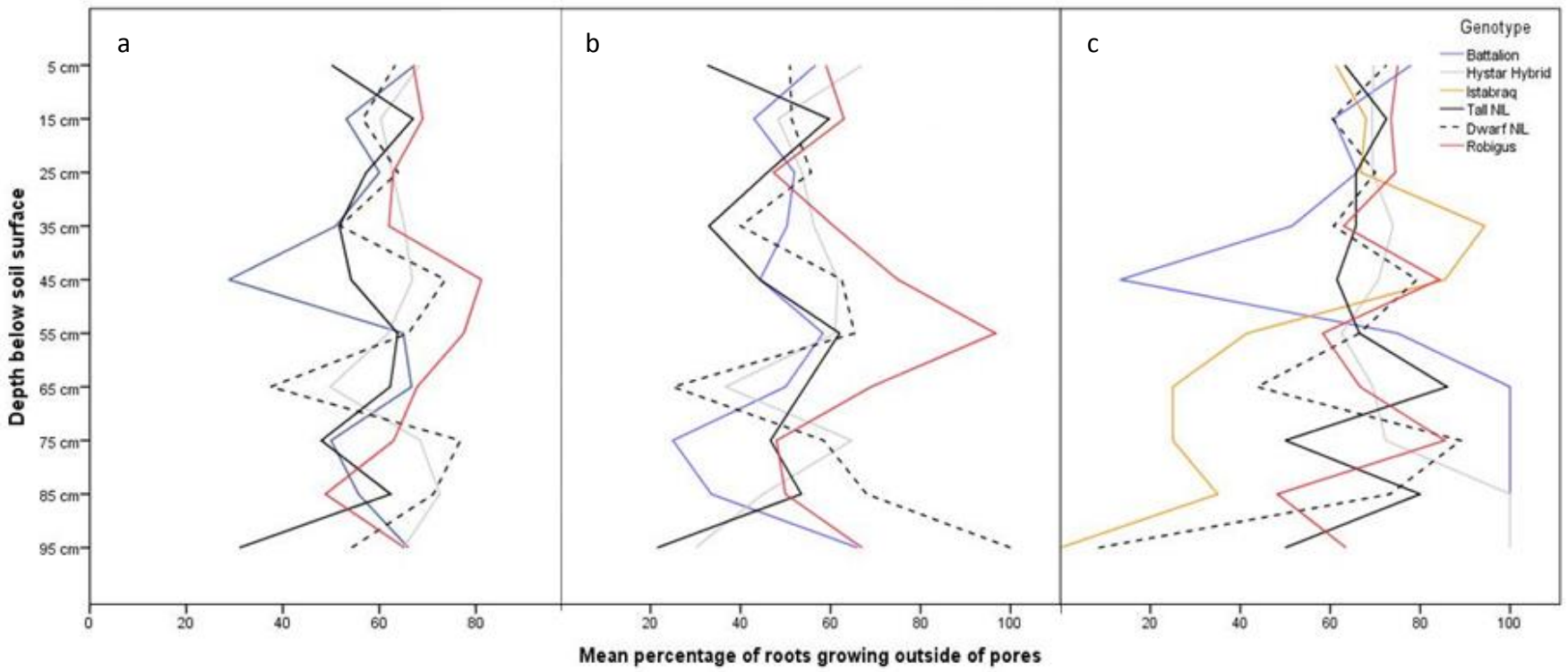


Figure 4.9a, b, c: Percentage of counted roots observed growing in bulk soil, outside of pores, in a) both years combined, b) 2014, and c) 2015, as counted during the core break method. Plotted data are the mean average of 7, 3, and 4 cores per genotype, respectively. Two-way ANOVA for percentage of pore occupancy by roots found significant differences between genotypes (p-value = 0.048) but not depth intervals (p-value = 0.357), nor was there a genotype*depth interaction (p-value = 0.183). SE not plotted because cores with no observed roots made calculating SE impossible at some depths.

4.3.4 Shoot growth and yield

LAI of all the genotypes were similar within each year but differed between 2014 and 2015 (Figure 4.10). In 2015 LAI increased over time, but at a slower rate than in 2014. In 2014, LAI peaked in mid-June for all genotypes except *Rob*, for which LAI had already started to decline, related to senescence. *Rob* is susceptible to yellow rust (*Puccinia striiformis*) and disease pressure was high in 2014.

Crop height was measured in 2015 (Table 4.3) but not 2014; visual observation in 2014 reflects the crop heights presented in Table 4.3, especially the small height of *Dwarf* in comparison with all other lines. Except for *Dwarf*, the yield was higher in 2014 than 2015 (Figure 4.11). Crop heights and LAI provided an estimate of canopy cover and health of the wheat plots.

Yield did not correlate with seedling root angle or mature rooting depth, for either year. In the wetter year (2015), when it was theorised a shallow rooting genotype like *Ist* might be better off than deep rooters, the yield was significantly lower than in the drier year that preceded it (Figure 4.11), and indeed the percentage yield drop was greater for *Ist* (-23.6%) than the average genotype (-15.4%). *Dwarf* was the only genotype to show an increase in yield in 2015 (+31.7%).

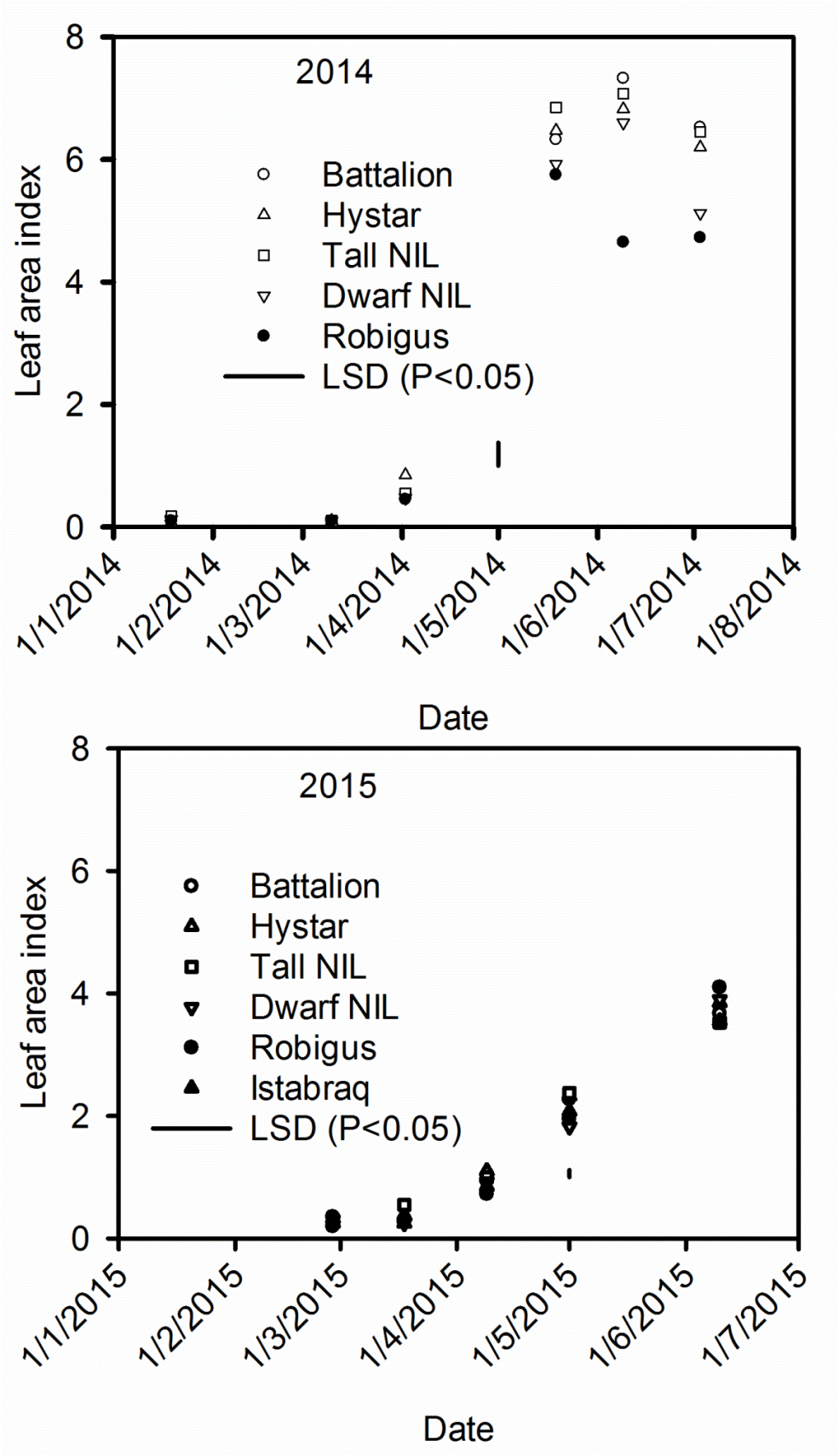


Figure 4.10: Leaf area index estimated with a ceptopmeter. Symbols represent means of four replicate plots.

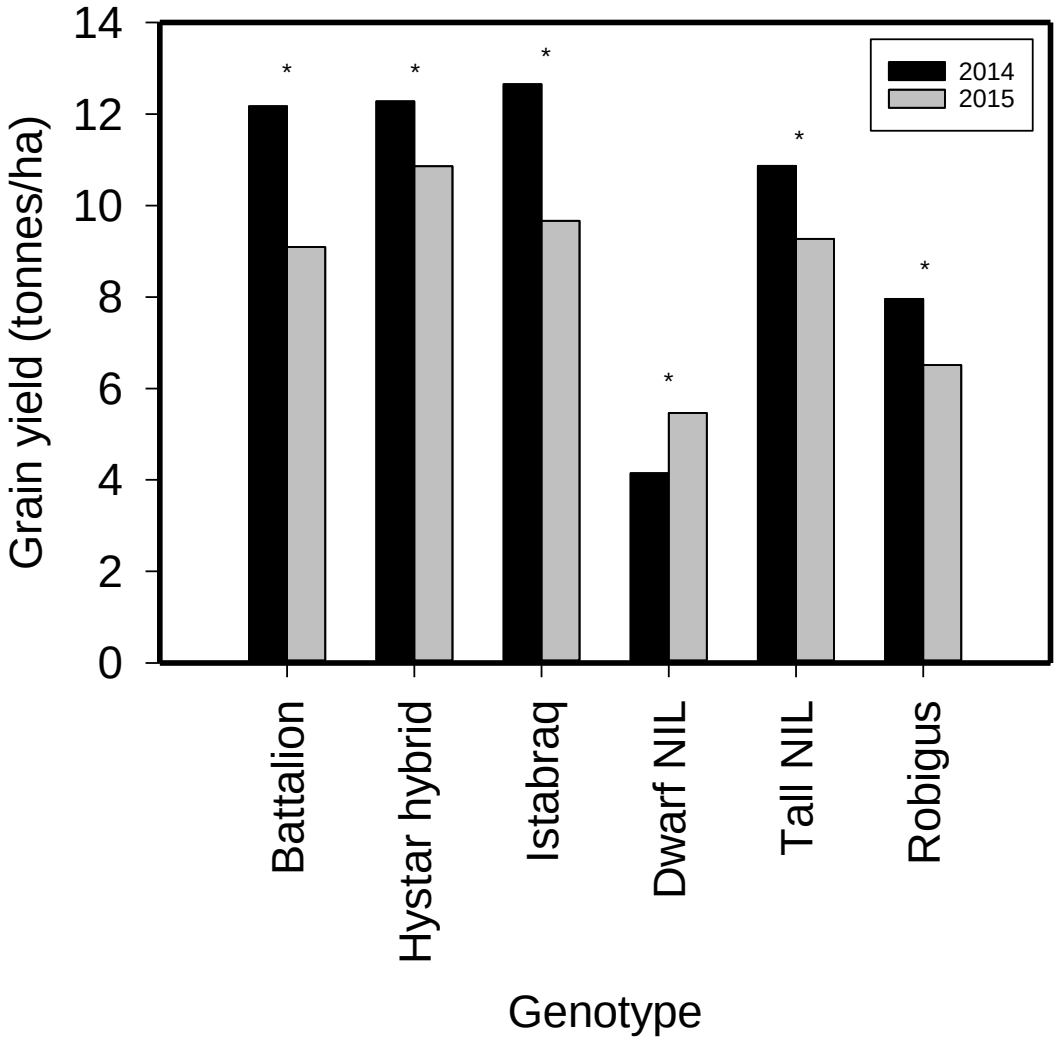


Figure 4.11: Yield data for 2014 and 2015. Bars represent the mean of four replications. The SED values for 2014 and 2015 were 0.54 and 0.89 Tonnes/ha respectively. * indicates significant differences in yield between years, p-value < 0.001 in all cases.

Table 4.3: Crop height measured on 19/06/15. Data are means ± SE of 4 replicates. No statistical analysis, data illustrates similarity to other wheat cultivars (Addisu *et al*. 2010).

Genotype	**Mean crop height (cm)**
Bat	73.1 ± 0.32
Hys	80.3 ± 1.32
Ist	80.3 ± 0.51
Dwarf NIL	44.4 ± 0.61
Tall NIL	80.8 ± 1.44
Rob	74.4 ± 1.43

4.4 Discussion

4.4.1 Why does root length density decline with depth?

In rhizotrons, wheat plants can have very high root length densities at depth (Gao *et al*. 2016, Manschadi *et al*. 2008). In contrast, when soil cores were taken from the field to estimate root distribution and the wheat was at heading stage (when root dry weight is reportedly at or near its maximum and root length distribution with depth has reached a steady state (Gregory *et al*., 1978a)), the root length density decreases rapidly with depth (Figures 4.7 and 4.8). Indeed, empirical root length density models are based on this relationship (e.g. Gerwitz and Page 1974, Fan *et al*. 2016) and similar root length distributions have been reported in field-grown winter wheat (Gregory *et al*. 1978a, Lupton *et al*. 1974; White *et al*. 2015). At depths below 40 cm when the soil is well-watered (Figure 4.1), or below 20 cm following soil drying by transpiration (Figure 4.2), penetrometer resistance exceeds 2.5 MPa, a value associated with very low rates of root elongation for maize (*Zea mays* L.) (Bengough and Mullins 1991), cotton (Taylor *et al*.1966), barley and wheat (Martino and Shaykewich 1994). Below 35 cm, the root length density is greatly reduced compared with the surface layer (Figure 4.8). The reduction in root length density with depth is likely a response to increasing penetrometer resistance (Figure 4.1). At a depth of approximately 35 cm, the numbers of root and pores are comparable (Figure 4.7). In deeper layers, there are more pores than roots whereas in the surface layers there are more roots than pores (although it should be noted that there appeared to be fewer pores at depth in cores with a sandy consistency, and as such this finding might not hold true across different soil types).

The relationship between rooting and soil pores at depth is hard to interpret. One definitive result is that at depth there are many empty pores. An extreme example is for one of the *Hys* cores where at a depth of 95 cm there was one root and 20 empty pores. At first glance, the limited number of roots at depth compared to the number of pores appear to support the conclusion of White and Kirkegaard (2010) that at depth roots are confined to pores. Penetrometer readings (Figure 4.2) suggest there should be no roots growing in bulk soil 20 – 30 cm below the soil surface, although in Canadian field trials wheat roots were able to grow into soil with mechanical impedance greater than 2 MPa, possibly by making use of biochannels and spatial and temporal heterogeneity in the soil structure (Martino and Shaykewich 1994). However, Figure 4.9 shows that roots were seen outside of pores, below 30 cm, for all cultivars. *Ist* is the only genotype to display the expected decrease in root growth through bulk soil, but even in this genotype roots are equally likely to be observed in bulk soil as soil pores up until 50 cm beneath the soil surface (Figure 4.9).

Since roots can expand in pre-existing pores to fill them, inspection of photographs is inconclusive and depends on the threshold size used to define pores. For this study, a pore size of 0.7 mm diameter was used because most wheat roots are smaller than this (e.g. Jin *et al*. 2015). However, roots can elongate in pores smaller than the nominal root diameter with relative ease, because their elongation rate is most sensitive to axial pressure and not the radial confinement that would be applied by pores (Bengough 2012, Bengough and Mackenzie, 1994, Jin *et al*. 2013). Thus, observing a root that is in intimate contact with soil cannot be taken as evidence of root penetration by deformation. It is unlikely that field studies alone will be able to determine whether deep rooting can be achieved by soil deformation. Given the increased soil strength at

depth, it does seem improbable. At depths below the first 30 cm, the number of roots in each broken core face decreased rapidly. While the average percentage of roots observed outside of pores remains fairly stable between both genotypes and depths at between 50 – 60 % of roots growing in bulk soil, in core faces from deeper in the soil profile this may equate to only one or two roots due to low total root counts. With fewer roots to observe, this makes it harder (both observationally and statistically) to distinguish roots that have grown in bulk soil and those that have grown into pre-existing pores and expanded.

A reduction in pore density with depth was also found in Australia (White and Kirkegaard, 2010) for pores greater than 0.2 mm in diameter and Germany (Athmann *et al*. 2013) for pores greater the 5 mm in diameter. In addition to fewer pores at depth, an incomplete level of pore occupation by roots at depth (Figure 4.7) contributes to a sparse root length density in deep soil layers. In this respect, the data are consistent with those published by White and Kirkegaard (2010) for Australian grown wheat (5% of pores contained roots at a depth of 1 m) and for barley grown in Germany (Athmann *et al*. 2013) (85% of pores contained roots at a depth of 1 m). Part of the reason for the low level of pore occupancy was likely due to high levels of root clustering, with 57% of roots in the subsoil sharing a pore with at least one other root (White and Kirkegaard 2010); while the higher level of pore occupancy by German barley may be due to the observed fibrous root system, with many thin, vertical roots growing into pores (Athmann *et al*. 2013). The larger pore size of the barley study also increases the likelihood of a root growing into that pore, relative to the smaller pore sizes investigated in the wheat study. While White and Kirkegaard (2010) justified their counting of several pores of different class sizes, from 0.2 mm to

over 1 mm, Athmann *et al*. (2013) do not explain their decision to consider large pores with a diameter of >5 mm.

Although all available pores were rarely completely occupied at depth, there was some limited evidence for root clustering in subsoil core sections; 25% of cores from 2014 were found to contain at least one pore with more than one root growing in it at depths below 50 cm, while in 2015 20% of cores contained pores exploited by multiple roots below 50 cm. The only genotype for which root clustering was not observed was *Ist*; the other genotypes had almost equal likelihood of having two or more roots sharing a pore at depth. Figure 4.7 does not suggest that *Ist* had fewer roots at depth than any other genotype, and Figure 4.9 shows that *Ist* was unusual in being the only genotype to have almost all roots deep in the soil profile growing within pores; so, the reason for why *Ist* is the only genotype not observed to have multiple roots sharing pores is not immediately apparent. The root counts do not suggest that *Ist* differs significantly to other genotypes in rooting habits, so it may be assumed that this is purely a chance finding. Regression analysis finds no significant (p-value = 0.997) difference in the number of roots relative to the number of available pores between *Ist* and other genotypes, although unlike the other genotypes, soil cores for *Ist* were not taken for 2014. If available, this comparison from another site might have offered further insight into *Ist*'s root clustering behaviour.

4.4.2 Genotypic effects

There was a significant interaction between genotype and depth in 2014, but not in 2015 (Figure 4.7, Table 4.2). In 2014, the genotypic differences in root length

distribution are correlated with soil water measurements in the surface layer. *Bat*, with more surface roots, is one of the most effective wheats at drying the upper soil layers. In contrast, *Dwarf* had the fewest surface roots and was less effective at drying the surface soil. In the top 25 cm, the root count explained 76% of the variation in soil drying measured with the neutron probe (p-value < 0.003). In 2015, there were no significant effects of genotype on either water uptake or on root distribution. Root count data shows that there were more roots at depth in 2014 compared with 2015 (Figure 4.7), which is supported by root length data (Figure 4.8) as well as the finding of deeper soil drying in 2014 (Figure 4.5). These data support the use of soil water content measurements as proxy for root length as previously discussed (Wasson *et al*. 2012). However, at depth there was limited water uptake from any of the genotypes studied (see also Whalley *et al*. 2017).

A large number of roots compared with the number of pores in the surface layers for some genotypes, particularly *Bat* (Figure 4.7a), suggests improved root proliferation in the non-structured pore space (i.e. soil where pores are smaller than 0.7 mm). This could either be due to a greater number of roots or the roots are better at deforming soil. In a laboratory screen, which grew wheat in sand columns with thin layers of wax impeding root growth (Whalley *et al*. 2013), *Bat* had more roots than all the other wheats studied here, except *Hys* which was not included in the laboratory screen. *Bat* was also better at penetrating strong layers, which has been confirmed by subsequent investigations (unpublished data of Whalley).

While the numbers of roots at depth for all wheats is small in 2014, *Dwarf* had a greater number of roots at 95 cm than *Tall*. In fact, they are the extremes, with *Tall*

having the fewest deep roots. One possible explanation for the deeper rooting of *Dwarf* compared to *Tall* in 2014 (Figure 4.7) is the finding and exploitation of pores is related to the number of roots. At 95 cm, 85% of pores were filled by *Dwarf* roots but only 20% for *Tall* in 2014 (2014, Figure 4.7). Laboratory studies have shown that *Tall* has fewer root axes compared with *Dwarf* when grown in a low impedance environment (Coelho Filho *et al*. 2013). When the substrate impeded growth, the number of roots was similar for both NILs, and indeed in the surface layer of the field both NILs have a similar number of roots (Figure 4.7a). A greater number of roots might explain a greater likelihood of pore location (Hewitt and Dexter 1979). Moreover, dwarf wheats generally have a greater root mass and length in comparison with taller wheats (Miralles et al. 1997), as observed here (Figure 4.7a).

The greater root density at depth of the dwarf wheat was not reflected in greater water uptake. This is possibly because even for *Dwarf* the root density at depth was low. Although the LAI is not affected by dwarfing genes (Figure 4.9), the shorter canopy of *Dwarf* (compared with all the other wheats we studied) made comparisons of water uptake more difficult (Table 4.3). Below 40 cm depth, the root length density of all lines does not change greatly with depth in both 2014 and 2015 (Figures 4.7 and 4.8). However, in the same depth interval (40 cm to 95 cm) water uptake by roots depends strongly on depth (compare Figures 4.4 and 4.5 with Figures 4.7 and 4.8). It seems that in this region, the ability of the roots to dry soil is only weakly related to root length density. A possible explanation is that with increasing depth, a greater proportion of roots are found in pores and hence root orientation varies with depth. At first this seems contradictory of the data in Figure 4.9, which suggests that up to approximately half of all roots found at depth are growing in bulk soil. However, it is

possible that these were roots growing into small cracks and pores, that then expanded to fill the pore space, hiding the pore during the core break analysis.

The growth of roots in pores versus bulk soils seems important in determining water uptake, since the ability of roots to dry soil depends also on their geometrical arrangement in soil, irrespective of soil to root contact (Passioura 1991). Vertical roots, which probably occur increasingly in the deeper layers, provide the least effective geometry for enabling roots to dry soil. A further complication is poor contact between roots in pores and the bulk soil (e.g. White and Kirkegaard. 2010). It is widely reported that flux of water through bulk soil does not appear to explain poor water uptake (Deery *et al*. 2013a; b) and radial hydraulic resistance between the root and soil is thought to limit water uptake (e.g. Herkelrath *et al*. 1977). From the results of this chapter, it is difficult to make a case for using water uptake to measure differences in root zone shape and size, at least in damper British soils. However, measuring water uptake from different parts of the soil profile may still be of value in drier regions, were improved access to even small amounts of water can have a dramatic impact on crop performance (Kirkegaard *et al*. 2007).

In contrast to root length densities estimated from the core break method, root washing showed no genotypic effects in either 2014 or 2015 (Figure 4.8). The main effects of year were similar with data obtained from both methods (Figures 4.7 and 4.8), although magnified in the data from the core-break method. It is difficult to be certain why the core break method discriminated differences between genotypes with respect to root growth in 2014 while the root washing method did not. It is possible that some roots were lost in the root washing method, which made it less sensitive.

Alternatively, the root core break method may have been less effective at detecting horizontal roots branched from the vertical axis.

The genotypes chosen for this trial were mostly selected from lists of high performing cultivars, already popular commercial choices for UK wheat crops. From the two years studied it appears that climate had a greater impact on yields than genotype (with the expected exception of *Dwarf*). A potential explanation for the relative lack of genotypic variation in yield may be that the root systems for these cultivars are all similarly adapted to take up adequate water under UK growing conditions (perhaps assuming no severe droughts), and therefore there is no further scope for root system improvement, at least not for this region, for these cultivars (Richards *et al*. 2002). There is no conclusive evidence that any of the examined genotypes are better at exploiting water at depth (Figure 4.5). However, this appears to be dependent on soil moisture conditions in the given growing season – in 2014, the profile of soil water extraction under wheat plots was shallower than in 2015, reflecting higher rainfall, and reduced need for deep stored water.

One the aims of this field trial was to establish if there was any consistency between genotypic seedling root angles measured in the laboratory, and patterns of root growth in the field. The inconsistency of genotypic differences in rooting patterns between the two field sites, and the significant environment effects, makes drawing any comparisons between the results presented Chapter 2 and Chapter 4 difficult. However, it is interesting to speculate on two key points that have been previously related to either laboratory trials or arid climate field studies:

1) *Bat*, identified as a consistent deep rooter in Chapter 2 and Whalley *et al.* 2013, and also identified as being better able to penetrate wax layers than some other cultivars, had the highest root counts of all cultivars in shallow soil layers. This is contrary to the main hypothesis that deep rooting seedlings will have more roots at depth, but does suggest the alternative hypothesis that deep rooting seedlings are better at soil penetration, thus increasing the root length of *Bat* in the top layers of the cores to be encountered and counted.

2) *Ist*, identified as an unusually shallow rooter in Chapter 2, was the only cultivar not found growing outside of pores at depth in the soil cores in 2015 (Figure 4.9). It would have been enlightening to make a comparison with another year of data for cores taken from *Ist* plots, but these were not available. However, from the data presented here, it could be hypothesised that roots growing at a shallower angle have a reduced ability to penetrate strong soils, so to reach deeper layers of the soil profile shallow root systems are dependent on exploiting pre-existing cracks and bio-pores.

It was not possible to incorporate basket pots into the field soil at the point of seed drilling, nor is shovelomics (digging up the soil crown to phenotype surface root traits) as effective for wheat as it is for thicker-rooted cereals like maize (Trachsel *et al*. 2011). Therefore, there is little direct evidence from these field studies to support the original hypothesis that seedling root angle directly influences root system depth; however, the two points above in combination provide some circumstantial evidence in favour of an alternative hypothesis: that genotypic differences in seedling root angle influence how easily roots are able to penetrate structured field soils. The data from

this trial (see also Whalley *et al*. 2013) suggest that genotypes with steeper seedling rooting angles find it easier to penetrate hard soil layers than genotypes with shallow seedling root angles. Another study investigating the responses of maize roots to hitting hard layers and objects found that the steeper the angle of growth before colliding with an obstacle, the less the root's direction of growth would be deflected once it cleared the obstacle that had interfered with its original trajectory (Bandara and Fritton 1986).

4.4.3 Differences between 2014 and 2015

Large differences in the root length distribution between 2014 and 2015 (Figure 4.7) are likely related to differences in the saturation of the soil profile (Figure 4.3). In 2015 there was limited soil drying until the beginning of April. It is possible the shallower rooting in 2015 compared with 2014 is related to limited oxygen availability due to higher levels of soil saturation (Gliński and Stępniewski, 1986; Blackwell and Wells 1983). Rainfall is unlikely to explain the wetter conditions in 2015, as accumulated rainfall between sowing and 1st April in 2014 and 2015 was 490 mm and 374 mm respectively. The most likely explanation for the wetter soil profile in early 2015 is differences in drainage between the adjacent fields.

When grown in the field (Thorup-Kristensen *et al*. 2009, Fan *et al*. 2016, Gao *et al*. 2016) or in rhizotrons (Manschadi *et al*. 2008, Jin *et al*. 2015), wheat roots can grow to depths of 100 cm or more, as observed in 2014. However, shallow rooting depths (< 60 cm) for wheat, as in 2015 (Figure 4.7b), are also commonly reported when a water table is present, thus reducing the depth of the soil profile (e.g. Xue *et al*. 2003,

Brisson *et al*. 2002). Wheat root growth is greatly affected by the presence of a water table, and a shallow water table (approximately 60 cm deep) limited root growth below 40 cm at 38 days after sowing (Zuo *et al*. 2006).

4.4.4 Relationship between yield, shoot growth, and rooting

The lower yield in 2015 (Figure 4.11), except for *Dwarf*, is consistent with a smaller LAI (Figure 4.10) and a shallower root system (Figures 4.7 and 4.8). Although the dwarf phenotype of Rht-B1c (*Dwarf*) was observed (Table 4.3) the effect on LAI was minimal, although in 2014 it was somewhat smaller than *Tall* and all other wheats (except *Rob* which had yellow rust). Although *Tall* does not contain the dwarfing gene, in a Mercia background the Tall *Rht* allele is comparable in height to the commercial semi-dwarf lines (Table 4.3). Yield is more closely related to plant height than to the particular allelic dwarfing nature (Addisu *et al*. 2010). The optimum plant height for a maximum yield is approximately 80 cm (Addisu *et al*. 2010); this is comparable to the height of the studied lines (Table 4.3), with the exception of *Dwarf*. At this height, harvest index, interception of photosynthetically active radiation and radiation use efficiency are maximised, and the risk of stem lodging is reduced. The effect of *Rht* genes on yield is related to relatively complex pleiotropic effects on spike fertility, grain number and grain size; including an interaction between grain number and size (Youssefain *et al*. 1992; Flintham *et al*. 1997). This is consistent with the observation the extreme dwarf NIL has sufficient rooting (Figure 4.7) and leaf area (Figure 4.10) to capture water, nutrients and light when compared with the other genotypes in this study.

4.5 Conclusions

The evidence presented in this chapter supports the hypothesis that the general and well-documented shape of the relationship between root length density and soil depth in UK grown winter wheat is related to the increase in soil strength with depth, and both the distribution of root-sized bio-pores with depth and/or the ability of roots to locate them. In the two years of this study:

- Effects of the soil environment on root length distribution were greater than genetic effects, consistent with previous trials conducted on six different field environments (at three separate sites) in Australia, which found that environment and environment x genotype interaction accounted for 48% and 40% respectively of the sum of squares for rooting depth among 24 diverse wheat cultivars.

- Genotypic effects in rooting density and soil drying were found in the driest of the two years: *Bat* had more roots in the surface layers than other genotypes, and the surface layers under *Bat* experiencing greater soil drying, whereas *Dwarf* had fewer surface layer roots than other genotypes and the least surface layer drying.

- Data from the *Tall* and *Dwarf* NILs suggests that deep rooting may be inversely related to the number of roots in soil surface layers (Figure 4.7), rather than genetic differences in seminal root angle (Fig. 2.15), as the

genotypes with fewest roots counted in the surface soil layers show a greater number of roots at depth, in both years.

Chapter 5: Discussion and Conclusions

5.1 General Discussion

The study of the potential agricultural benefits of roots to crop species has long been of interest (Weaver 1926). However, studying root traits in the field has always presented difficulties, and in the laboratory it has been necessary to establish which (ideally easily measured) root traits can be used as proxy variables. Proxies are needed to estimate variables of interest, such as rooting depth (Manschadi *et al.* 2008), horizontal root system spread (Richard *et al*. 2015), or lateral branching habits (Lamb *et al*. 2000). This study expands on previous work, which established that seedling root angle could predict rooting depth and root length density in Australian wheat cultivars (Manschadi *et al*. 2008), by determining whether UK wheat cultivars also show variation in seedling root angle (Chapter 2) and whether it was physiologically important (Chapters 3, 4).

Variation in root angle has already been established in wheat cultivars from diverse regions of the world, including Japan (Oyanagi 1994) and Australia (Manschadi *et al*. 2006), and in Mediterranean and North American durum wheats (Sanguineti *et al*. 2007). When this study commenced, there was little information on root angle spread among UK wheat varieties. Nevertheless, a study that demonstrated differences in the ability of selected UK, CIMMYT, and dwarf gene NIL wheat cultivars to penetrate thin wax layers also recorded variation in angular spread of these cultivars (Whalley et al. 2013). This study first highlighted that *Bat* and *Rob* had significant differences in seedling root angle (see also Chapter 2, Figure 2.8), but there was little variation in the

ability of wheat varieties to penetrate strong wax layers, due to lack of genotypic variation in root diameter. However, other studies have positively correlated the ability to penetrate hard soil layers with angle of root growth, with steeper roots showing greater ability to penetrate hard layers (Kubo *et al*. 2006, Lynch 2013). Steeper root angles may also help prevent inter-plant competition in densely planted field crops (Lynch 2013). Thus, studies such as Whalley *et al*. (2013) and the work in Chapter 2 may help reveal useful genotypes and genetic material for developing cultivars capable of growing on compacted land, as the ability to penetrate deeper into the soil profile to access stored soil water can benefit crop yields, even in the UK's temperate climate (Dodd *et al*. 2011).

Seedling root angle has long been proposed to positively correlate with adult plant root angle and rooting depth, such that a steep seedling root angle is now used as a proxy indicator of a deep rooting cultivar (Oyanagi 1994, Manschadi *et al*. 2008, Manschadi e*t al*. 2010). There are other traits that have been correlated with deep rooting, including a 'vigorous' fast-growing root system which accesses deeper layers of the soil profile more rapidly (Palta *et al*. 2011), and increasing the time period between germination and flowering, granting the root system more time to develop and access water before the grain-filling stage (Richards 2006). Whichever trait is under study, it is a recurrent oversight that the potential benefits of deep rooting are poorly explored outside of regions prone to terminal droughts. In any region where there is residual water available after grain-filling, selecting for deep rooting may increase water uptake and therefore yield (Richards *et al*. 2002). When high-performing UK cultivars were grown in the south east of the country under optimal conditions, there was unextracted water in the soil profile after harvest below 100 cm

in 2014 and 80 cm in 2015 (Figures 4.4 and 4.5). Thus, greater root exploration of these deep layers of the soil profile may enhance yield of UK wheat cultivars.

Establishing a healthy root system is vital to healthy crop development, successful growth, and good yields (Weaver 1926). Whereas the importance of deep rooting to the mature crop is widely discussed and has been demonstrated through both modelling (Semenov *et al*. 2009, Manschadi *et al*. 2006) and experiments (Kirkegaard *et al*. 2007, Manschadi *et al*. 2008), there has been scant focus on the potential benefits of rooting angle on the young seedling and developing plant. This is surprising since roots play key roles in anchoring, water uptake, nutrient foraging, and phytohormone synthesis (Schiefelbein and Benfey 1991, Hodge 2009), and these processes are vital at all stages of growth (Tian *et al*. 2014, Andresen *et al*. 2016).

Roots in drying soil synthesise the 'drought hormone' ABA, which is believed to be transported to the shoots and leaves, causing stomata to close thereby maintaining plant water potential under water-limited conditions (Wilkinson and Davies 2002). Plants with roots in the upper soil layers are more prone to having those roots experience drying conditions, due to higher rates of evaporation (Saradadevi *et al*. 2016). Therefore, root positioning in soil may be very important, even in younger plants. Chapter 3 tested this theory by growing wheat cultivars with contrasting shallow and deep seedling root angles in large pots with distinct vertical soil moisture gradients. The hypothesis was that *Ist* (shallow root angle) would grow better than *Bat* (deep root angle) in a soil moisture gradient where the surface was wetter than the base of the pot; conversely, *Bat* would grow better when the pots were wetter towards the base. Analysis of leaf ABA concentration revealed that genotype (seedling root

angle) did not significantly affect foliar ABA levels, but both watering location and watering availability did significantly influence leaf ABA levels (Table 3.3).

The only consistent difference between genotypes in Chapter 3 was *Ist* having higher average stomatal conductance than *Bat*. This could not be linked to seminal root angle phenotype; if root angle were responsible, then it would have been expected that there would also have been corresponding changes in leaf ABA and Ψ_{leaf}. An alternative hypothesis was put forwards to explain the higher stomatal conductance in *Ist*: *Ist* has higher numbers of stomata per unit area than *Bat* (Franks *et al*. 2009). Taking leaf impressions and counting stomata under a microscope confirmed that *Ist* has significantly (p-value = 0.002) more stomata per unit leaf area, approximately 19% more, than *Bat*. When phenotyping and investigating root traits it is still important to look at the physiology and morphology of the whole plant, to put the results in proper context.

The other notable observation raised by Chapter 3 is the apparent divergence of water deficit responses between the two cultivars. Raised ABA levels and decreased stomatal conductance in *Bat,* to some of the treatment combinations, suggest isohydric behaviour by closely regulating stomatal opening to conserve plant water status. On the other hand, *Ist* does not show significant increases in ABA even under soil drying, but does show significantly reduced Ψ_{leaf} under soil drying, which is more typical of an anisohydric response (Gallé *et al*. 2013). It could be hypothesised that plant species or cultivars with shallower root systems (like *Ist*) would tend towards anisohydric behaviour, otherwise accumulation of ABA through roots being surrounded by drying

soil could inhibit aboveground plant growth. However, there is insufficient data from the experiments described in Chapter 3 to do any more than speculate on this.

Using rooting depth as a parameter in computer simulation models has provided evidence of the potential to increase yield of UK wheat cultivars (King *et al*. 2003, Semenov *et al*. 2009). In the UK climate, there would be small positive effects from doubling root vertical extension rate; mean yields would increase by 0.6% due to increased water uptake (Semenov et al. 2009). Decreasing the vertical extension rate had greater influence even under only mild drought, because the roots failed to grow sufficient length to access deep soil water. The vertical profile of root length density is important; since having proportionally more roots in the upper layer of soil profile, while having few at depth, may predispose the crop to greater reliance on future rainfall to ensure optimal water uptake (Dardanelli *et al*. 1997). A more homogenous spread of roots in the soil profile allows access to deep stored water and results in more efficient water uptake (while also being theorised to optimise nutrient uptake (King *et al*. (2003)). In the UK, this difference in water uptake is minimal compared to the difference it makes for Australian cultivars; however, it still exists and could be a trait to take into consideration when looking for future breeding goals.

This is especially pertinent when considering the likely future weather patterns under climate change, as it is predicted that patterns of rainfall are going to vary dramatically between the north and south of the UK (Ekström *et al*. 2005), and thus the effect of the spread of roots in the soil profile on crop yields could be important in terms of crop adaptation and yields. Scotland and northern England are predicted to experience more frequent severe rainfall events, with Scotland 30% more likely to

experience long lasting (> 10 days) severe rainfall events, whereas Wales and southern England are less likely to experience long lasting severe rainfall events, and have only a very small increase in the likelihood of one-day severe rainfalls (Ekström *et al*. 2005). Perhaps most importantly, these events are expected to occur mostly in autumn and winter (a trend that has already become apparent in the last 60 years and is set to continue (Osborn and Hulme 2002, Jones *et al*. 2013)), when wheat root systems will still be relatively shallow. Less frequent rain events during summer emphasises the importance of access to stored water. Rainfall events are predicted to become more frequent in winter and less frequent in summer, especially in the south of the UK (Jones *et al*. 2013), and so deep roots may become more important in terms of water extraction. Under current rainfall patterns, water is still available for plants beneath ≈ 100 cm in the soil profile (Figure 4.5), however for all the cultivars sampled in Chapter 4, relative root density at these depths was very small (< 3% of roots counted) (Figure 4.8).

Dwarfing genes were once theorised to diminish wheat root length and biomass (Manske and Vlek 2002), but studies conducted after the extensive commercial uptake of dwarf cultivars suggested this was not the case (Lupton *et al*. 1974, Manske 1997, Miralles *et al*. 1997). Similarly, our own field trials found no evidence of the dwarf NIL having less root material at depth than the tall NIL (Figure 4. 7a).

One of the more surprising findings of Chapter 4 was that roots were apparently growing outside of soil biopores at depths below 50 cm, when soil penetrometer resistance exceeds 2.5 MPa. Soil this strong would have been expected to severely slow root growth, even if it did not completely inhibit it (Bengough and Mullins

1990). While the amount of root material at depth was expected to be positively correlated to the number of pores, as roots would be expected to use these to bypass hard layers and access deep soil (Lipiec and Hatano 2003), some roots were found in bulk soil at high soil strengths and not in pre-existing pores (Chapter 4). However, for *Ist* it appears that having pores available is vital for growing any deep roots at all, thus underlining the importance of field management in efficient crop growth. In both Australia (McCallum *et al*. 2004) and Germany (Gaiser *et al*. 2012), growing wheat after a rotation with a deep rooting perennial, usually alfalfa (*Medicago sativa*), improves yields. Water extraction from deeper soil layers is often significantly higher and likely plays a part in these increased yields. It is believed that the alfalfa rotation enhances access to deeper soil layers by improving soil structure and the creation of new deep pores (Gaiser *et al*. 2012). However, it can be difficult to separate the yield effects of soil pores from the other benefits of an alfalfa rotation, such as soil N enrichment and reduced compaction from heavy machinery during pasture growth (McCallum *et al*. 2004).

Aside from utilising pre-existing pores, there are other mechanisms by which roots can be found in even in very strong soils. At least under field conditions, as in Chapter 4, it is possible for the root system to grow into soils while they are damp, only to have the soil dry out later in the season, presenting a penetrometer reading that does not accurately represent the impedance on the root at time of growth (Clark *et al*. 2003). Also, the way in which a root grows into the soil is different to the way in which a penetrometer will be pushed through the profile (Whalley *et al*. 2005); with the root's ability to exploit patches of higher moisture and pores they can continue to

grow into strong soils, and would not experience friction in the way the penetrometer needle does.

Root interactions with their environment, such as utilisation of pre-existing pores, highlight that while breeding for improved root traits, e.g. rooting depth, may offer the opportunity of increased yield potential, the yields may not be achieved under certain environmental conditions. For example, Wasson *et al*. (2012) describes two hypothetical genotypes, one with deep-growing, highly branched roots, and the other with limited branching that does not grow as deep as the first. Under optimal conditions with good distribution of water and nutrients in the soil, the second genotype may have equal or higher yields since it avoids unnecessary investment in root material. However, water-limiting conditions (for example, a hotter climate reducing the duration of the grain filling phase (Shpiler and Blum 1986, García *et al*. 2016), or inefficient hydraulic regulation of the roots and shoot (Vadez 2014)), may favour the selection of the genotype with the deeper growing, highly branched root system (Wasson *et al*. 2012), with the second genotype suffering a yield decrease through inadequate water supply. The field trials presented in Chapter 4 have shown that in the UK, wheat does not have the requirement for very deep roots to access water in the way that some Australian cultivars would. The cut off depth for water extraction appears to be at approximately 100 cm (Figure 4.3), so using Wasson *et al*.'s (2012) example, we could hypothesise that in wet years, a root system that grew 80 cm deep would be adequate, but in dry years this smaller root system would not provide enough water to a UK grown wheat plant. Therefore, the ideotype rooting depth for UK wheats would be around 100 cm, to account for the possibility of a dry summer.

5.2 Methodological issues

A drawback of the phenotyping method described in Chapter 2 was the difficulty in disentangling the effects of soil moisture and soil strength on seedling root angle. Both can independently affect root angle (Oyanagi *et al*. 1995, Jin *et al*. 2015), and since soil strengthens as it dries (Weaich *et al*. 1992), both biophysical variables (soil strength and matric potential) may interact to determine seedling root angle.

Although the pot experiments of Chapter 3 successfully created soil moisture gradients, water loss from surface-watered pots was greater than from basally-watered pots (Figure 3.5). Basally-watered pots maintained a higher soil water status at depth than pots that were surface-watered maintained at the top. Although all pots were always watered back to their starting weight (except for water withheld treatments), plants in surface-watered pots experienced higher levels of soil drying in the 24-hour watering cycle, with each surface-watered pot required 48% more water, on average, to return to start weight. There are two potential explanations for why the surface watered pots lost more water in each 24-hour cycle. Firstly, watering from the surface made water available for plants to transpire, and the extra water was taken up by roots in the upper section of the pot by the plants. Or secondly, water was lost more readily at the soil surface than from the base of the pot, thus pots receiving water at the surface lose it again at a faster rate than the basally-watered pots. Looking at the data in Figure 3.13, the second theory appears to better explain the average stomatal conductance readings across the eight treatments. Comparing well-watered *Ist* pots that are basally and surface watered, stomatal conductance is significantly lower in the surface-watered treatment; this is the same in the other three pairs of treatment

combinations. The lower stomatal conductance in the surface watered pots suggests that the water has evaporated from the soil, rather than being used by the plants.

This greater loss of soil water from the surface than the base does mean that the gradients created in the pots are not fully reversed; the soil moisture levels at the base of a basally watered, well-watered pot was not equal to the soil moisture content of surface watered, well-watered pot. Nor were the ratios of surface soil water content to base soil water content equivalent between the two water availability treatments as the pots dried. Equivalency would have allowed for a fairer test of the hypothesis, but maintaining it in the pot system used in Chapter 3 was not possible.

Neutron probes measured water extraction at depth in Chapter 4. Dardanelli *et al*. (1997) suggest that measuring water extraction can act as a proxy for measuring root material at depth. Both water extraction (Figure 4.4) and root biomass washed out of soil core samples (Figure 4.7) at different depths showed no genotypic differences, thus these data cannot indicate the relative value of each technique in determining genetic variation.

The limited depth of soil coring precludes a determination of genetic differences in maximum rooting depth. Since all genotypes had roots in the last sampled layer at ~ 95 cm, it can be assumed that genotypes were capable of reaching depths beyond 100 cm in the soils on the Rothamsted Research farm fields. Although total root mass at these depths would be severely limited (and therefore the neutron probes, which were recording changes in water availability to 145 cm, would likely not have detected significant changes through root uptake) it is a possibility that some genotypes could

have grown much deeper than others, but the method used was not able to provide that observation.

5.3 Conclusions and thoughts for future work

In conclusion, this body of work has provided new insights into the interactions between seedling root angle, rooting depth of mature plants, and water uptake in wheat cultivars grown in a temperate climate. Although well researched in arid regions around the globe, these factors have been poorly quantified by physical trials in the UK. Computer models (King *et al*. 2003, Semenov *et al*. 2009) have predicted the potential benefits and drawbacks of deeper root systems for UK cultivars. Model studies have limitations though; to build a workable biological model it is necessary to use assumptions that can sometimes prove too restrictive, for example King *et al*. (2003) had to build their model with the assumption of soil profile homogeneity. Chapter 4 provides evidence that the soil profile is far from homogenous, in terms of soil water content, soil strength and root distribution. By running physical experiments in the field, like those presented in Chapter 4, the gaps in the model systems can begin to be addressed.

The main conclusions of this thesis are:

- There is variation in the 3D seedling root angle of a selection of commercial UK wheat cultivars (Chapter 2). The range of angles was found to be between 35° and 52° from the vertical. In comparison, seminal root angle in a selection of 12 Japanese cultivars ranged from 28° to 46° (Oyanagi 1994), and in a

selection of Australian wheats the range was 36° to 56° (Manschadi *et al*. 2008). The range in seedling root angles in UK wheats is similar to that found in Australian cultivars, and suggests that there is adequate genetic material to select for root angle phenotypes, if they were believed to have the potential to improve yields.

- Some cultivars show notable levels of phenotypic plasticity in root angle under different soil environments, suggesting that a cultivar's ability to grow a root system suited to the local environment is genetically controlled (Chapter 2), raising the potential of highly adaptable, high-yielding genotypes in future breeding programmes.

- Soil moisture gradients have a greater impact on the developing plant than between-cultivar differences (Table 3.3, Chapter 3).

- There is variation in water use behaviours between UK commercial wheat cultivars, with some showing more isohydric behaviours whereas others show water deficit responses more in keeping isohydric behaviours (Chapter 3). Where in-season rainfall is likely, or crops are irrigated, then anisohydric behaviours may increase carbon assimilation without leaving the plant completely depleted of water in the grain filling stage and may, therefore, be more beneficial in wetter soil profiles in the UK than conservative isohydric behaviour.

- Soil pore availability in the soil profile plays a more important role in access to deep strata of the soil profile than genotypic variation in root seedling root angle (Chapter 4).

- Even in a temperate climate, the surface soil layers dry out before anthesis and grain-filling, making access to deeper stored water vital for good yields in rainfed wheat crops (Chapter 4).

Interesting future research questions that could build on the findings of this thesis include:

- Mechanistic studies of root angle plasticity. Root angle is genetically controlled, with species and cultivars having a 'default' angle of growth. However, a broad range of environmental stimuli can trigger a change in the angle of growth, even without a physical touch stimulus (e.g. Oyanagi *et al.* 1995). The active gravitropism response is believed to be triggered by the actions of multiple mechanisms, including auxin gradients in root tips and starch-statolith accumulation in cells (Sato *et al*. 2015). As other stimuli, such as salt concentration, can divert root angle away from the downwards force of gravity, it would be highly interesting to investigate whether these other stimuli interact with the gravity sensing apparatus of roots to change root growth angle.

- While this thesis focuses mostly on the relationships between seedling root angle, water uptake, and yield, in Chapters 2 and 4 it was established that soil

drying almost inevitably also strengthens soil, which in and of itself can cause changes in root angle (Jin *et al*. 2015). Developing an experimental soil-based system that allows for the isolation of these two variables would allow greater understanding of the true impact of each variable on root angle and consequent effects on plant growth, as well as, potentially, deepening mechanistic understanding of how the two interact to bring about changes in root angle.

- No significant differences in yield were observed between commercial wheat cultivars in Chapter 4, despite the cultivars possessing diverse seedling root angle phenotypes. Two of these cultivars were identified as having highly plastic root angle phenotypes (*Ist* and *Rob*), but root angle plasticity is rarely investigated outside of a laboratory setting, and then usually in response to nutrient availability rather than water or soil strength (e.g. Liao *et al*. 2001) It is likely a heritable trait, but what effects it has on plant growth, survival and/or yields remain largely unexplored. Large scale field trials could investigate both the genetics of plasticity, and the impact plasticity can have on mature plants.

In a commercial setting, seedling root angle remains of interest for crop breeders, and new techniques for investigating this will continue to be developed. Comparing the consistency of root angle results across 2D and 3D techniques may establish the usefulness of the different methods. Being able to select for easily identifiable morphological traits is attractive to commercial breeders, especially if that trait can be observed early in the plant's lifespan (Richards *et al*. 2002); this could potentially

reduce the cost and time span of breeding trials, as fewer generations would require growing to crop maturity.

Plant breeders should consider measuring changes in the soil water profile over the course of the growing season to identify efficient root systems, especially if paired with total grain yield. Screening hundreds of lines in a traditional field breeding system would be a laborious undertaking, especially if using methods like the neutron probes used in Chapter 4. However, recent technological developments may provide more practical solutions; for example, electromagnetic induction (EMI) surveys of the soil beneath wheat plots were able to determine the electrical conductivity of the root zone. Soil electrical conductivity and soil water content have a proven positive correlation, allowing for extrapolation from the field measurements of conductivity to field soil moisture (Shanahan *et al*. 2015).

While the limited selection of cultivars featured in this study did not show conclusive yield differences over the two years of the field study (Figure 4.11) it does not preclude the possibility that other UK wheat cultivars and future hybrids may have root systems that are more efficient in extracting soil moisture, thus significantly improving crop yields.

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