# University of Reading



# Quantifying rooting at depth in a wheat doubled haploid population with introgression from wild emmer

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### **Declaration of original authorship**

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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### Abstract

Wheat root systems may not be optimal for the acquisition of subsoil water, due to excessive root growth in surface layers and inadequate soil exploration at depth. The aim of this project was to study the phenotypic and genetic diversity of rooting at depth within the wheat doubled haploid population of Shamrock x Shango. Shamrock has recent introgression from wild emmer (*Triticum dicoccoides*) and exhibits a non-glaucous trait mapped to the short arm of chromosome 2B (2BS). The population was genotyped using the Wheat Breeder's 35k Array, and a linkage map produced to facilitate study of the association of rooting and wild emmer introgression. Rooting in doubled haploid lines was measured in the field (below 50 cm), at and shortly after anthesis, in addition to canopy traits of photosynthetic capacity, spectral reflectance indices and canopy temperature. Root traits of field-grown plants were compared to lines grown in a seedling screen and within rhizotrons to the end of tillering.

Shamrock had greater root length density (RLD) than Shango at depth in the field and within the rhizotrons. The DH population exhibited diversity for rooting traits within the three environments studied. QTL were identified on 5D, 6B and 7B, explaining variation in RLD post-anthesis in the field. Effects associated with the non-glaucous trait on RLD interacted significantly with depth in the field and some of this interaction mapped to 2BS. The effect of genotype interacted greatly with the context of root assessment: e.g. glaucousness expressed in the field was negatively associated with RLD in the rhizotrons, but positively associated with length in the seedling screen.

Non-glaucous lines yielded more than glaucous lines, associated with a stay green trait inherited from wild emmer. RLD in the field at anthesis had a significant negative association with canopy temperature. Significant relationships were found for spectral reflectance indices measured at ear emergence and rooting at depth. However, these relationships were dependent on the glaucousness of the canopy.

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### **Chapter 1. Literature review**

#### **1.1. Introduction**

Wheat is grown on about 230 million hectares of land worldwide (Reynolds et al., 2009), with 730 million tonnes of grain harvested every year (FAO, 2016). Its popularity as one of the top three cereals, grouped with rice and maize, originates from its contribution of carbohydrates, fibre, essential amino acids, vitamins and minerals to the human diet. The gluten protein component of the grain imparts the viscoelastic properties to doughs, necessary for multiple food products such as bread, pasta, noodles and biscuits (Shewry, 2009). Wheat provides one-fifth of the world's calorie intake and farm yields can exceed 10 t/ha if sufficient water, nutrients, and crop protection measures are provided (Reynolds et al., 2009; Shewry, 2009). However, average global yields are around 3 t/ha due to shortages of water, soil resources and susceptibility to pests and disease (FAO, 2016). As well as its unique processing properties, the success of wheat production worldwide can also be attributed to its adaptability to a wide range of growing environments. Wheat is cultivated from the Arctic Circle, 67° N in Scandinavia and Russia, to south of the equator, 45° S in Argentina, and from sea level to 3000 m altitude (Gooding and Davies, 1997; Shewry, 2009). This is due to the crop being able to survive and develop in temperatures ranging from 3-4°C to 30-32°C and precipitation ranging from 250 to 1750 mm (Curtis et al., 2002). With year-to-year fluctuations in growing conditions predicted to increase, yield stability is a desirable feature to cope with unpredictable growing season weather patterns (Calderini and Slafer, 1998; Porter and Semenov, 2005).

Current wheat yields are threatened by future climate change, particularly because

summer drought is predicted to increase (Jenkins et al., 2010). In the UK, nearly 0.5 million hectares of the 1.9 million hectares grown is sown on shallow or sandy soils prone to drought (Foulkes et al., 2001). Although winter rainfall typically replenishes soil to field capacity in early spring, these drought-prone soils have limited reserves of soil water and expose the crop to water-stress conditions if precipitation is low over the summer period (Foulkes et al., 2001). In the UK it is estimated that 1-2 t/ha or 10% of yield potential is lost due to insufficient water supplies during crop growth (Foulkes et al., 2007). Temperatures have risen by approximately 1°C since the 1980s with summer temperatures predicted to rise by up to 4.2°C by 2080 (Jenkins et al., 2010, 2008). Seasonal precipitation in the UK has decreased in the summer and increased in winter. Specifically, between 1961 and 2006 the contribution of high rainfall events has increased in the winter and decreased in the summer in all regions of the UK except the north east (Jenkins et al., 2008). This change in precipitation pattern is projected to increase in the UK, with a rise in winter between 10 and 30%. Summer precipitation changes have a north south gradient with decreases of as much as 40% in the south west to almost no change in Shetland and the north of the UK (Hulme et al., 2002; Jenkins et al., 2010).

Yield potential of wheat is gradually increasing with the introduction of new cultivars through breeding. Improving yield through faster growth or an extended growth season may raise the water use demand of the crop and increase the risk of a significant water deficit occurring during crop growth, compounding the negative impact of any reduction in summer rainfall (White et al., 2015). The timing of any water deficit is pertinent: some growth stages are more sensitive than others, including booting, anthesis and grain fill, and all occur at the later stages of growth (Dodd et al., 2011). This is an issue not limited to the UK, with other countries in Europe also being

affected. Average farm yields in the Mediterranean are 2.3 t/ha with the major limiting factor to yield being post-anthesis terminal drought (Carvalho et al., 2014). Gooding et al. (2003) found that drought 1-14 days after anthesis severely affected grain fill with a reduction of grain dry matter yield of 55%. This is because cell expansion is driven by water uptake so that if drought interrupts water uptake, more shriveled grains are produced (Gooding et al., 2003). The effect of limited access to water also exposes the crop to more significant heat stress (Alghabari et al., 2014). Therefore, through improving the allocation of crop biomass for higher densities of deeper roots, access and uptake of subsoil water can be increased which directly benefits yield outputs at later growth stages (Wasson et al., 2012). This is because translocation processes and grain filling can be maintained during late season drought, helping to stabilise yields in unfavourable growing seasons (Kirkegaard et al., 2007).

#### **1.2. Wheat root form and function**

#### 1.2.1. Root system architecture

The acquisition of essential resources from the soil, is highly dependent on the architecture of the root system (Lynch, 2013). Root architecture refers to the various aspects of root form contributing to the spatial distribution of the root system. Including: first, morphology – the shape and surface features of this plant organ such as root hairs, root diameter and the root cap; second, topology – the pattern of root branching; and third, distribution - root biomass or total root length within a volume of soil, or distance of roots from neighbouring plants (Lynch, 1995). Root architecture characterises these terms, and Gregory (2006) identifies the major determinants of root architecture in cereals to be the number of root axes, the order and density of root branching, the degree of downward growth in response to gravity and the deflection index i.e. the tendency of a root to stray from its current index of growth. Genetic

determinants play a significant role in wheat root form and function, in addition to the amount and distribution of a range of essential nutrients and water within the soil. The interaction of genotypic and environmental influences gives the root system its high plasticity and is a major determinant of the ability of the plant or crop to exploit soil resources. In addition to this complexity there is a huge diversity of soil microbiota which interact with crop root systems and root system exudates within the rhizosphere (Huang et al., 2014; Rascovan et al., 2016). This further interaction of roots and the soil microbiome is not discussed in this report due to the focus on root architecture. However, the importance and relevance of this topic is briefly mentioned in the final discussion (refer to chapter 4).

Much of the complexity of plant root systems originates from the variation of root architecture between species and among genotypes within a given species. Root traits that have been identified to show genotypic variation, specifically within wheat crop plants, include root elongation rate and rooting depth, root distribution at depth, root to shoot ratio and xylem vessel diameter (Manschadi et al., 2008). However, studying the genetic variation of these root traits is complicated by the interaction of the root system with environmental conditions (de Dorlodot et al., 2007). Important soil environmental factors known to interact with roots include soil structure, bulk density, water availability, concentration of nutrients and temperature, which all vary in time and space. Understanding this variation within genotypes and the interactions with environment provides an opportunity to exploit crop root architecture to improve the efficiency of agricultural land use and increase yields. Potential improvement in yields through manipulating root architecture may not have been fully exploited in the past because root traits were not major selection criteria in breeding programmes. Consequently, roots systems have been blindly selected within the optimal environment

of a breeder's field and may be sensitive to more unfavourable conditions such as drought or heat stress prone growing seasons. Large improvements in the potential yields of crops during the *green revolution* appear to have been derived from canopy architecture manipulation rather than root traits (Smith and De Smet, 2012). The *green revolution* was the production of high yielding wheat varieties through the introduction of reduced height (Rht) genes, which allowed higher nitrogen fertiliser applications without lodging. This progression doubled cereal production between 1960 and 1980. Norman Borlaug was instrumental in breeding new semi-dwarf and dwarf wheat cultivars in CIMMYT, Mexico (Borlaug, 1968).

#### **1.2.2.** Wheat root system and development

Cereal root systems have two origins, identified as seminal and nodal roots; they are subsets of the root system as a whole and operate in a complementary fashion (Manske and Vlek, 2013). Seminal roots, sometimes referred to as primary roots, develop from the scutellar and epiblast nodes of the embryonic hypocotyl and generally grow deeper into the soil horizons than nodal roots (Manske and Vlek, 2013). The seminal root system comprises a primary root and a first and second pair of seminal roots which originate from nodes within the embryo (Nakamoto, 1994). Wheat plants typically have an average of four to six seminal roots which emerge before the second leaf stage and are active throughout the vegetative growth period (Araki and Iijima, 2001). Nodal roots, also referred to as secondary, adventitious or crown roots, are produced from primordia which develop after germination. These roots primarily appear on the crown, which is typically 1 to 2 cm below the soil surface, at approximately the same time as tillers appear. Nodal roots then continue to develop until stem elongation transports the higher nodes above the soil surface (Fig. 1.1).



Figure 1.1. Simplified schematic of a wheat plant with its first tiller and seminal and nodal roots.

Klepper et al. (1984) studied root and shoot development in winter wheat and found that nodal roots developed from the leaf bearing nodes, with the first four nodes of the main stem remaining in the crown after stem elongation. Nodes six and seven were generally found above the soil surface with node five either being within the crown or slightly elevated. Therefore, nodal roots are largely present in the upper soil surface and the number of these roots is positively correlated with leaf number and the tillering ability of the plant (Manske and Vlek, 2013). The number of seminal axes present has been shown to vary with seed size (R A Richards and Passioura, 1981). A wheat plant with five leaves on the main stem (typical of a plant shortly before stem extension) could be expected to have all its seminal axes (pairs one to three), a pair of coleoptile axes with second order laterals and pairs of nodal axes at nodes one to three (refer to red line Fig. 1.2). There may also be a coleoptile tiller and three tillers on the main stem (Klepper et al., 1984).



Figure 1.2. Diagram showing the coordinated development of wheat leaves, root axes and tillers. Initiation of growth for tillers, root axes and root axis branching is shown relative to the growth of leaves on the main stem. Seminal (sem), coleoptile (col), leaf node (n) and pair (pr). Original research of Klepper et al. (1984), reproduced with permission of Gregory (Gregory, 1994).

The rate of wheat root growth depends on environmental factors, as well as on plant developmental stage. Root extension rate slows in the winter when temperatures are lower and then increases in the spring when temperatures are warmer. Gregory et al. (1978) calculated root penetration rates of 6 mm/day between sowing at the end of October and early April and 18 mm/day between April and June, in the UK. The absolute rate of extension of spring wheat is greater than winter wheat because of the warmer temperatures and greater incidence of radiation. However, Thorup-Kristensen et al. (2009) estimated that winter and spring wheat achieved the same extension rate when temperature differences were included (1.3 mm °C/day), this was calculated using accumulated average daily temperatures with a base temperature of 0°C. Due to greater accumulation of temperature before anthesis, winter wheat rooted to 2 m, twice that of

spring wheat (Thorup-Kristensen et al., 2009). Roots also grow quickly during stem extension and then slow after anthesis when grain filling takes place and the crop is partitioning its energy to yield outputs (Gregory, 2006).

Morita and Okuda (1995) studied the developmental times of seminal and nodal roots by measuring the root distribution in the top 30 cm of soil of the Japanese wheat cultivar Norin 61, under field conditions. Soon after planting the number of seminal root axes reached a stable number and the total number and length of nodal roots increased during the more advanced growth stages. More specifically, Gregory et al. (1978) found that production of six seminal roots in Maris Huntsman winter wheat was completed before spring and these roots were active throughout the crop's life cycle. Nodal root axes on the other hand were produced in mid-February, but it was not until mid-April that they rapidly began to branch and extend, reaching around 20 nodal axes in total by May.

A recent study by White et al. (2015) measured root length densities at anthesis from 17 different field experiments. Average root length density (RLD) down the soil profile was found to be 1.94 cm/cm<sup>3</sup> in the top 20 cm of soil, reducing to 0.46 cm/cm<sup>3</sup> at 80-100 cm below the soil surface (White et al., 2015). Figure 1.3 shows these RLD's down the soil profile, compared against more dated published values.



Figure 1.3. Graph showing the mean RLD of winter wheat (filled circle, solid line) from 17 different experiments between 2007 and 2013 across the UK. Compared against published reference values (open circles, dashed lines (Barraclough et al., 1991, 1989; P.J. Gregory et al., 1978)). The critical RLD of 1 cm/cm<sup>3</sup> for wheat is shown by the dotted line. Box and whisker plots at each soil depth show the median, mid-line; interquartile range, boxes; and the minimum and maximum, 'whiskers' (White et al., 2015).

The large differences between RLD values from the study by White et al. (2015) and previously published values may be methodology. White et al. (2015) used soil core diameters of 2.6 or 1.9 cm whereas Gregory et al. (1978) used 10 cm diameter cores and Barraclough et al. (1989) 7 cm. Core size is known to influence RLD values obtained and should be representative according to row spacing of the wheat plants, as rooting densities will change based on distance from within the row to midway between the row (Kumar et al., 1993). This is discussed in more detail in the methodology section 1.5.

#### 1.2.3. Distribution of root length with depth in the soil profile

The majority of a crop root system is found within the top soil layers as a result of the mathematical rules of a branching system and the origin of seminal and nodal roots

occurring from the seed and the stem base. Better aeration and a higher availability of nutrients aid the proliferation of roots in the top 30 cm of soil. Generally about 70% of the total root length is found in the top 30 cm of soil (Manske and Vlek, 2013). Winter wheat crops grown in the field, studied by Barraclough and Leigh (1984), had more than half of their root system in the top 20 cm of soil and only 8% or less below a depth of 80 to 100 cm. Entz et al. (1992) similarly found 60 to 80% of the total root length of two winter and two spring wheat cultivars to be present in the top 50 cm of soil.

Hoad et al. (2004) studied the root and shoot growth of two different winter wheat varieties and found root length density to vary between 3 to 6 cm/cm<sup>3</sup> in the plough layer (0-20 cm), with less than 1 cm/cm3 being found below 40 cm, using 8 cm diameter soil cores at anthesis. This study was completed in multiple UK field site locations using UK recommended winter wheat varieties (Consort and Malacca) and therefore provides relevance to this study. The reduced root growth at depth has been suggested to limit nutrient and water uptake in these deeper soil layers (Hoad et al., 2004). Watt et al. (2008) identified the type of root growing in these deeper soil horizons in field grown wheat, barley and triticale; using the number and diameter of xylem tracheary elements (XTE, specialised cells within the xylem which transport water up the plant) to distinguish between root types. Between 1.3 m and 2 m deep, there were no nodal roots present. Half of the roots found at this depth were the smallest order branch root, with less than 10% of the roots being seminal roots. According to Hagen-Poiseulle law, which states that flow in a tube is related to the radius of that tube to the fourth power, the smallest order seminal branch roots may have 1000-fold less axial water flow compared to seminal roots. The high number of branching roots at the deepest soil layers is potentially reducing water flow from the soil to the plant due to

small diameter XTE. However, this may be a technique to reduce soil drying and preserve water for important growth stages (Watt et al., 2008).

Gerwitz and Page (1974) found that a simple exponential model explained more than 80% of the variance in 71 studies of root density distribution with depth. This model described an exponential decrease of root density with depth. Although the root distribution of crop plants through the soil profile can be successfully predicted by such general models, they are less successful in heterogeneous soils with soil management and varying concentrations of soil water and nutrients having large effects. These factors were identified by Gerwitz and Page (1974) as 'exceptions' to their model and involved: poor nutrient status of the soil, depth of cultivation and poor moisture. Adiku et al. (1996) constructed a model to assess the effects of varying soil moisture on the pattern of root growth. They assumed that the maximum root length density was the same for all soil depths and that under water-limiting conditions more dry matter would be partitioned to the wetter regions of soil and that growth would slow in the dry regions. These assumptions were similar to the observed growth patterns of a wheat crop, which had greater root length density at depth compared to the surface soil, based on the distribution of soil moisture (Adiku et al., 1996).

#### 1.2.4. Metabolic cost of roots

Respiration in the wheat root system uses 52 - 67% of the carbohydrates transported from the shoots (Lambers et al., 1996). The metabolic cost of soil exploration is high and larger root systems may become yield limiting if they do not result in improved water and nutrient acquisition (Lambers et al., 1996; Manske and Vlek, 2013). Therefore, there is a trade-off between the size of the root system and root respiration. This is the functional equilibrium which refers to root system size and activity determining and being dependent on shoot system size and activity: root mass x specific root activity = shoot mass x specific shoot activity. Where specific root activity is the absorption of soil resources and specific shoot activity is the rate of photosynthesis (Gregory, 2006). In stressed conditions such as limited water, the partitioning of photosynthetic assimilates will change from the shoots to the roots to allow continued soil exploration and root elongation to find water and consequently shoot mass will reduce (Gregory, 2006). Root growth will continue in dry soils if another part of the root system has access to water, using water supplied by the phloem for growth (Boyer et al., 2010).

The metabolic cost of roots can be reduced by producing roots of a smaller diameter or with more aerenchyma (plant tissue containing air spaces; Fig. 1.4). Aerenchyma improve soil exploration through maintaining root size and growth in soils with low resource availability (Lynch, 2013). These adaptive traits have been shown to improve phosphorus uptake efficiency as a greater specific root length (m/g) increases the volume of soil per unit of root surface area as well as the reduced carbon cost of root production (Sandana and and Pinochet, 2014).

RCA



Figure 1.4. Image of root cortical aerenchyma (RCA) in two different maize genotypes with the left image showing greater aerenchyma due to higher volume of air spaces (Lynch, 2015).

#### **1.2.5.** Growth angle of seminal axes

As seminal roots originate from the embryo and develop throughout a crop's vegetative period, they tend to grow deeper into the soil horizons. They are also better at penetrating more compact soil than nodal roots, especially in shallow rooting wheat cultivars (Araki and Iijima, 2001); therefore these roots play a significant role in the uptake of water from the subsoil (Manschadi et al., 2008). The growth angle of the seminal root system is an important aspect of root architecture and has been recognised to have a significant association with the acquisition efficiency in crop plants, because the seminal growth angle is significantly related to rooting depth (Manske and Vlek, 2013; Oyanagi et al., 1993). This root trait shows considerable genotypic variation between wheat cultivars with different drought tolerance. Manschadi et al. (2008) suggested that the angle of seminal root axes in the seedling stage plays a large role in defining the architecture and functioning of the mature root system. In their study, 27 Australian and three CIMMYT wheat genotypes were studied and the growth angle of the first pair of seminal roots varied significantly between these genotypes, ranging from 36 to 56 degrees. However, the growth angle of the second pair of seminal roots did not differ significantly between these wheat genotypes with an average of 78.4 degrees. The same characteristic of a wider growth angle in the secondary seminal root was found in Japanese wheat cultivars (Morita and Okuda, 1995). Manschadi et al. (2008) found that the more drought tolerant genotypes, adapted to deep clay soils in northern Australia, had a more compact root system with smaller primary seminal root growth angles that resulted in more roots occupying the soil directly under the crop. The wheat genotype SeriM82, was identified as having a narrower root angle: this genotype was grown in root observation chambers in a separate study where its root system was less-laterally spread and more compact compared to a standard wheat

genotype, Hartog (Fig. 1.5). SeriM82 therefore produced 3.8 times more root length at depth (90-112.5 cm) and subsequently extracted more water, particularly during grain filling. Modelling SeriM82 water uptake efficiency suggested an additional 0.55 t/ha grain yield gain per mm of water extracted post anthesis (Manschadi et al., 2006). However, it is difficult to compare this study to the field environment and therefore how SeriM82 would perform in these conditions due to Manschadi et al. (2006) growing single plants of the crop genotypes in 240 cm wide and 10 cm thick chambers, eliminating any competition the plant would experience in the field.



Figure 1.5. Diagram showing the spatial root configuration of the drought tolerant wheat cultivar SeriM82, the standard wheat cultivar Hartog and the barley variety Mackay, grown in root observation chambers at crop maturity. Numbered squares indicate the  $30 \times 22.5$  cm sections of the transparent sides used for root digital imaging. Symbol (diamond) represents the plant base (Manschadi et al., 2006).

De Dorlodot et al. (2007) stated that more drought tolerant crop species have vertically inclined root systems. Seminal roots are known to be sensitive to the soil water environment. Oyanagi et al. (1993) studied root characteristics of 133 native Japanese wheat cultivars and 51 showed a stimulated gravitropic response, more vertically

inclined root growth, at low soil water potentials, possibly due to ABA concentrations. Although the angle of root axes changed during the course of root growth due to environmental conditions, it is thought that root angle is primarily controlled by genetic factors (Oyanagi et al., 1993). This root trait has been proven to be more heritable than other traits such as length within wheat root screens (Christopher et al., 2013; Richard et al., 2015; Sanguineti et al., 2007).

#### **1.3.** Root characteristics important for resource acquisition

#### 1.3.1. Root shoot biomass allocation

The domestication and modern improvement of crop plants has shifted the root v shoot biomass relationship in wheat genotypes, a consequence of greater biomass allocation to aboveground components in more modern wheats. Siddique et al. (1990) compared root to shoot ratios of old and modern wheat cultivars and tall and dwarf isogenic lines. In the modern varieties, the shift towards greater allocation of biomass to the shoots compared to the roots occurred 35 days earlier than in the older variety, 55 days after sowing (DAS) compared to 90 DAS. The old variety Purple Straw was widely grown in Australia for 30 years from 1860 and the modern variety Kulin was registered in 1985 by the Western Australian Department of Agriculture (Fitzsimmon et al., 1986; Simmonds, 1925). Maximum root biomass was observed at anthesis in all varieties, with Purple Straw having significantly more root length density and dry matter in the top 40 cm and higher root dry matter at a depth below 30 cm (Siddique et al., 1990). However, water uptake did not vary between the varieties. Greater allocation of biomass to yield components, and a reduction of root dry matter, improved the crop harvest index of Kulin and may have caused the improved water use efficiency (grain yield/water use) of the grain (Siddique et al., 1990). Qin et al. (2012) compared root and shoot biomass allocation in two older (diploid and tetraploid) and two modern

(hexaploid) wheat genotypes during the initial 30 days of growth. The two older genotypes allocated almost equal biomass to their shoot and root systems whereas shoot biomass allocation surpassed root allocation in the two modern genotypes. Qin et al. (2012) concluded this to be a result of intensive agricultural practices which provide moderate nutrient and water supplies to the soil, thereby promoting a larger above ground biomass and a root system that does not need to forage for resources. The increase in harvest index and reduction of root biomass with chromosome ploidy was confirmed by Li et al. (2014) who studied above and below ground biomass in diploid, tetraploid and hexaploid wheat. Aboveground biomass decreased from diploid to tetraploid wheat but then increased to the maximum value from tetraploid to hexaploid. This included significantly higher photosynthetic rates of flag leaves and significantly greater grain weight and number per spike. Li et al. (2014) concluded that root biomass reduced with chromosome ploidy and domestication lowered competition for soil resources between individual plants and allowed more dense plants of higher yields, contributed also by reduced tiller numbers. Li et al. (2014) reported reduced aboveground biomass when comparing diploid and tetraploid wheats whereas Qin et al. (2012) reported the same root and shoot biomass in diploid and tetraploid wheat. This may be due to Qin et al. (2012) only studying the first 30 days of growth and Li et al. (2014) measuring shoot dry weight at maturity. Additionally, the number of genotypes for each ploidy wheat was low in both studies, ranging between one or two genotypes for diploid and tetraploid wheat.

Allocation of biomass to the above or belowground components of a crop plant may be modified by the type and degree of environmental stress. Hamblin et al. (1990) studied spring wheat in two successive years in western Australia, average rainfall within the growing season was 209 mm in the first year and 178 mm in the second year. However,

in the first half of the year 216 mm of rainfall fell before sowing at the end of May in the first year compared to 82 mm before sowing in the second year. In the first year, the root to shoot ratios at harvest were smaller at 0.1 whereas in the second year the root to shoot ratios varied between 0.4 and 0.8, due to root length densities being significantly greater in the drier year. Kadam et al. (2015) drought stressed rice and wheat plants for 30 days and assessed rooting traits in the plants 45 DAS. Drought stressed wheat plants allocated more biomass to the roots, resulting in increased maximum root length, root length density and root volume. These plants also showed increased metaxylem diameter and lower metaxylem number at the root tip and the reverse at the root-shoot junction. Higher axial conductance with increased metaxylem number at the root-shoot junction but a lower axial conductance close to the root tip with reduced metaxylem number can conserve water in deeper soil profiles and is thought to facilitate efficient water use up until anthesis and grain fill (Kadam et al., 2015; Wasson et al., 2012). Alternatively, applying stress to the aboveground part of the plant reduces the allocation of dry matter to roots as seen by Welbank et al. (1973). Dry weight of barley roots decreased by 25% when the plant underwent 50% shading 49 days after sowing. An additional 14 days of shading reduced root dry weight by a further 50% compared to the controls due to shaded plants diverting more resources to aboveground growth at the expense of root growth, suppressing root growth at depth (Welbank et al., 1973).

This root to shoot biomass ratio can be manipulated through restricting the tillering ability of a wheat crop. A wheat plant can produce up to 15 tillers even at conventional sowing rates. However, up to 75% of these tillers senesce before anthesis, due to the restricted availability of resources within the plant (Berry et al., 2003). Consequently, the production of tillers which do not succeed to reproductive growth stages cause a loss of water and nutrient resources which cannot be sufficiently replaced through

translocation in the plant (Berry et al., 2003). Through restricting tillering, with the use of a tillering inhibition (*tin*) gene, the plant root to shoot ratio is weighted more towards the roots and significant increases in rooting and yield components can be accomplished (Hendriks et al., 2016). Two near isogenic lines (NILs) were studied (Banks and Kite) alongside a free tillering variety (Seri) and root: shoot ratios were found to increase significantly when comparing a free tillering plant (6-15 tillers per plant) with a manually detillered plant (just main stem) and a plant containing the tin inhibition gene (3-4 tillers for Banks and 2-3 tillers per plant for Kite; Fig. 1.6). The *tin* inhibition gene caused up to 60% reduction in leaf area and aboveground biomass and increased total root length and biomass by 120 and 145% respectively. In the field, wheat varieties with restricted tillering had significantly deeper maximum rooting depths at 135-147 cm compared to the tillering crop at 117 cm. The reduced aboveground biomass of nontillering wheats resulted in slowed water use which prolonged post-anthesis green leaf area, facilitating grain fill (Hendriks et al., 2016). The partitioning of biomass resulted in fewer spikes and less grains per  $m^2$  in restricted tillering crop plants but this was compensated for by a higher harvest index, more grains per ear and a greater thousand grain weight, resulting in a higher grain yield (Hendriks et al., 2016).



Figure 1.6. Graph showing the root-to-shoot ratio at anthesis for three wheat genotypes: NIL pair (B=banks, K=kite) and a free tillering variety (Seri) under different treatments which include a free-tillering 'control', a manual 'detillered' treatment and a NIL plant containing the tin allele. Detillered plants and those with the tin allele (+) had significantly higher root: shoot ratio compared to the control. Standard error bars given (Hendriks et al., 2016).

These studies focused on the balance of biomass allocated to the shoot and roots of a wheat crop have demonstrated the decline in root biomass through recent breeding efforts. This is due to focus on traits to improve yield output and reduce neighbour competition suitable for higher stand densities (Qin et al., 2012). Reduced root biomass resulted in higher harvest indexes (Li et al., 2014) due to high resource application preventing the need for the crop to forage for soil nutrients. However high rooting densities were observed in deeper soil layers in wheat varieties with larger root systems (Siddique et al., 1990) and deeper rooting was achieved through manipulation of the root: shoot ratio (Hendriks et al., 2016). Therefore, reducing biomass allocation to the wheat root system overtime may have also resulted in a reduced rooting depth. Preventing unnecessary loss of crop biomass is important in drier growing

environments with limited rainfall, in addition to conservative traits such as manipulating metaxylmen number and diameter (Kadam et al., 2015). This ensures more economic biomass allocation and resource use efficiency. However, in a more temperate climate such as the UK agronomic decisions such as sowing rate can determine tiller number and the number of ears in a metre square (AHDB, 2015).

#### **1.3.2.** Improving rooting at depth

In current cropping environments, non-uniform root systems are resulting in a high density of roots in the top 30 cm of soil where cultivation reduces soil bulk density and nutrient availability is high. Consequently, water stressed crop plants can leave substantial amounts of available water in the subsoil at maturity (Passioura, 1983) which can directly hamper yield due to a reduced potential of water uptake (White et al., 2015). A more evenly distributed root system throughout the soil profile would reduce the path length for subsoil water to reach the plant, with the potential to increase plant biomass and harvest index with this extra supply of water (Passioura, 1983). Gregory et al. (1978) showed the importance of deep roots, when rainfall is limited, in a field grown wheat crop. Just 3% of the crop's root dry weight was found below 1 m depth, but when upper soil layers dried out these roots supplied 20% of the evapotranspiration.

Another possible reason for this underutilisation of subsoil water is that around anthesis root growth ceases due to carbohydrate reserves being translocated to the grain, causing the roots to run out of time and resources to reach deeper soil layers (Palta and Watt, 2009). Increased root growth rates prior to grain filling can promote deeper rooting depths and improve water use efficiency. Wheat cultivars with higher root growth rates were associated with higher leaf growth rates and leaf area ratios (Van Den Boogaard et
al., 1996). Multiple studies have concluded that maximum rooting depth is better associated with water loss in the soil profile than total root length, with fine roots being the major sites for water uptake (Hamblin and Tennant, 1987; McCully, 1999; Richards et al., 2002).

Richards et al. (2002) suggested that the simplest way to improve rooting at depth is to increase the vegetative period, growth before anthesis (Zadoks growth stage; GS 61). Significant differences in root traits and nitrogen uptake have been identified in early and late senescing wheat lines (Hebbar et al., 2014). Root biomass at booting was 42% less in early senescing lines compared to late senescing lines (Fig. 1.7); at this growth stage early senescing lines had almost 40% senescent leaves while this was negligible in late senescent lines. Within the doubled haploid lines studied, booting differed by a maximum of three days. The early senescence of some wheat lines was thought to be a result of poor root biomass significantly reducing nitrogen uptake. Total plant nitrogen in early senescing lines was less than half of that in the late senescing lines (Hebbar et al., 2014). Root biomass and nitrogen uptake had a strong correlation with plant senescence when comparing the early and late senescing groups, but within groups the relationship seems weaker. This suggests that additional factors, other than root biomass, are influencing plant senescence when comparing less extreme phenotypes (Fig. 1.7).



Figure 1.7. Correlations between extent of plant senescence with plant N uptake ( $R^2 = 0.872$  P<0.001) and root biomass ( $R^2 = 0.746$  P<0.001) in early (circles; DH2 and DH8) and late (triangles; DH9 and DH13) wheat senescing lines at booting. (Hebbar et al., 2014).

Root vigour is a characteristic which causes early and fast root extension, subsequently increasing root biomass and density, which allows for the expansion of the root system during the vegetative period (Palta et al., 2011). Root vigour may promote the use of soil water before grain filling takes place, negatively effecting yield in environments where crops rely on stored soil water. Therefore, this trait is more valuable in rain fed environments. Breeding selection for root vigour is mainly advantageous for crop establishment and early season water and nitrogen capture, particularly in dry

environments to reduce soil evaporation. Early and rapid root growth has the potential to continue plant growth through drought conditions even if the crop is not resistant to desiccation, as a result of improved establishment; contributing further to crop yield (Hurd, 1974; Palta et al., 2011). Palta and Watt (2009) also found that vigorous roots in wheat plants are less costly in terms of carbon to maintain than less vigorous roots. These roots are more efficient for water and nitrogen capture because they consume less carbon in respiration per unit of nitrate uptake, which is potentially why this characteristic produces greater root biomass and length (Palta and Watt, 2009). Wheat lines bred for early vigour, based on leaf breath and width in early establishment (varying between 18 and 44 DAS (Rebetzke and Richards, 1999)), had 50 to 70% greater biomass of roots and 33 to 83% more root length compared to less vigorous wheats at stem elongation (GS 31; Palta et al., 2011). Although the maximum rooting depth was similar between less vigorous and vigorous wheat roots, branching on the seminal axes occurred 5 to 7 days earlier in vigorous roots which resulted in a greater number of root tips, allowing 42 to 60% more nitrogen uptake by stem elongation (Palta and Watt, 2009).

### 1.3.3. Influence of subsoil water use on wheat yield

The advantage of improved crop rooting at depth can be significant for yield, due to the beneficial access of subsoil water. This is particularly relevant in rain fed environments where the occurrence of subsoil water can provide a source of water late in the season when grain growth is sensitive to drought stress (Lilley and Kirkegaard, 2007). The increase in cereal crop biomass obtained as a result of subsoil water access was determined by Hammer et al. (2009) who studied the historical maize yield increase in the US Corn Belt. The yield increase was attributed to a change in root architecture and water capture as a result of narrower and deeper root systems (>2 m). Sowing at higher

plant densities caused this change in rooting and subsequently the increase in water use from the subsoil layers increased crop biomass once the surface soil layers dried out (Hammer et al., 2009).

Lilley and Kirkegaard (2007) specifically modelled the effect of deeper wheat roots on subsoil water use. Restricting rooting depth to 0.8 m compared to 1.2 m reduced mean grain yield of wheat by 0.7 to 0.4 t/ha. Simulating rooting to 1.8 m resulted in water extraction of 14 to 27 mm in the 1.2 to 1.8 m soil zone (Lilley and Kirkegaard, 2007). This study indicated the value of rooting at depth, where water extraction is particularly significant to grain yield around anthesis (Kirkegaard et al., 2007). In a separate study completed within a field environment, the use of 10.5 mm of additional subsoil water from the 1.35 to 1.85 m soil layer during the post anthesis period increased grain yield by 0.62 t/ha, due to greater grain size. Water use efficiency was increased during these periods of growth as it contributed directly to yield components; in this specific study water use efficiency was 59 kg/ha of yield achieved per mm of water used by the crop post-anthesis (Kirkegaard et al., 2007).

The advantage of deeper root systems in dry seasons was shown by a comparison of the yield of bread wheat and durum wheat in South Australia (Zubaidi et al., 1999). Over a period of three years, durum wheat yielded less than bread wheat during dry years when rainfall was less than 450 mm. The differences in yield were thought to be due to bread wheat having better early vigour which resulted in more tillers and kernels per m<sup>2</sup> and between 14 and 22 weeks after sowing the bread wheat varieties had 50 to 100% greater total root length throughout the soil profile, compared to the durum wheat genotypes (Zubaidi et al., 1999). Under non-limiting water conditions, durum wheat produced 20% higher yield compared to bread wheat due to larger kernels, suggesting that the

loss of yield in the drier seasons was due to insufficient root length at depth (Zubaidi et al., 1999).

Use of available subsoil water can also be promoted through an irrigation strategy, as shown by Misra and Chaudhary (1985) who used four different limited water treatments to study the effects on root growth, water use and grain yield of wheat. It was found that a split water treatment of 14 cm of water at sowing and at nodal root initiation increased rooting density by 31% compared to just 14 cm of water at sowing. Irrigation at the initiation of nodal roots is known to promote wheat root growth and those plants that avoided water stress during early growth had better root development. Therefore, by promoting growth before heading using limited irrigation, grain yield was increased despite water stress in later growth periods as the crop was forced to use the available water in the subsoil (Misra and Chaudhary, 1985).

Improving rooting at depth can include higher root length densities or greater maximum rooting depth. Deeper rooting is thought to be better associated with soil water loss compared to total root length (McCully, 1999; Richards et al., 2002). Richards et al. (2002) suggested that increasing the vegetative period (before anthesis) can help improve rooting depths as post-anthesis the crop assimilates resources to the grain. Additionally, maintaining green leaf area during anthesis and before grain fill can help improve rooting depths and biomass to maintain water uptake during growth stages sensitive to drought (Hebbar et al., 2014). A number of studies also identified early vigour as an important trait to improve root biomass and drought resilience, this can reduce soil evaporation, specifically in drier environments (Lopez-Castaneda and Richards, 1994). Palta and Watt (2009) reported vigorous wheat plants had higher root length densities at depth but maximum rooting depth was similar between vigorous and less vigorous wheat types. However, Zubaidi et al. (1999) found bread wheat yielded

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higher than durum wheat cultivars in drier growing seasons due to better early vigour which promoted tillering and subsequently root biomass. Therefore, early establishment may be a more beneficial trait than crop vigour to ensure good accumulation of crop biomass to improve survival to early stress compared to vigorous roots at later growth stages being susceptible to using up resources before anthesis and grain fill.

#### **1.3.4.** More efficient root systems

It is difficult to define the most efficient root system size as there are likely to be tradeoffs for water and acquisition of different nutrients. Differing root ideotypes were defined by White et al. (2013) for phosphorus, potassium and nitrogen uptake in cereal crops (Fig. 1.8). These three alternate root systems are relevant for more extreme environments where soil is highly deficient of a particular substance, whereas for the majority of scenarios the crop is required to access all of these resources from the soil. There are identified architectural traits that are common in the three scenarios which include: early root vigour, large root biomass, more cortical aerenchyma, large root surface area and high root length density. Common bean genotypes (Phaseolus vulgaris) differing in rooting depth were studied by Ho et al. (2005) under phosphorus stress, water stress and combined phosphorus and water stress. The shallow rooting genotypes coped best under phosphorus stress and the deeper rooting genotypes coped best under water stress. A dimorphic root system permitted dense rooting throughout the soil profile within the combined stress treatment. The genotype with the greatest root biomass was found to grow best in all stress treatments; this was a shallow rooting genotype because it allowed early seedling growth as a result of phosphorus acquisition, subsequently improving water uptake in drying soils (Ho et al., 2005).



Greater association with organisms fixing N<sub>2</sub>

Figure 1.8. Root ideotypes defined by White et al. (2013) for acquisition of (A) phosphorus (B) potassium and (C) nitrogen.

In lupin, early production of high density roots in the top soil and a vigorous tap root was also shown to be the most efficient for capture of nitrate and stored subsoil water. However, there is a limit to the efficiency of high density rooting in the surface soil layers due to an increase in overlap of depletion zones (Dunbabin et al., 2003). Lynch (2013) presented a maize root ideotype for water and nitrogen acquisition, concluding that a root system which vigorously explores deeper soil layers would be more sought after due to water and nitrogen resources more commonly travelling down the soil profile. More specific rooting characteristics which would enhance resource uptake were abundant root cortical aerenchyma, large cortical cell size and high cortical senescence; a moderate number of crown roots with narrow growth angles and few but long laterals; and a lowered response of root branching to localised resource availability. Cortical aerenchyma and a lowered stimulated root response to resources reduces the metabolic cost of roots and Lynch (2013) suggests few but long laterals increases soil exploration of areas accessible to the mass flow of water.

White and Kirkegaard (2010) studied the root-soil interface of wheat plants growing on red Kandosol soil in southern Australia. Cross-sectional areas of soil were studied within 40 cm soil cores collected post-anthesis in a wheat field. Pore distribution and size was studied as well as root growth vertically down these pores. Red Kandosol soils have intact subsoils of high soil strength (5 MPa) causing 30 to 40% of the wheat roots to be present in soil pores and cracks in the upper horizons (<0.6 m) and up to 85 to 100% in the subsoil (>0.6 m). This indicates that soil pores aid root growth in soils of a high bulk density. However, it was concluded that root length densities were sufficient to extract subsoil water but there was resistance caused by poor root-soil contact. Figure 1.9 is data taken from a UK agricultural soil, indicating that high soil strengths (>4 MPa) are seen at depth in this environment also (Gao et al., 2016). Valentine et al. (2012) measured seminal root elongation in barley seedlings grown in field soil collected from the surface 10 cm or manually packed soil at 1.06 g/cm<sup>3</sup> over a 2 day period. Field soil penetration resistance varied between 1 and 3 MPa whereas packed soil was less than 1 MPa. Consequently, root elongation in the field soil was one third of that compared to the packed soil. Pore volume reduced with penetration resistance and within the higher bulk density field soils root elongation was closely related to pore diameter size, even within this short time period (Valentine et al., 2012). McKenzie et al. (2009) demonstrated how soil bio pores can aid root growth by placing a mesh

horizontally 20 cm below the soil surface with a differing number of 2 mm diameter holes to replicate biopores. Five barley plants were assessed up until 110 days after sowing (DAS) and plants with unrestricted access to the subsoil were taller and had significantly greater leaf area, which was reduced in other plants depending on restriction (0, 100, 200, 583 or 1167 holes/m<sup>2</sup>). Significant differences between treatments occurred 41 DAS with a steady decline in plant height with amount of restriction to the subsoil, varying between 37 cm and 27.7 cm. Soil water content was associated with amount of root access, indicating the importance of subsoil water access to crop growth (McKenzie et al., 2009).



Figure 1.9. Penetrometer profiles on a silty clay soil at the Rothamsted experimental farm in Bedfordshire, UK. The increases in penetrometer resistance between March 3<sup>rd</sup> and April 30<sup>th</sup> are because of the effects of soil drying by wheat roots (Gao et al., 2016).

These studies show that the physical properties of agricultural soil strongly influence root growth ability. White and Kirkegaard (2010) suggested that breeding strategies to improve root proliferation to utilize these pores, and dense root hairs for water uptake would be a more productive route compared to breeding for better penetration ability. However, penetration ability is still important in root growth as root elongation through soil has improved root-soil contact which aids resource uptake, compared to growth through soil pores (Gao et al., 2016).

The density of the root system is also important for the acquisition of soil resources and its efficiency varies depending on the resource being acquired. Van Noordwijk (1983) calculated root lengths sufficient, in terms of volume of soil, to acquire nitrogen, phosphorus and water. The root length sufficient to acquire water and nitrogen were similar at 0.1 to 1 cm/cm<sup>3</sup> but rose to 1 to 5 cm/cm<sup>3</sup> if there was soil root contact resistance (most commonly a root-soil air gap preventing resource uptake). Phosphorus on the other hand had a higher necessary root length for acquisition at 1 to 10 cm/cm<sup>3</sup>. This value was supported by Barraclough et al. (1989) who used a mobile shelter to drought a wheat crop in the field from tillering to maturity for 100 days. Droughted plants with sufficient nitrogen extracted all available water to 80 cm depth with an average root length density of 1 cm/cm<sup>3</sup>.

Postma et al. (2014) modelled efficient lateral root branching in maize for nutrient uptake using the plant model SimRoot. Optimum lateral root branching density changed depending on the resource, the availability of the resource and a trade-off between branching density and root elongation. Long lateral branching was optimum for nitrate acquisition with 1 to 3 branches/cm in low nitrogen soil and 5 to 8 branches/cm for higher nitrogen available soils. Short and densely spaced roots were optimum for phosphorus acquisition with branching density as high as 21 branches/cm (Postma et al., 2014). Hamblin and Tennant (1987) studied the root length per unit ground area and its relation to water uptake, as the two are considered to be directly related, in wheat, barley, lupin and field pea plants. The legumes appeared to have a more efficient root system size for water uptake as water loss from the soil profile was very similar for

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wheat and lupin plants despite cereals having a root length per unit ground area that was 5 to 10 times larger. The cereals in this study had root length densities of 1 cm/cm<sup>3</sup> in the bottom third of the rooted profiles which may have been inadequate to access all the available soil water. Therefore the cereal root systems were inefficient as root length densities were unbalanced throughout the soil profile, with much larger root length densities nearer the surface (Hamblin and Tennant, 1987).

To define the economic optimum of a wheat root system, in terms of size and distribution for the capture of soil water and nitrogen, King et al. (2003) used a quantitative model. The investment of fine roots at depths and a smaller abundance of surface roots would give a greater economic return in terms of accessing more water and nitrogen, during grain fill. The economic return of root investment for water capture is double that compared to the same amount invested in nitrogen capture. A more efficient root system would have a lower concentration of roots in the upper soil layers to reduce overlap and inter-root competition, therefore promoting the economical resource capture of the roots (King et al., 2003). Lynch (2013) also stated that rapid foraging in the deeper soil layers would optimise water and nitrogen capture, as these resources are quickly depleted in the upper soil layers and can be transported down the soil profile. Simulation modelling is a valuable tool in root system studies but further research is needed to improve root system modelling in terms of the cost of root systems for resource capture, specifically in terms of carbon (Dunbabin et al., 2003; King et al., 2003).

White et al. (2015) collected root length density values to a depth of 1 m, for commercially grown winter wheat and oil seed rape crops in the UK. These values were used in a model described by King et al. (2003) to assess water resource capture achievable per unit root length density of the crop. Averaging over 17 crops the root

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length densities of winter wheat in the top horizon (0-20 cm) was 1.94 cm/cm<sup>3</sup> and 1.18 cm/cm<sup>3</sup> in the 20-40 cm layer. These values fell to 0.74, 0.52 and 0.46 cm/cm<sup>3</sup> in the lower three horizons (40-100 cm). The critical root length density for water capture was reported at 1 cm/cm<sup>3</sup> based on past studies (Barraclough and Leigh, 1984) and the King et al. (2003) model showed that root length densities above this value are only associated with small increases in water capture. Predicted yields therefore ranged from 7.5 t/ha on low available water content soils with the lowest root length densities and to 12.5 t/ha on high available water content soils with the highest reported root length densities. White et al. (2015) concluded that root systems were not adequate for full water capture due to root length densities being less than the critical value below an average depth of 0.32 m, subsequently causing a shortfall in grain yield of 3.5 t/ha.

When considering a wheat crop at mature growth stages in a managed environment there are a multitude of beneficial root architectural traits for resource uptake taken from the studies mentioned. Water acquisition is particularly important post-anthesis to maintain green leaf area, carbon fixation and resource allocation to the grain (Farooq et al., 2014). Consequently, higher rooting densities in deeper soil layers is beneficial for access to subsoil water and additionally nitrogen, due to this soluble nutrient commonly travelling down the soil profile (Lynch, 2013). A more efficient root system would have lower densities of roots within the top 30 cm of soil as concentration of resources is high here and additionally to reduce inter-root competition (King et al., 2003). This would allow more root biomass further down the profile and optimally a root system with longer root branches and narrow growth angles, with root length densities greater than 1 cm/cm<sup>3</sup> (Barraclough et al., 1989; Lynch, 2013; Postma et al., 2014; Van Noordwijk, 1983). In environments with dry or highly compacted soil, greater root penetration resistance or more plastic roots which can take advantage of soil biopores

and cracks can promote root growth at depth (Gao et al., 2016; White and Kirkegaard, 2010). In addition to these traits early root vigour was identified as beneficial to a more efficient root system, to access resources in early growth, and increased cortical aerenchyma to aid greater root surface area (Lynch, 2013; White et al., 2013).

#### **1.3.5.** Root characteristics for water limited environments

The grain filling process in wheat plays a significant role in determining the yield and quality of the crop. This growth stage is sensitive to drought stress due to the time of year it occurs and the need of the crop to take up water to aid grain filling. Gooding et al. (2003) found drought severely affected the grain filling process 1 to 14 days after anthesis, reducing grain dry matter yield by 55%. Drought specifically reduces cell expansion as it is driven by water uptake, cell expansion is important to allow starch and protein deposition in the expanded grain and to increase dry matter accumulation (AHDB, 2015). Therefore water stress slows down the rate of grain filling causing the production of more shrivelled grains (Gooding et al., 2003; Passioura, 1983). A few crop water use efficiency strategies have been identified to prevent the reduction of grain yield and shrivelled grains in water stressed environments. Water use efficiency is defined by grain yield/total water used (Ram et al., 2013) and is most important in environments where crops rely on water stored in the soil and therefore cannot be permitted to use up all the available soil water before grain filling takes place (Passioura, 1983). Seminal root morphology has been identified as being important in soil water conservation as increased axial resistance to water flow in the roots can ensure water is not used up before critical growth periods. This can be achieved by reducing either the number of seminal axes or the diameter of the xylem vessels (Passioura, 1983; R A Richards and Passioura, 1981). The grain yield of wheat plants in northern Australia benefited by about 500 to 700 kg/ha for each week's supply of water still left in the soil at ear emergence, indicating the benefit of conserving stored soil water (R. A. Richards and Passioura, 1981). Diameter of the xylem vessel was proven to be more genetically heritable than the number of seminal axes in a screening of over 1,000 wheat genotypes from a variety of wheat growing areas (R A Richards and Passioura, 1981). This trait was successfully bred into Australian wheat cultivars, reducing xylem diameter from 65  $\mu$ m to 55  $\mu$ m. Differences in yield were not significant compared to plants with larger xylem diameters in wetter seasons. However, in dry seasons yield was improved by 3 to 11% through increased harvest index, greater biomass and grain number (Richards and Passioura, 1989).

Manschadi (2006) also identified that conservation of water early on in the season for use during the reproductive phases in wheat improves drought tolerance. However, this was achieved through a different aspect of root architecture, which involves a compact and uniform root system that promotes growth at depth. The drought tolerant wheat variety SeriM82 produced less horizontal growth and 3.8 times more root length at depth (90 to 112.5 cm) as well as 19% more post anthesis root growth, which allowed 27.2% more soil water extraction from the subsoil, compared to a standard wheat variety, Hartog (Fig. 1.5; Manschadi et al., 2006). The slow establishment and less vigorous shoot growth of SeriM82 reduced water use early in the season, benefiting the crop at later growth stages. This genotype also maintains green leaf area during postanthesis drought, subsequently allowing a longer grain filling period to give better yields. This may be an effect of improved rooting at depth and better access to deeper subsoil water (Manschadi et al., 2006). These results contradict those of Palta and Watt (2009) who suggested that root vigour can improve rooting at depth. Root plasticity is also an important trait under water stressed conditions; altering the partitioning of biomass from the shoots to the roots as well as improving the efficient use of biomass, such as fine roots to increase soil exploration. Carvalho et al. (2014) studied barley and durum wheat in soil columns under well-watered and drought conditions and found the two cereals responded differently under drought stress. Above ground biomass was reduced by 46-47% over two years in barley compared to 26-30% in durum wheat. Differences in total root length were significant with barley reducing total root length by an average of 40% under water stress but durum increased its total root length by 49%, with a 90% increase in root length density in the 125-150 cm soil layer (Carvalho et al., 2014). Subsequently, barley had a reduced grain yield of 47% compared to durum wheat with 30%, indicating the greater root plasticity of durum wheat improved water capture under drought conditions (Carvalho et al., 2014).

## 1.3.6. Selecting for improved root systems

Selection criteria to improve wheat yields in water stressed conditions should focus on improved water uptake during the grain filling period which involves increasing maximum rooting depth and reducing root vigour at later growth stages which can deplete soil resources early in the growth season. Improved water use efficiency during this growth stage can improve grain yield to 55 kg/ha per mm of water used (Hamblin and Tennant, 1987; Manschadi et al., 2006). Hurd (1969) recognised the importance of root architecture in breeding and stated that parents selected for crosses, to be grown in a semi-arid climate, should have a dense root system with greater rooting at depth as well as the ability of the roots to penetrate the soil rapidly. Currently, this is done by selecting well adapted parents with diverse origins and testing as many lines as practically possible at several locations over several years. Mapping populations are used once a donor for a specified trait has been identified. Mapping populations are an important tool for breeding programmes and are usually the F<sub>2</sub> progeny of two genotypes, which differ for an identified trait/traits. Other examples of mapping

populations include backcross lines and near isogenic lines for a specific trait of interest, recombinant inbred lines and doubled haploid lines (Singh and Singh, 2015). Genetic diversity within the population for the trait allows for identification of quantitative trait loci which can identify molecular markers that are linked to genes of interest and subsequently allow marker assisted selection. The sister lines share a common ancestry and this prevents genetic differences shadowing proxy effects of the identified trait (Wasson et al., 2012). A proxy effect is an easily measured variable which can serve as a substitute or indication of a variable which is unobservable or more difficult to measure; such as water uptake for deep rooting.

# 1.4. Genotypic differences in root morphology and physiology1.4.1. Origin of wild relatives of hexaploid wheat

Wild emmer (*Triticum dicoccoides*, AABB) is thought to have originated between 200 and 500 thousand years ago in the fertile crescent of the Near East (Huang et al., 2002). Produced from the natural hybridisation of two wild diploid grasses – an unknown relative of *Aegilops speltoides* and *Triticum urartu*, closely related to einkorn wheat. The domesticated version of wild emmer (*Triticum dicoccum*) is the progenitor of modern hexaploid bread wheat, having crossed with another wild grass (*Aegilops tauschii*, DD) about 6,000 BC to produce *Triticum aestivum* (AABBDD). Fertility and genetic stability of this hexaploid wheat is due to the presence of the Ph1 gene which prevents pairing of chromosomes from within the same homologous groups (Griffiths et al., 2006). The diversification of *T. dicoccum* formed the modern pasta wheat durum, *T. turgidum* subsp durum (Van Ginkel and Ogbonnaya, 2007; Zhao et al., 2005). Due to the origin and expansion of the wheat crop lineage in a semi-arid climate, wild emmer has a high drought tolerance with some genotypes known to thrive in arid desert

environments (Budak et al., 2013). Natural populations of *T. dicoccoides* can be found in Israel, Syria, Jordan, Lebanon, northern Iraq and western Iran, and constitute a rich genetic resource for wheat improvement with physiological traits which aid resistance to abiotic stresses such as drought (Nevo, 2014; Xie and Nevo, 2008).

The domestication of crop plants from their wild progenitors has led to the erosion of original genetic resources, potentially limiting the genetic diversity of modern crop species (Singh and Upadhyaya, 2015). The polyploidisation of hexaploid wheat from the chance hybridisation of wild emmer and A. tauschii has been estimated to have resulted in the loss of 10 to 17% of genes (with an estimation of the hexaploid wheat genome containing between 94,000 and 96,000 genes) compared to the three diploid progenitors; AA from T. urartu, BB from an unknown A. speltoides relative and DD from A. tauschii (Brenchley et al., 2012). Modern cultivated species are better suited to managed environments and have a lessened capacity to adapt to stress conditions (Budak et al., 2013; Xie and Nevo, 2008). Waines & Ehdaie (2007) compared the root systems of landraces and early, mid and late green revolution bread wheat cultivars. The difference between these old and modern cultivars was significant, with the root biomass of early green revolution wheats being less than two-thirds that of some of the landraces. Zhao et al. (2005) studied root systems of six wheat genotypes with different ploidy chromosome sets and showed that area and length of roots decreased with increasing chromosome ploidy, also demonstrated using root biomass by Li et al. (2014). In addition to this, hydraulic conductivity of the wheat root system increased during wheat evolution, with water uptake ability from wild to modern cultivars strengthening. However, this makes modern wheat varieties vulnerable to water stress. In dry environments crop plants must save available soil water for the important growth stages such as anthesis (R. A. Richards and Passioura, 1981). The increase in water uptake ability and economic use of water for yield components between modern and landrace wheats can aid genetic breeding to improve crop water use efficiency (Zhao et al., 2005).

Analysis of the genetic diversity within wild emmer populations occurring along a natural aridity gradient in Israel and its surrounding regions, identified a 56% genetic variation among 145 genotypes from 25 different populations and a 44% genetic variation between populations. This diversity was found to be independent of geographical location (Nevo and Chen, 2010). However, a study utilising the Cultivated Wheat Collection (CWC) at CIMMYT (International Maize and Wheat Improvement Centre) consisting of 297 genotypes from a wider geographic range of 27 countries, found that rooting at depth varied significantly depending on the country of origin (Fig. 1.10). Spring wheat varieties from Australia, the Mediterranean and west Asia (including Turkey, Jordan and Iraq, where wheat originated), rooted deeper than cultivars from south Asia, Mexico and Canada (Narayanan et al., 2014). This study indicates the opportunity of locating useful genotypes within certain geographic regions to improve adaptive traits of modern cultivars.



Figure 1.10. Average rooting depth of spring wheat genotypes (A) originating from different regions of the world. (B) Dry region included Argentina, Armenia, Australia, Chile, Egypt, Germany, Iraq, Japan, Jordan, Kenya, Lebanon, Libya, Mexico, Pakistan, South Africa, Turkey, and USA states of Arizona, California, Colorado, Idaho, Montana, Nebraska, Nevada, Oregon, Utah, and Washington. Humid region included Bangladesh, Brazil, Canada, Colombia, Guatemala, India, Nepal, Paraguay, Russia, Uruguay, and USA states of Indiana, Minnesota, North Dakota, Oklahoma, South Dakota, Vermont, and Wisconsin. Error bars are standard error (Narayanan et al., 2014).

## 1.4.2. Genetic diversity of wild relatives

Wild wheat relatives related to modern cultivated varieties hold much potential to improve agronomic traits of current wheat crops, such as for abiotic and biotic stress tolerances, protein quality and quantity and micronutrient contents (Xie and Nevo, 2008). Peleg et al. (2005) characterised the genetic diversity for drought tolerance in wild emmer wheat by examining 25 populations of wild emmer under well-watered (650 mm) and water-limited (250 mm) irrigation regimes. The results indicated a large

genetic diversity within and between wild emmer populations, with the majority of accessions having greater productivity in terms of spike and total dry matter, compared to their cultivated durum wheat counterpart controls in water-limited conditions. Of the 25 wild emmer populations, seven exhibited low drought susceptibility with high performance in both the dry and wet irrigation regimes (Peleg et al., 2005). Early reproduction (fewer days from planting to heading) and a longer time period from heading to maturity was well correlated (P < 0.05) with high spike and total dry matter; this is thought to aid terminal drought escape. Wild emmer accessions also exhibited greater plasticity for water use efficiency as indicated by greater carbon isotope discrimination in the dry treatment, compared to the cultivated controls (Peleg et al., 2005). Carbon isotope discrimination is the ratio of stable isotopes of carbon ( $^{13}C/^{12}C$ ) in plant material relative to the atmosphere. Discrimination of  $^{13}C$  decreases with water stress because of lowered stomatal conductance (Farquhar et al., 1989)

Jing et al. (2007) studied the diversity in agronomic traits of the wheat landrace einkorn (*Triticum monoccocum*). Einkorn wheat was one of the earliest domesticated wheat species, abandoned during the Bronze Age and now grown in detached areas of Europe and Morocco for its high nutritional value and pest resistance (Zaharieva and Monneveux, 2014). By analysing 30 accessions of *T. monoccocum* from a variety of geographical origins, large genetic diversity of multiple traits was found, such as grain storage protein, germination under drought and salt stress and endosperm texture. Identified genetic diversity had a limited relationship with geographical origin indicating that although this landrace has been widely spread since domestication, it hasn't evolved genetically to a large extent in the past 10,000 years (Jing et al., 2007). As well as wild emmer, einkorn wheat is an important source of alleles for future wheat breeding. Loci of *T. monoccocum* have been successfully introduced into bread wheat

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cultivars to improve traits such as pre-harvest sprouting, leaf rust and powdery mildew resistance as well as increasing zinc uptake efficiency (Jing et al., 2007). In a two year field study comparing several wheat genotypes, the underutilised diploid and tetraploid wild relatives *T. monococcum* and *T. timopheevii* showed greater potential and plasticity in rooting density compared to seven durum wheat varieties. These genotypes had larger rooting densities throughout the soil profile in well-watered conditions and partitioned a greater proportion of roots to deeper soil layers under drought conditions. This is thought to have been achieved through their lower specific root volumes (root dry mass/root volume) thereby lowering the metabolic cost of the roots and allowing increased soil exploration (Nakhforoosh et al., 2015).

Genome analysis and identification of quantitative trait loci (QTL) in these wild progenitors has furthered the genetic bases and associations for these agronomic traits. Peleg et al. (2009) studied the genetic and physiological basis of a tetraploid wheat population (152 recombinant inbred lines) produced from a cross between durum wheat and wild emmer. Multiple QTL were identified under water-limited and well-watered conditions with major genomic regions on chromosomes 2B, 4A, 5A and 7B involved in productivity and its related traits. Productivity was defined in terms of grain yield, spike dry matter, total dry matter and harvest index, with genomic regions on 2B and 7B being specifically associated with higher grain yield under water limited conditions (Peleg et al., 2009).

# **1.4.3. Synthetic hexaploids**

Production of synthetic hexaploids is a method of introducing the genetic diversity from *A. tauschii* (DD) and *Triticum turgidum* (AABB) into modern wheat cultivars. This process recreates the chance hybridisation event that occurred around 10,000 years ago,

by crossing a modern wheat species (a tetraploid such as domesticated emmer or durum wheat) with A. tauschii (a diploid) (Kihara, 1944). Chromosome doubling then takes place to produce a hexaploid which can be crossed with any modern wheat variety (Ram et al., 2007). This is an important advantage of synthetic hexaploid wheats, as they can be backcrossed with elite wheat cultivars to produce synthetic backcross lines that eliminate unfavourable alleles (Zhang et al., 2005). The genetic diversity of the wild types, can be successfully introgressed into synthetic hexaploids as shown by Lage et al. (2003) who studied the genetic diversity of synthetic hexaploids derived from T. diccocum and A. tauschii parents. The genetic diversity of the synthetic hexaploids, determined using polymorphism markers, was 39% compared to the 12% found within winter and spring wheat controls derived from the Pacific Northwest United States. The markers found groupings related to the geographic origin of the T. diccocum parent, but no groupings related to the A. tauschii parent. The geographical location of the lines allowed them to be grouped according to traits such as height, number of spikelets per spike, time to anthesis, kernel weight and length and size of flag leaves (Lage et al., 2003).

Synthetic hexaploid wheats have proved beneficial in improving quality traits of modern wheat cultivars, indicating the potential that these wild crosses can contribute to wheat breeding. Lage et al. (2006) evaluated the grain quality of 58 synthetic hexaploid wheats with a wild emmer parent. Compared to bread wheat controls the synthetic hexaploids had significantly higher protein contents and sodium dodecyl sulphate (SDS) sedimentation values. The SDS test is based on evidence that wheats of different baking quality differ in the swelling, viscosity and sedimentation of its gluten (Morris et al., 2007). These traits were specifically linked with the wild emmer parent as regressions of the synthetic hexaploid quality traits and the corresponding quality traits

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of the wild emmer parents explained 8.7% of the variation in protein content and 28.8% of the variation in the SDS sedimentation values (Lage et al., 2006). Similar findings for these wheat quality traits were found by Ram et al. (2007), except the parent of these synthetic hexaploids was *T. turgidum*. Average protein content of the 75 synthetic hexaploid lines was 13.3% and positively correlated with grain weight and diameter, which is important for wheat breeding as protein content can potentially be improved without a negative effect on grain weight (Ram et al., 2007).

CIMMYT has played a large part in trialling and providing successful synthetic hexaploid wheats to breeders around the world. Trials in 2001 to 2003 identified globally competitive synthetic hexaploid wheats that matched or exceeded yields of the highest performing common wheats (Van Ginkel and Ogbonnaya, 2007). Under drought conditions, synthetic hexaploids increased yields in India, Pakistan, Ecuador and Argentina by 5 to 40% (Van Ginkel and Ogbonnaya, 2007). Yield advantages were also found among 134 synthetic backcross derived lines and ten Australian wheat cultivars studied in a diverse range of environments in Australia. Yield advantages of the synthetic backcross derived lines were very dependent on the environment but ranged from 8 to 30% greater than the best local cultivars, demonstrating the potential of synthetic hexaploids to significantly increase modern crop yields (Ogbonnaya et al., 2007). CIMMYT has been specifically exploiting the D genome of A. tauschii as it is thought that during the initial hybridisation of hexaploid wheat, few accessions of this wild goat grass contributed to the genetic makeup of modern wheat. The study of the diverse range of A. tauschii accessions has the potential to add new genetic material to the wheat genome (Gooding, 2009; Lage and Trethowan, 2008).

Lopes & Reynolds (2011) studied the response of four elite synthetic derived lines with their corresponding parents in irrigated and drought conditions. Variation was seen in the response of synthetic lines to drought which included earliness to flowering, greater root mass at depth and greater water use efficiency at anthesis, which contributed to the 26% increase in yield of the synthetic derived wheat lines compared to the parental wheats (Lopes and Reynolds, 2011). Vorobey was one of the best performing lines and the only elite line to have significantly greater root density at depth (90 to 120 cm). Vorobey was bred by crossing conventional hexaploid wheat with non-elite synthetically derived wheat lines in order to produce a cultivar with stress adaptive traits that still permits a high yield in favourable seasons. Compared to its parents Vorobey exhibited an additional 3.5 g/m<sup>2</sup> root biomass at depth which allowed it to increase its water uptake by 4.7 mm at anthesis compared to its parents (Lopes and Reynolds, 2011). In a separate study Becker et al. (2016) studied six synthetic wheat lines and four locally adapted wheat cultivars in drought stressed and well-watered conditions in soil columns. Their results agreed with those of Lopes and Reynolds (2011), synthetic wheats had improved rooting at depth, particularly of fine roots, in the drought stress treatment (Fig. 1.11). Synthetic wheat line SYN-172 had relatively small reductions in above ground biomass with large increases in root biomass. Stomatal conductance and water use in the drought treatment had a significant positive relationship with fine roots; total root biomass in the bottom section of the tube did not show a significant relationship with stomatal conductance in either treatment. This indicates the importance of fine roots in water uptake in dry conditions (Becker et al., 2016).



Figure 1.11. Distribution of root biomass in the top, middle and bottom root tube sections for each treatment in the well-watered (WW) and drought stressed (DS) treatments. TRBM; top root biomass (0-33 cm), MRBM; middle root biomass (33-66) and BRBM; bottom root biomass (66-98). SYN; synthetic hexaploid wheats and Byrd, GS, Hatcher and Ripper are locally adapted wheat cultivars (Becker et al., 2016).

# 1.4.4. Breeding for improved rooting at depth

Breeding focused on improving rooting in wheat first occurred in the 1960s to produce a drought tolerant wheat variety for the semi-arid growing areas of the Canadian prairies. The cultivar Pelissier had higher yields under water stressed conditions attributed to its reduced rooting in the top 7.5 cm of soil and more extensive rooting deeper in the soil profile (Hurd, 1974). Subsequently Pelissier was used in a breeding program to produce a new variety, Wascuna, which had a similar rooting pattern to Pelissier but out-yielded two Saskatchewan local check varieties by 15 and 9%. It also out-yielded Pelissier by 9% but had equal yields in drought conditions (Hurd et al., 1972).

To improve rooting of wheat cultivars under water-stressed conditions, Placido et al. (2013) introgressed chromosome 7E from the wild relative *Agropyron elongatum* into cultivated wheat to produce the 7DL.7EL translocation line. This introgression had

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been previously used to improve leaf rust resistance, but has also been shown to increase biomass and yield in numerous wheat backgrounds. Translocation lines grown for 18 days had significantly higher root and shoot biomass under water stressed conditions and less of a reduction in photosynthetic rates and stomatal conductance compared to the parent line and negative control. Placido et al. (2013) also identified the down-regulation of the KNAT-3 gene in the translocation line compared to the parent line and negative control. This gene has been identified in Arabidopsis to negatively regulate lateral root development so that the translocation lines produced more lateral roots and increased root biomass (Placido et al., 2013). Improved rooting has also been observed in 18-day-old wheat plants with the identification of a major QTL for root length in common wheat. qTaLRO-B1 has been mapped to a 0.9 cM interval on the short arm of chromosome 2B. Isolines differing for the qTaLRO-B1 allele were developed and showed that the presence of the allele caused increased root length, biomass and subsequently improved phosphorus uptake in a hydroponic system. Root surface area differed significantly between isolines with  $(69.7 \text{ cm}^2)$  and without  $(36.7 \text{ cm}^2)$  the allele (Cao et al., 2014).

Breeding for deeper rooting in rice was achieved with the successful identification of a QTL promoting narrow root growth angles, subsequently leading to greater maximum rooting depth which can improve drought tolerance of modern rice cultivars (Uga et al., 2013). Near isogenic lines (NIL) containing the DRO1 QTL had more than twice the maximum rooting depth of shallow rooting rice but no significant differences in shoot characteristics or root dry weight, indicating that DRO1 only influences changes in root distribution. DRO1 is associated with cell elongation and causes asymmetric root growth at the root tip; increased expression of DRO1 causes more downward bending of the root (Uga et al., 2013). Under moderate drought stress, DRO1-NIL had similar

grain weights to unstressed conditions whereas shallow rooting rice had grain weight reductions up to 43%. Additionally, in severe drought the percentage of filled grain in shallow rooting rice was close to zero whereas DOR1-NIL rice had more than 30% filled grain (Uga et al., 2013).

# **1.5.** Methods for the analysis of wheat root systems

#### **1.5.1.** Key root system parameters

Methods used to study the root systems of crop plants include both destructive and nondestructive approaches. More recently developed methods try to address the time consuming issue that partners root work and to reduce the error associated with uncovering the root system. This includes efforts to image roots in-situ, within the soil, using x-ray microtomography (Hargreaves et al., 2009) and root observation chambers, which reduce some of the limitations of excavation methods (Zhu et al., 2011). When investigating the properties and functions of a root system available time, equipment and labour may be limiting factors. Therefore it is important to measure parameters that relate to the functional objectives of any project as well as those parameters which have the ability to be converted into useful quantities such as combining root length and soil volume measurements into root length density (Atkinson, 2000).

It is important to identify root system parameters that are related to functional significance and therefore allow a link between root measurement and function. Some examples of important root system parameters include root length (the basis for most functional calculations including the inflow rate of water and nutrients); root dry mass (which indicates partitioning of carbon to the root system as well as soil exploration); and branching pattern (which can be combined with root diameter and length to give a

more comprehensive view of a plant's root architecture) (Atkinson, 2000; Bengough et al., 2000).

#### **1.5.2. Field methods for root analysis**

Methods for studying roots in the field are either disruptive or destructive but due to the plasticity of the root system it is difficult to reproduce the root system of natural crop systems with confidence in the lab or in artificial growth media. Therefore, observing the root status in its natural functioning environment is advantageous when applying the data and conclusions to commercially relevant systems. Such methods produce inherently variable data due to the spatial variability of root systems as well as error added during the extraction of roots from the soil. Experimental errors can be reduced through increasing replication and careful sampling (Bona et al., 2000). In addition to sampling errors, the root system is affected by genotype x environment interactions. This effect on rooting traits was assessed by Acuna and Wade (2012) who studied root depth in 24 wheat genotypes in six different field environments differing in soil physical properties. Genotype accounted for only 12% of the variance in rooting depth with the genotype x environment interaction accounting for 40%. When the different field environments were grouped by soil physical characteristics (low, medium and high soil strength) the wheat genotypes could be clustered into six groups that accounted for 72% of the genotype x environment interaction. Principal component analysis with soil physical characteristics accounted for 54% of the variation in root depth (Acuna and Wade, 2012).

The main root parameters obtained from field measurements are usually biomass and root length per volume of soil. The most popular method for estimating the volume of soil exploited by the plant root system, throughout the soil profile, is the auger method. This involves coring a soil sample, usually of 5 to 8 cm diameter, by hand or mechanically to a desired depth in the soil profile. Roots are hand washed from the soil, which can cause error due to loss of fine roots or incomplete separation of roots. Some authors add a correction factor of between 1.4 to 2 which can negate dry weight losses of between 30 and 50% (Bona et al., 2000). The measurements obtained are typically the balance between new roots produced and older roots that have died so that the destructiveness of field measurements and the spatial variability of root systems do not allow estimates of root turnover from frequent measurements (Bona et al., 2000).

An alternative method of measuring root parameters in the field involves observation techniques, which attempt to quantify root system properties and characteristics in situ. The profile wall method is not practical on smaller sites as it requires digging a wide trench into the soil and from visible observations it is difficult to distinguish between live and dead roots. However, profile walls do give a good indication of soil exploration as well as the interaction of the roots with the soil environment, such as the chemical, biological and physical characteristics (Van Noordwijk et al. 2000). This method only observes a small aspect of the root system but through excavating shallow boxes from multiple locations in the profile wall, the root length density (RLD) behind the wall may be estimated (Van Noordwijk et al. 2000). An alternative method of root observation in the field is the use of mini-rhizotrons, a transparent tube inserted into the soil at an angle in which observation equipment can be placed to view plant roots. The advantage of this method is that roots can be observed over time, allowing information on elongation rate and root turnover to be obtained. However, issues arise due to the disruption to the soil profile through inserting the mini-rhizotron, such as a change in soil structure and the formation of voids which can aid root growth (Smit et al., 2000; Zhu et al., 2011). These factors have contributed to the commonly observed result that

mini-rhizotrons demonstrate lower root lengths in the surface soil and greater root lengths at depth, compared to soil cores (Wiesler and Horst, 1994).

The use of isotopes in root studies allows more detailed detection of the transport of nutrients and in particular the partitioning of carbon, which gives information on plant resource allocation. In addition to this, isotopes are able to identify the detailed location of roots within the soil profile through distinguishing the root tissue from the surrounding soil (Bingham et al., 2000). The measurement of stable isotopes, such as  ${}^{13}CO_2$ , commonly uses mass spectrometry which is a very sensitive technique that ionises a sample and uses its mass-to-charge ratio to identify isotopes and their quantities (Bingham et al., 2000). This technique can also be used to measure root water uptake as shown by Wang et al. (2010) who used the measure of oxygen ( $\delta^{18}O$ ) and hydrogen ( $\delta D$ ) isotopes to assess the location of water uptake in the soil profile for maize and cotton crops in China. Stable isotopes of oxygen and hydrogen do not fractionate during root water uptake; therefore the source of water obtained in the plant xylem can be deduced with this technique by comparing hydrogen and oxygen isotopes in potential water sources with that found in the xylem (P. Wang et al., 2010).

# **1.5.3.** Sampling strategies for estimating field crop root distributions

To ensure accurate and reliable field root distribution estimates, a good sampling strategy is necessary to minimise bias due to sample number and location; this is often neglected in many field root studies (Van Noordwijk et al., 1985). As previously mentioned, the heterogeneous nature of the soil environment and the plasticity of the root system makes it difficult to produce representative core samples in field row crops. Van Noordwijk et al. (1985) was one of the first to suggest the minimum number of replicate samples necessary to identify significant differences between two sets of treatments. This was based on the equation:  $d/m = [(s/m)2^{1/2}t]/n^{1/2}]$  where d is the difference between the two means and m is the mean. The coefficient of variation is s/m (standard deviation divided by the mean) and t is the critical value of t in a t-test for n number of replicates. This equation estimates the minimum number of samples necessary to achieve a 50% chance of obtaining a significant result if the difference between two means is of size d (Van Noordwijk et al., 1985). A normal coefficient of variation between root samples of grasses and cereals is around 40%, which according to the above equation means that a minimum of 10 samples are needed to identify a 35% difference between two means (Van Noordwijk et al., 1985).

Sample location is also important when trying to produce representative root core samples. It is suggested that for a wheat crop in rows 22 cm apart, two auger samples of 7.5 to 10 cm diameter extracted from within the row and midway between the rows, will give the best approximation of mean root length density. This is based on a bias between actual and observed root length densities obtained using soil monoliths and soil cores (Kumar et al., 1993). However, by simply averaging root length densities obtained from cores within and between rows, the actual value can be overestimated by as much as 30% (Bengough et al., 2000). A lower bias can be calculated when a weighted scheme of 1:3 is used for root length densities (RLD) within rows and between rows: mean estimated root length density = (RLD within row + (RLD between row x 3)) / (1+3), first suggested by Van Noordwijk (1985). Using this ratio to estimate root length densities, Buczko (2009) concluded that an average of eight soil cores are necessary to reasonably estimate root length density in maize crops (Fig. 1.12).



Figure 1.12. Schematic diagram showing hypothetical within and between row auger samples for wide (horizontal blocks 2 and 5) and narrow (horizontal blocks 2 and 4) row spacing, with respect to the location of soil monolith sample (grey filled cubic boxes). The location of crop plants is denoted by black-filled rhombic symbols for the wide and by white-filled circular symbols for the narrow row spacing (Buczko et al., 2009).

# 1.5.4. High-throughput field root analysis methods

Due to the substantial time commitment required for root studies in the field, alternative methods have been developed for more high throughput measures of root systems in this environment. One of these methods involves quantitative real-time polymerase chain reaction (qPCR) to measure plant root DNA concentrations directly from soil samples. This allows estimation of root dry weight and root distribution through the soil profile. It has proven to correlate strongly with measured root fresh weight and is also highly reproducible (Huang et al., 2013; Mommer et al., 2008). This method also reduces the error of including dead roots in field measurements as more than 90% of plant DNA degrades within 10 days after death in soils (Huang et al., 2013). The qPCR method involves amplifying plant DNA target sequences. Huang et al. (2013) used internal transcribed spacers (ITS), which are non-functional RNA, located between

structural rRNA and occur in tandem repeats. This is a suitable technique to measure plant DNA in the soil as these ITS exhibit large levels of variance between species (Huang et al., 2013; Mommer et al., 2008). Huang et al. (2013) used this DNA based method to study the response of different wheat genotypes to drought stress in a field environment. The qPCR method and ITS showed significant genotypic differences, with a reduction in root DNA density in the drought treatment compared to the wellwatered treatment. However, careful and site specific calibration is needed to estimate root biomass and root length from root DNA density values as the distribution of live plant root cells will change with soil depth, plant age and field site. Additionally, the genotypes studied had diverse genetic backgrounds; therefore the use of this method to compare a mapping population is questionable (Huang et al., 2013).

The core break count (CBC) method described by Van Noordwijk et al. (2000) and used by Wasson et al. (2014) can speed up root length studies in the field to allow for more samples to be taken to reduce error or to study field rooting traits in mapping populations. The method involves breaking cylindrical soil cores at different depths and counting the visible roots on each side of the break, as the same root cannot appear on both sides of the break (Fig. 1.13). This is due to root break most likely occurring within the core rather than in the plane of observation (Van Noordwijk et al., 2000). It is important to break the core rather than slice as this will only leave very small crosssections of roots which are difficult to see. The colour and characteristic of a root determines whether it is from the current crop, as a live root is paler and more flexible than a dead root (Wasson et al., 2014). These values can be correlated with actual root length density to calibrate the CBC values and convert to cm/cm<sup>3</sup> (Van Noordwijk et al., 2000). Wasson et al. (2014) converted CBC values to RLD in cm/cm<sup>3</sup> using the equation: n(corr(CBCn+CBCn+1, RLDn)) where the sum of CBC of depth n and the CBC at depth n+1 was correlated with the RLD for depth n. Wasson et al. (2014) assessed a diverse set of germplasm of spring wheats in three different field environments using the CBC method. The correlation of CBC and RLD differed between sites with an r<sup>2</sup> value of 0.8 being found at one site but only 0.53 at a site close by. However, with the use of the CBC method significant variations were found for the rooting traits of maximum depth and descent rate. Although genotypes differed between sites, genotypes that performed consistently well or badly on each site were identified (Wasson et al., 2014). Rich et al. (2016) also used the CBC method to compare rooting traits in 49 high yielding Indian wheat varieties and 26 high yielding Australian varieties from the wheat growing areas of the north and south west. Using this method significant differences between genotypes were found at two different sites for rooting at depth, with the Indian varieties achieving greater total root length, root penetration rate and rooting depth (Rich et al., 2016).



Figure 1.13. Coring and the core break method. (A) Root sampling with a 2 m long steel coring tube driven into the ground using a tractor mounted push press. (B) A soil core emptied into a cradle, the core has been scored every 10 cm to aid breakage. (C) The broken face of a soil core segment, the number of visible roots are highlighted with white arrows (Wasson et al., 2016).

Shovelomics is an additional high-throughput root analysis method which allows the study of root systems in the field. This technique derives its name from the use of a shovel to excavate the top 10-20 cm of a crop's root system to study branching patterns, root number and root angle. Trachsel (2010) introduced this technique to compare rooting in three maize recombinant inbred line populations. Excavation and visual measurement of the maize root crowns took 5-10 minutes per sample to assess branching of brace roots and the number, angle and branching of crown roots. Crown root angle had a repeatability of 67% between the three sites studied, indicating the stability of this method across environments. The method identified differences between the populations with shallow crown root angles being found to be associated with high branching and density of brace roots (Trachsel et al., 2010). Based on this method Bucksch et al. (2014) designed an image and analysis system which speeds up the process and also identifies additional crown root traits (such as diameter) in maize plants. This involves placing the crown root on an imaging board with a specific sample number barcode and a circle of known diameter which allows calculation of units from the image. Traits which can be assessed using this imaging technique are nodal root length and diameter, average lateral length, diameter and branching (Bucksch et al., 2014).

#### 1.5.5. Root growth in different media

Laboratory root studies have grown plants in different media to improve screening for root phenotypes. Specifically, agar gel is favoured because of its transparency and ease of production. However, the phenotypic plasticity of root systems in different environments makes it difficult to compare results from these studies to the field, or even to plants grown in soil media in the lab (Bengough et al., 2004). The significant growth difference, in terms of root length, between soil grown plants and those grown in gel chambers was shown by Wojciechowski et al. (2009) who measured the effect of dwarfing genes on seedling root growth of wheat. Dwarf lines, compared with semidwarf controls, grown in gel chambers showed a significant 40% increase in root length but in soil dwarf lines had a reduced root length of 24-33% (Wojciechowski et al., 2009). Similar changes in rooting extent were seen in barley seedlings grown in gel chambers and soil sacs (Hargreaves et al., 2009). Seedlings exhibited significantly greater total root length and average diameter when grown in a gel chamber, compared to those grown in soil. However, number of vertical axes produced by seedlings was unaffected by the growth media and barley seedlings had the same number of roots and angular spread in both the gel chamber and soil sacs (Hargreaves et al., 2009). These differences are thought to be due to soil causing greater physical impedance on root growth in addition to a lower nutrient concentration in gels, promoting root length (Hargreaves et al., 2009; Wojciechowski et al., 2009).

A recent development in growth media used for root studies is the development of 'transparent soil'. Presented by Downie et al. (2012), the medium is described as heterogeneous, transparent and porous, mimicking properties of soil which isn't possible using gel. The substrate is made from the polymer Nafion which has a similar refractive index to water and therefore allows the addition of an aqueous nutrient solution with a matching refractive index, without causing refraction of light between the boundary of materials when imaging (Downie et al., 2012). Fluorescent dye can be added to the transparent 'soil' to study pore spaces and geometry, which can be applied to monitor the characteristics of root growth. The growth media was tested using lettuce roots and it was found that plant growth in the transparent medium was similar to that in
soil with a mean root diameter of 0.24 mm in soil, 0.24 mm in sand, 0.18 mm in phytagel and 0.28 mm in the transparent medium. Comparison of growth between media found that plants grown on transparent 'soil', soil, or sand had significantly more lateral roots and a higher biomass than plants grown on phytagel. However, lettuce roots grown in the transparent medium had 40 mm less lateral root length as well as about 15 mm greater primary root length compared to those plants grown in soil (Downie et al., 2012). Therefore, although plants grown in transparent 'soil' show similar characteristics to those grown in soil, it doesn't solve the problem of increased root length in this growth media.

Growing cereal genotypes within tall columns or boxes is advantageous to study the root system throughout the soil profile and allows the manipulation of growth conditions within the soil. Carvalho et al. (2014) grew durum and barley cultivars in 0.15 m diameter x 1.5 m tall columns to study the response to drought. The columns allowed the manipulation of soil bulk density and irrigation treatments based on the evapotranspiration estimated through gravimetric analysis. Water use efficiency of the crop plants could be measured based on the soil moisture at different growth stages, assuming soil evaporation and drainage were zero. Total root length, volume and density were measured through destructive sampling of the plants (Carvalho et al., 2014). Alternatively, root observation chambers, rectangular root boxes with a clear front, allow the rooting profile to be mapped or imaged over time to study and compare specific root architectural traits. Liao et al. (2006) used glass-walled growth boxes, which were 1 m deep, 0.24 m broad and 0.1 m wide, to study the effect of vigorous roots on nitrogen uptake (Fig. 1.14). Soil nitrogen content was controlled in different soil layers, separated with waxed paper supported by a wire mesh, to study the location of nitrogen uptake and effect on root biomass. Laying the boxes at a 30° angle caused the roots to grow against the clear front and they could then be traced every few days to monitor growth and architecture (Liao et al., 2006). This growth box method was also used to study the effect of reduced tillering in wheat on root biomass and early nitrogen uptake. Root length, density and number were compared between tillering and reduced tillering genotypes through tracing rooting through the clear front of the box. Root shoot relationships and nitrogen content of the canopy were also studied and compared between genotypes (Palta et al., 2007).



Figure 1.14. Diagram of glass-walled root box used by Liao et al (2006).

#### **1.5.6.** Root screening methods

Currently, the most advanced screening method for root phenotypes is x-ray microtomography, which can produce 3D images, non-invasively, of root systems growing in soil. However, this method is not able to measure a large number of root systems at a time because of constraints on scanning, caused by the time taken to obtain

a necessary signal to produce a fine image. Therefore this method is not suitable for high throughput screening, such as for mapping populations (Gregory et al., 2009). A more suitable screening method for root phenotypes within a large population involves a paper seedling screen which can correlate with plants in the field and identify quantitative trait loci (QTL) for root traits. This method uses germination paper rolls wetted with a nutrient solution in a controlled environment (Bai et al., 2013; Watt et al., 2013). Although these seedling root screens may not correlate with mature or flowering plants in the field, they can provide information on early root architectural traits such as total root length, root dry weight and seminal root architecture (Bai et al., 2013). Watt et al. (2013) tested the correlation between seedlings in the root screen and plants from the same population grown in the field. Root traits exhibited at the two leaf stage within the seedling screen were able to select for early vigour and biomass of plants in the field up to the early stem extension stage, and root length within the seedling screen correlated well to plants in the field at the two leaf and the five leaf stage (Watt et al., 2013). Bai et al. (2013) used a seedling root screen to identify the effect of *Rht* genes on seedling root growth in a diverse set of wheat germplasm. Significant differences were found between lines in early seminal root architecture, and seedling root QTL correlated with mature plant height and thousand grain weight QTL determined in lines grown in the field (Bai et al., 2013). Atkinson et al. (2015) designed a high throughput root phenotyping pipeline to study seedling root QTL in wheat populations. Seeds were grown in paper growth pouches supported within a frame where the pouches were held within a nutrient solution in a growth chamber, and could be transported to a copy stand for imaging. The design allowed for 360 plants to be imaged in 3 hours and these images were analysed using RootNav software, taking 2 minutes per image. Seedling traits were analysed by the software and included length of seminal and lateral roots,

maximum depth of root system, angle of emergence between seminal root pairs at the first, second and third quartile of the total length and the maximum width of the root system (Atkinson et al., 2015). The same population was grown in the field over two seasons and in three different sites but seedling root traits did not show strong relationships with mature traits of height, grain yield and nitrogen uptake. However, one QTL for nitrogen uptake did co-locate on 7D with total root length and total length of seminal roots (Atkinson et al., 2015).

A recent alternative high throughput seedling root experimental method has been designed by Richard et al. (2015) which involves growing seeds in a soil substrate in clear pots, specifically to measure number and angle of seminal roots. In a 4 l clear pot filled with soil/compost, 24 seeds were sown around the edges and after 11 days seedlings were imaged to analyse root angle. Seedlings were removed from the soil to measure the number of seminal roots; this approach proved more accurate than counting from the images, as the soil can cover some of the roots. Roots can clearly be distinguished from the darker soil and seminal root angle was highly correlated between experiments ( $r^2$ =0.82) and was more heritable ( $h^2$ =0.65) compared to a growth pouch method on the same wheat material ( $h^2$ =0.52) (Richard et al., 2015). This method has been successfully implemented to study root angle of barley seedlings as a proxy for improved drought tolerance. Seven QTL for root angle and number of seminal roots were identified with a strong QTL on 5HL explaining a significant proportion of variation in root angle and number (Robinson et al., 2016).

A contrasting high throughput phenotypic screening method for root traits measures canopy properties associated with the functioning root system in the field. The importance of trying to screen for desired root traits in cereals derives from the fact that water uptake during the reproductive growth stages determines seed set and grain filling. Water absorbs energy at specific wavelengths, thought to be specifically associated with light absorption at 970 nm. Therefore, specific reflectance indices have been suggested as means of predicting crop water content within the canopy which can help to identify those plants with a deeper root system accessing subsoil water (Peñuelas et al., 1993b). Gutierrez et al. (2010) tested the ability of different water spectral indices to estimate plant water content and subsequently identify wheat plants better adapted to drought conditions. Water spectral indices are based on near infrared (NIR; 700-2500 nm) wavelengths, which penetrate deeper into the crop canopy than other wavelengths in the visible spectrum and can therefore estimate crop water content. Gutierrez et al. (2010) identified a normalised water index (NWI) that exhibited strong relationships with soil and leaf water potential as well as canopy temperature. This NWI used the calculation [R<sub>970</sub>-R<sub>880</sub>]/[R<sub>970</sub>+R<sub>880</sub>] and could distinguish genotypic differences in wheat germplasm in terms of drought resistance under different levels of water stress during reproductive growth stages. With more negative values being associated with lower reflectance of light at these wavelengths and increased water content (Gutierrez et al., 2010). The correlation between the NWI and soil water potential shows how this high throughput phenotyping technique can be used to estimate the crop's ability to exploit the soil and soil water.

# **1.6. Introgression of wild emmer** (*Triticum dicoccoides*) into Shamrock

#### 1.6.1. Non-glaucous trait

Shamrock is a UK group 1 bread making wheat, derived from a cross between a *Triticum dicoccoides* derivative and adapted UK Germplasm; it is an example of using genetic material from wild wheat relatives to produce modern cultivars (Simmonds et al., 2008). Shamrock is known to exhibit traits derived from the wild emmer

introgression which includes a non-glaucous trait that is regularly seen in wild emmer but uncommon in domesticated emmer and modern cultivars. It causes reduced cuticular wax on the spikes, leaves and stem and gives Shamrock a characteristic green colour to its canopy (Fig. 1.15). Simmonds et al. (2008) mapped this phenotypic trait to the short arm of chromosome 2B, the same location as the dominant non-glaucous gene Iw1. The non-glaucous trait is associated with delayed senescence due to a stay-green effect, this was identified through QTL analysis on a doubled haploid population of Shamrock x Shango, which segregates for this trait (Simmonds et al., 2008).



Figure 1.15. Picture of Shamrock and Shango field plots. Shamrock is easily identifiable due to its bright green non-glaucous canopy, taken from Simmonds et al. (2008).

In hexaploid wheat glaucousness is controlled by the wax producing dominant alleles W1 and W2, mapped to the short arm of chromosomes 2B and 2D respectively (Lu et al., 2015; R Nishijima et al., 2014). Non-glaucousness is controlled by Iw1 and Iw2 which act as inhibitors of W1 and W2, also found on 2B and 2D. The presence of a single Iw allele will cause the reduction of epicuticular wax if the wheat plant contains W1 or W2. Iw1 on 2B is inherited from wild emmer and Iw2 on 2D is inherited from *Aegilops tauschii* through polyploidisation (R Nishijima et al., 2014; Xu et al., 2015).

The wax present on the leaves and stem mostly consist of  $\beta$ -dikotones, primary alcohols and n-alkanes (Adamski et al., 2013; Xu et al., 2015). The main function of Iw1 and Iw2 is the reduction of the  $\beta$ -diketone component of the plant wax. Xu et al. (2015) studied the durum wheat cultivar Langdon and wild emmer 2B chromosome substitution lines of Langdon to compare the wax content of glaucous and non-glaucous plants. In glaucous plants, 60% of the total wax content was  $\beta$ -dikotones whereas in w1w2iw1iw2 containing plants it was 8% of the total wax and in W1W2Iw1iw2 and W1W2iw1Iw2 containing plants it was undetectable (Xu et al., 2015).

Adamski et al. (2013) studied the Shamrock x Shango doubled haploid population to assess the genetics of the Iw1 locus and its effect on the wax content of the leaves. The location of the Iw1 locus was described on chromosome 2BS as in Simmonds et al. (2008). Shamrock was also crossed with six glaucous hexaploid UK wheats to assess the genetic control of the Iw1 locus. It was concluded that Iw1 is a dominant gene as the resulting six crosses displayed the non-glaucous trait (Adamski et al., 2013). By measuring the amount of leaf wax it was found that Shango contained twice as much wax as Shamrock with 67% of this consisting of  $\beta$ -dikotones. Shamrock had 62% less wax on its leaves and 87% less wax on the peduncles compared to Shango (Fig. 1.16). The presence of epicuticular wax reduces transpiration (R Nishijima et al., 2014) but it is unclear what effect non-glaucous leaves have on the heat tolerance of a plant. No effect was found for the non-glaucous trait on the water use efficiency of field grown plants, assessed using the  $\delta^{13}$ C values of plant biomass to determine carbon isotope discrimination (Adamski et al., 2013).

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Figure 1.16. Cryo-scanning electro micrographs of Shamrock (a, c and e) and Shango (b, d and f) exposed peduncles (a, b) and abaxial (c, d) and adaxial (e, f) flag leaf blade surfaces; tubular structures on b, d and f are presence of wax (Adamski et al., 2013).

#### 1.6.2. Stay green effect

The delayed senescence and prolonged post anthesis green leaf area duration in Shamrock has been studied in other winter and spring wheat populations as it is regarded as an adaption to stress (Lopes and Reynolds, 2012; Verma et al., 2004). Lopes and Reynolds (2012) used the normalised difference vegetation index (NDVI) to measure crop greenness between grain fill and physiological maturity in two different wheat populations in the field. The crops were stressed with heat, drought and combined heat and drought stress. The rate of crop senescence and crop greenness at physiological maturity explained 8% of yield variation in the heat stressed and heat and drought stressed environments. The stay green traits combined with canopy temperature at grain fill explained an average of 30% of yield variation in the same environments (Lopes and Reynolds, 2012). Christopher et al. (2008) suggested that root system traits could be a possible cause of this stay green effect. The CIMMYT wheat line SeriM82 and the northern Australian cultivar Hartog were grown in six field environments differing for water availability. SeriM82 maintained green leaf area in its canopy for longer than Hartog in most of the environments. SeriM82 achieved the highest grain yield difference compared to Hartog (28%) in an irrigated environment and the lowest (14%) when rainout shelters during the post-anthesis period excluded rain. Where soil moisture was depleted by a previous summer crop, SeriM82 did not yield more than Hartog or exhibit the stay green trait (Christopher et al., 2008). This was thought to be due to a lack of deep soil moisture, which promotes late season water uptake and therefore delays crop senescence, prolongs grain fill and increases grain yield. SeriM82 has a narrow root system which can extract more soil moisture at depth post-anthesis (Manschadi et al., 2006).

Genetic analysis of delayed canopy senescence has identified multiple quantitative trait loci (QTL), although these differ between environments and populations. Verma et al. (2004) identified QTL on chromosomes 2B and 2D in a doubled haploid population of a cross between a photoperiod sensitive wheat and a photoperiod insensitive wheat. The QTL on the long arm of chromosome 2D explained 25.7% variation in yield and 32.9% variation in the percentage of flag leaf remaining green 35 days after anthesis, in an unirrigated environment. To a lesser extent QTL identified on 2B explained 11.4% yield variation and 10.2% variation in the flag leaf area remaining green 14 days after anthesis (Verma et al., 2004). Over a two year field trial Kumar et al. (2010) identified three QTL in a recombinant inbred population (RIL) of a cross between a stay green and a non-stay green wheat variety. A QTL on 1A was identified in both years, explaining 24.8 and 11.1% of the variation in stay-green in each year. QTL on 3B and 7D were found in subsequent years with all QTL contributed by the stay green parent Chirya 3 (Kumar et al., 2010). Naruoka et al. (2012) found that green leaf area duration after heading was associated with higher seed weight and seed diameter in drought and heat stress conditions, indicating the importance of this trait in maintaining yields in less favourable environments. Using a RIL population of long and intermediate green leaf area retaining parents, a QTL was found on 4A, which explained 11 and 20% of the phenotypic variation in green leaf area duration post-heading; this was an allele derived from the stay green parent. A co-located QTL for xylem exudate, a proxy for root activity, was also found on 4A and explained 10% of the variation in this trait. Xylem exudate is the water and ions transported by the xylem and is measure by collecting on a cotton bud after the stem is cut (Naruoka et al., 2012). Root dry weight from the same plants where xylem exudate was assessed was found to significantly correlate (r=0.76) with this trait (Naruoka et al., 2012).

#### **1.6.3.** Improved rooting at depth

Another important characteristic found in Shamrock is its greater rooting at depth compared to other winter wheat cultivars (Ford et al., 2006). Wheat genotypes are known to vary for many root traits and in a study of six winter wheat cultivars (Claire, Consort, Hereward, Malacca, Savannah and Shamrock) there were significant differences in post anthesis root mass and length. Over two seasons Shamrock had the largest root length and mass below 40 cm depth (Fig. 1.17; Ford et al., 2006). It was not known whether this greater rooting at depth was associated with the wild emmer introgression, but it shows consistency with past work by CIMMYT on synthetic hexaploids, where modern cultivars are bred with wild relatives (Lopes and Reynolds, 2011).



Figure 1.17. Graph showing root length density (RLD) of field-grown Shamrock winter wheat (O) taken during anthesis with the mean of five other elite lines (Other;  $\bullet$ ) in (A) 2001 and (B) 2002 (B) (Ford et al., 2006). Error bars are + and – SED, \* P<0.05.

#### Aims of research project

The aim of this thesis was to study the diversity of rooting at depth in the wheat doubled haploid population of Shamrock x Shango from both a phenotypic and genetic perspective, with the following research hypotheses:

#### **Research hypotheses**

1. Shamrock has greater root length densities at depth in the field, at anthesis, compared to Shango

2. The non-glaucous trait, inherited from wild emmer, is associated with increased rooting at depth

3. Root architecture traits studied in controlled environments at the seedling and late tillering stage relate to improved rooting in the field at anthesis

4. Photosynthetic capacity and water content of the canopy can act as proxies for increased rooting at depth in the field at anthesis

#### **Outline of the thesis**

This thesis reports the findings of a study of the wheat doubled haploid population Shamrock x Shango, known to differ for certain traits which Shamrock inherited from introgression of wild emmer. Shamrock has been shown to exhibit significantly greater root length densities at depth (below 40 cm) compared to other UK grown elite wheat cultivars (Ford et al., 2006). In chapter 1 a review of the literature regarding the function, importance and measurement of wheat root systems is given. In chapter 2 the diversity of root architectural traits within the doubled haploid population is studied at different growth stages and in both field and controlled environment conditions. The phenotypic and genetic influences, which potentially cause these differences between doubled haploid lines, are assessed. Chapter 3 studies the root shoot relationship of doubled haploid lines in the field, in an attempt to identify any canopy characteristics that can act as a proxy for improved rooting at depth. Differences in canopy traits, which derive from the wild emmer introgression and their advantage to yield and adaptation to stress, are discussed.

A chapter for the proposed book 'Achieving sustainable wheat cultivation' edited by Professor Peter Langridge was completed with Professor Peter Gregory entitled 'Establishment and root development in wheat crops', due to be published at the start of 2017. Refer to appendices.

### Chapter 2. Investigating the genetic diversity of rooting at depth in the Shamrock x Shango doubled haploid population in the field and controlled environments

This chapter is an extended version of a research paper titled 'Quantifying rooting at depth in a wheat doubled haploid population with introgression from wild emmer' submitted to Annals of Botany.

#### 2.1. Introduction

There is a challenge to improve wheat yield and yield stability whilst the amplitude and frequency of weather fluctuations is predicted to increase (Hansen et al., 2012; Ray et al., 2015). Climate projections indicate rising temperatures and reduced summer rainfall in major wheat growing areas, in the UK and globally (Jenkins et al., 2010). A large proportion of the world's wheat crop is rainfed and water deficits from meiosis onwards reduce wheat productivity, as both grain set and grain filling are compromised (Barber et al., 2015; Gooding et al., 2003). Crop and field management influences the availability of soil water and soluble nutrients (Thorup-Kristensen and Kirkegaard, 2016) but deep rooting can also improve access to subsoil water (Lilley and Kirkegaard, 2016; Wasson et al., 2012). Wheat roots can potentially extend to 2 m below the soil surface (Thorup-Kristensen et al., 2009). In one experiment the 3% of the total root system length found below 1 m supplied nearly 20% of the water evaporated during grain filling (P. J Gregory et al., 1978).

Wheat improvement in breeding programmes of modern cultivars has focused on above-ground biomass accumulation and partitioning. In contrast, potential yield improvements through more efficient biomass allocation in root systems have not been much explored (Smith and De Smet, 2012). Increasing harvest index and breeding concentrated on shoot traits, to produce the varieties released since the 1960s as part of the so-called green revolution, may have reduced root biomass at maturity by over 50% compared to landrace wheats, confirmed in a study comparing mid-green revolution wheat varieties with tall landrace cultivars, grown in pots to maturity (Waines and Ehdaie, 2007). A reduction in root biomass may arise through an unconscious selection of root systems sufficient for good or near optimal growing systems but which may increase the vulnerability of the crop to heat or drought stress. Root length density (RLD) of modern UK wheats is calculated to be inadequate for water uptake in the subsoil because RLDs of less than 1 cm/cm<sup>3</sup> are frequently recorded below 40 cm depth (Hoad et al., 2004). A threshold of 1 cm/cm<sup>3</sup> has been determined as the critical RLD sufficient to acquire water and nitrogen resources (Barraclough et al., 1989; Van Noordwijk, 1983; White et al., 2015). Greater investment in fine roots at depth, at the expense of roots close to the surface, would be more efficient for acquiring water and solutes such as nitrate (King et al., 2003). Uptake of stored subsoil water can be particularly effective at stabilising yields if the duration of grain growth and retranslocation processes can be maintained whilst a terminal drought develops after meiosis (Kirkegaard et al., 2007; Wasson et al., 2012).

The high plasticity and low heritability of root system traits makes them difficult to study and therefore select for in breeding systems (de Dorlodot et al., 2007). Dry soil, coupled with the large size of the root system compared to early growth stages, means that the quantification of RLD at depth is particularly challenging during the later growth stages when terminal droughts are likely to develop. Nonetheless, an elite line of winter wheat, *Triticum aestivum* cv. Shamrock, was found to repeatedly exhibit greater root length below a 30 cm deep ploughed layer in UK field plots at and after

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anthesis (Ford et al., 2006). Shamrock has a notable pedigree: the cultivar was first recommended in the late 1990s and contains material recently introgressed from wild emmer (*Triticum dicoccoides*); derived from a cross between NW Europe germplasm (CWW 4899/25 - Moulin x Monopol) and a *T. dicoccoides* derivative (Comp Tig 323-1-3 M) (Simmonds et al., 2008). Wild relatives of wheat can provide a valuable germplasm resource for improving yield performance under abiotic stress, due to their origin and diversification in dry environments such as the Fertile Crescent (Nevo and Chen, 2010; Xie and Nevo, 2008). Shamrock inherited a non-glaucous trait, the reduction of epicuticular wax, through the wild emmer introgression, which is apparent at flag leaf emergence. The trait is associated with delayed senescence and causes a striking green colour of the canopy. The non-glaucous gene has been mapped to the short arm of chromosome 2B (Simmonds et al., 2008), associated with the wax inhibitor gene *Iw1* which reduces the  $\beta$ -diketone component of the plant wax (Adamski et al., 2013).

We are unaware of previous studies that have succeeded in identifying quantitative trait loci (QTL) amongst well-adapted elite germplasm that associate with differences in root traits in the field at depth, late in the growing season. The primary aim of this study was to use a doubled-haploid (DH) mapping population to investigate the genetic basis for improved rooting at depth of cv. Shamrock. Of particular interest were potential associations between single nucleotide polymorphism (SNP) markers on the short arm of 2B (2BS) and the non-glaucous phenotype from wild emmer, with RLD. Further, due to the challenges of washing and assessing roots from soil cores taken from the field at anthesis, we investigated the potential utility of assessing early root system growth in a seedling screen (Bai et al., 2013; Watt et al., 2013) and within 1 m tall rhizotrons (Liao et al., 2006) to predict deep rooting in the field.

#### **2.2. Materials and Methods**

#### 2.2.1. Plant material and genetic mapping

Eighty-seven lines of the Shamrock x Shango DH population, and the two parents, (Simmonds et al., 2008) were genotyped using the Axiom® Wheat Breeder's 35k Genotyping Array, (Affymetrix Inc., Santa Clara, CA; (Allen et al., 2016)), and Kompetitive Allele Specific PCR (KASPTM) genotyping chemistry (LGC Ltd., Teddington UK; (full methodology in appendix 2; Allen et al., 2011)). A linkage map containing 21 groups was produced using 3785 SNP markers and the program MapDisto version 2.0 (Lorieux, 2012). Total map length is 3126 cM with an average linkage group length of 148 cM and a median distance between markers of 2.3 cM (Fig. 2.1). Linkage groups were determined using a logarithm of odds (LOD) threshold of 3.0 and a recombination fraction of 0.3. Genetic distances were computed using the Kosambi (1943) mapping function and markers with significant segregation distortion values were removed from the map, assessed using the chi-squared test. SNPs were anchored to chromosomes using the consensus genetic map produced by merging five genetic maps from mapping populations genotyped using the Wheat Breeder's 35k Array (Allen et al., 2016). Duplicated markers were removed from the map based on agreement with the consensus map and previously genetically mapped SNPs from cerealsDB (Wilkinson et al., 2012). If a marker matched the position in the survey sequence it was retained but if no previous position was defined it was removed. Duplicates were removed at random if both agreed with the survey sequence position.

сM	1 <b>A</b>	сM	1B	сМ	1D	сM	2A	сM	3 <b>A</b>	сM	3B
0.0 4.6	AX-95653435	0.0 3.5	BS00080214	0.0 16.7	AX-95258374	0.0 10.5	BS00053661	0.0 4.6	BS00022985 AX-94669520	0.0	AX-94869603 AX-95142306
8.0	AX-94545275	26.6	AX-94570104	34.9	AX-95681643	11.6	AX-94875440	16.5 18.8	AX-94595620 AX-94569370	2.3 3.4	AX-94800960 AX-94520040
9.1 10.3	AX-94540853 AX-94765928	27.7 41.9	AX-94847672	49.0 50.2	AX-94413085 AX-95119988	16.2 24.2	AX-94684111 AX-94684375	37.0	AX-95127888	11.4	BS00023186
11.4	AX-95630149 BS00031289	46.4 52.1	AX-94629930 AX-94562740			25.4 26.5	AX-95255993 AX-95152008	42.8 45.0	AX-94591588 AX-94490447	13.7 44.3	_/ BS00022971 AX-94446828
38.3 41.8	AX-94657330	54.4 55.6	AX-94824369	сM	28	38.0 41.5	AX-94464525	59.0 67.0	AX-94421535 BS00025739	47.7	AX-94387053
44.1 45.2	AX-94793337 AX-95681746	56.7 57.9	AX-95208619 AX-94717933	0.0	BS00084668	46.0 47.2	AX-94998685 AX-94756068	87.9 93.8	BS00022711 AX-95075882	54.6 60.3	BS00022315 AX-95206732
46.3	AX-94974317	65.9	AX-94699684	3.5 4.7	AX-94777767 AX-94939920	48.3	AX-95098442	98.3 140.7	AX-94410643 BS00022103	61.4 62.5	AX-94492579 AX-94658662
49.8 50.9	AX-94392216 AX-95129506	67.0 72.9	AX-95215369 BS00010475	5.8 7.0	AX-94505732 AX-94579143	81.0 89.1	BS00021691	146.5	_) / AX-94660817	69.4	AX-94470136
52.0 53.2	AX-94906803 AX-94443825	78.7 81.0	AX-95199768	8.1 9.2	AX-94399171 AX-94645643	90.3 93.7	AX-94982825	151.0 152.2	AX-95154450	70.5 71.7	AX-94904639 AX-95107806
54.3 57.7	AX-95072103 BS00021740	82.1 83.3	AX-94744409	11.5 12.7	AX-95091124 BS00175222	115.5 116.6	AX-94766564	154.4 156.7	BS00022459 AX-94495683	76.3 77.4	BS00106922 BS00046052
62.3 64.6	BS00064197 AX-94921896	84.4 85.6	AX-94709629 AX-94981461	15.0	BS00070900	120.0 122.3	AX-94664024 AX-94535064	157.9 159.0	AX-95203473	102.0 108.8	→ T _ AX-94637675
65.7	_/// AX-94633263	87.8	AX-94620168	16.2 17.3	AX-94499963 AX-94529633	129.4	BS00009730	160.2	AX-94675271	110.0	AX-94771710 AX-94413295
66.8 73.7	AX-94643380	92.4 97.0	AX-94693862	20.7 21.9	AX-95197220 AX-94805858	140.2 141.3	BS00010014 BS00049644			112.2	AX-94692827
76.0 78.3	AX-95683697 AX-94831368	99.3 100.5	AX-94847341 AX-94801647	23.0 24.1	AX-94483531 AX-94802827	143.6 150.8	BS00022641 AX-94463649	сM	5B	113.4 122.6	AX-94492274 BS00030651
79.4 81.7	AX-94585769 AX-94997258	103.9 105.1	AX-95253773 AX-94677202	25.3 27.6	AX-94569439	151.9 153.1	AX-94860603 AX-95020355	0.0	AX-94634359 AX-94610974	124.8 129.4	AX-94603670
86.3 87.4	AX-94603312 AX-94489106	106.2 107.4	AX-94834787 AX-94726027	42.1 43.3	AX-94687086	159.9 163.3	AX-94536561 AX-94439270	21.0 28.4	AX-94931820	136.3 137.5	AX-94811024
88.6 94.3	AX-94394930	108.5	AX-94716273 BS00039135	59.9	_/ AX-94736574	166.7	AX-94776946 AX-94781832	30.8 35.4	BS00076782 AX-95091073	138.6 140.9	AX-94762065 AX-95162413
96.5	AX-94826839	113.1	_// \_ BS00022539	66.9 71.4	AX-94817182 AX-94437617	173.6	AX-94390510	36.6	AX-94757478	142.0	AX-94628943 AX-94662238
101.2 104.6	BS00077432	114.3 125.2	AX-94537059	76.0 77.2	AX-94946297 AX-95176720	178.1 179.3	AX-95166962 AX-95246552	38.9 45.7	AX-94623475	145.4	AX-94428563
110.4 144.4	AX-95166641 AX-94963312	130.9 140.8	BS00022609 BS00071895	81.8 82.9	AX-95115111 AX-94440078	181.5 182.7	AX-94992169 AX-94402040	48.0 49.1	AX-94862352 AX-94913780	152.4	BS00056737
152.4 154.7	AX-94503208	149.5 150.7	AX-95226038 AX-94629504	84.0 88.6	AX-94994885 BS00034314	183.8 185.0	AX-95630313 AX-94667635	50.3 53.7	AX-94882207		
155.8	AX-94551566	151.8 164.6	AX-95078982	89.8	J/ L BS00081757	186.1 187.3	AX-94479692 AX-94916544	56.0 58.3	AX-95073977 BS00022107	CM 0.0	6B BS00023196
		174.0	AX-94527502 AX-95134123	99.0 111.9	BS00026412 BS00022950	188.4	AX-94688452 AX-94567538	60.5 62.8	AX-94400196 AX-94813848	14.5 21.5	BS00033629 BS00110713
сM	2D	179.7	AX-94945293	113.1	BS00022487	191.8	AX-94672987	69.7	AX-94439232	28.8	AX-94943043
0.0	AX-95172975	180.9 182.0	AX-94656870 AX-94956073			194.1 195.3	AX-94402591	70.8 73.1	AX-94470072 AX-94612603	33.6 36.0	AX-94752582
22.5 25.9	AX-94860457 AX-95102138	183.2 191.2	AX-94608604	CM 0.0	4B AX-94681492	197.6 217.1	- AX-95004346 BS00023214	74.2 76.5	AX-95174893 AX-95011964	37.1 46.3	AX-94559717 AX-94549328
30.4 38.5	BS00065456	194.6	AX-94817190	2.3	AX-95629916 BS00022653	218.3 221.8	BS00023123	78.8 84.5	AX-95252378 AX-94390404	48.5 49.7	AX-94401844 AX-95082971
39.6 40.8	AX-94652914 AX-94431423			16.5	BS00067428	223.0 227.6	BS00021693	93.7 107.7	AX-94382905 AX-95660741	66.6 70.1	AX-94395490
41.9	AX-95165682	cM	3D	17.7 30.6	BS00058946 AX-94415673	228.7 229.8	AX-94874921 AX-94550638	111.1 122.8	AX-94582169 BS00108019	85.3 87.6	AX-95076851
45.3 46.5	AX-94765106 AX-94485462	0.0 8.1	BS00023210 AX-94499713	34.0 35.1	AX-95630372 AX-94599848	231.0 232.1	AX-94980851 AX-95205011	124.0 133.4	BS00034333 AX-94713015	88.7 91.0	AX-94768049 AX-95630592
51.1 52.3	BS0009606 BS00151318	9.3 12.7	AX-94989783 AX-94720192	36.3 50.3	AX-94616755 AX-94488964	236.7	AX-94827966	137.9	AX-94520860 AX-94628272	93.3 94.4	AX-94827069
67.5 75.5	AX-94485593	16.1 21.8	AX-94452681 AX-95189814		0	237.8 240.1	AX-94653560 AX-95160129	140.2 141.4	_\\\\  ///_ AX-94890972	96.7	AX-94510541 AX-94513671
79.0 82.5	BS00064538 AX-94603120	28.6 48.7	AX-94798292 AX-95185244				-	142.5 144.8	AX-94910642	104.7 107.0	BS00023032 BS00067161
	~		<u> </u>					147.2 148.3	- AX-95258348 - AX-95092867	110.5	BS00093914
								149.4	AX-94433869		

Figure 2.1. Genetic linkage map of Shamrock x Shango doubled haploid population, continued on next page



Figure 2.1. Genetic linkage map of Shamrock x Shango doubled haploid population

#### 2.2.2. Field Experiment

Two randomised blocks containing each DH line were sown at Reading University Crops Research Unit, Sonning, UK (0°54' W, 51°29' N) in each of the 2013/2014 and 2014/2015 growing seasons (15 October 2013, 25 September 2014); the parents were sown twice in each block (Image 2.1). The field was power harrowed after ploughing to a nominal depth of 30 cm. Seeds were drilled in 2 x 5  $m^2$  plots at 300 seeds/m<sup>2</sup> in 120 mm rows on a free draining sandy loam overlying coarse red-brown sand (Sonning series; (Jarvis, 1968)). In the first year, the wheat was the third cereal following a grass ley; in the second year the wheat was the first cereal after a 3-year grass plus clover ley. All plots received 16 kg S/ha in both years, and 200 kg N/ha in 2013/14 and 235 kg N/ha in 2014/15 as granular fertiliser during stem extension. Weeds and foliar pathogens were adequately controlled with standard herbicides and fungicides. Weather was recorded in both seasons using an on-site weather station. Average temperatures for winter (October to February) were 7.5°C for 2013/14 and 6.8°C for 2014/15, and for stem extension (March to May) were 10.5°C for 2014 and 9.4°C for 2015. Total rainfall for winter was 494 mm (2013/14) and 328 mm (2014/15), and for stem extension was 163 mm (2014) and 80 mm (2015). The plots were harvested using a combine harvester and grain yield determined.



Image 2.1. Randomised block design of field experiment at Sonning. Picture taken during grain fill in 2015.

Roots were sampled during the first three weeks after anthesis (growth stage (GS) 63: 2nd June 2014; 15 June 2015). In 2014, only the Shamrock and Shango parents were sampled to a depth of 70 cm using a steel hand corer of 80 mm diameter (Image 2.2). Cores were split into 15 cm sections in the top 30 cm (the plough layer) and 10 cm thereafter, generating six distinct soil horizons: 0-15, 15-30, 30-40, 40-50, 50-60 and 60-70 cm deep. Each core section was placed in a sealed bag and stored in a cold room (2-4°C) prior to washing. Five cores were taken between the rows and three cores within the row due to multiple studies stating cores solely taken within the row, in addition to small auger sizes, can overestimate real RLDs (Buczko et al., 2009; Kumar et al., 1993; Van Noordwijk et al., 1985). A pumped root washing system was used to separate roots from the soil over a 550 µm mesh collection filter (Image 2.3; Root Washer, Delta T, Cambridge UK). Roots were hand separated from organic debris: washed samples from the top 30 cm were subsampled due to large amounts of debris. Roots were then scanned using a flatbed scanner (Expression 1600 XL-PRO, Epsom UK Ltd) at 300 dpi resolution and assessed using *WinRhizo* (Regents Instruments Inc., QC, Canada). Samples were dried at 80°C for 48 h and weighed. In 2015, roots from all lines were measured in soil collected between 50 and 80 cm depths. These samples were collected with a 73 mm diameter window sampler driven into the ground using a tractor mounted hydraulic static pile driver (Image 2.4; model number MCL2, Norsk Hydro, Geonor, Norway). Three samples were taken per plot: two between the row and one within the row (Fig. 2.2). Samples were analysed as in 2014 after splitting cores into 10 cm sections (50-60, 60-70 and 70-80 cm).



Figure 2.2. Schematic diagram of soil core positioning in the field (eight cores per plot in 2014 and three cores per plot in 2015).



Image 2.2. Steel hand corer with T- handle in the field in 2014.



Image 2.3. Root washer apparatus. A) Side view of root washer showing collection filters and buckets on table surface, containing soil samples. B) Top view of root washer showing jet pumps filling buckets containing soil samples. Water containing wheat roots passes through hollow cylinder in bucket and into collection filters.



Image 2.4. Soil coring in the field in 2015. A) Window sampler attached to hydraulic static pile driver after being driven into the ground to 80 cm. B) Window sampler attached to tractor mounted hydraulic static pile driver.

#### 2.2.3. Seedlings

A paper roll system was used to grow the DH population to seedling stage, as described by Bai et al. (2013). Seeds of uniform mass (0.05 g  $\pm$  0.005 g) were surface-sterilised in 0.5% calcium hypochlorite (Ca(ClO)<sub>2</sub>) solution for 30 minutes before being rinsed with sterilised water and then placed in a cold room at 4°C overnight. Seeds were then pregerminated at 10°C on paper wetted with sterilised water for 72 hours. Three pregerminated seeds from each line were placed in a roll of germination paper (Anchor Paper Company, Saint Paul, MN, USA) 2 cm wide and 38 cm tall. Germination paper rolls were supported within a wire-lattice on a tray of nutrient solution in four randomised blocks, giving 12 replicates of each DH line (Image 2.5; (Bai et al., 2013)). Half strength solution was used for the first three days and thereafter replaced with full strength for the remainder of the experiment, which was changed every day. The trays were placed in a controlled environment cabinet (12 h day, light intensity 500  $\mu$ mol/m<sup>2</sup>/s, 70%/80% day/night relative humidity 20°C/16°C day/night temperatures (Bai et al., 2013). After 11 days the paper rolls were placed in sealed plastic bags and stored at 3°C until root analysis. The whole experiment was repeated to achieve 24 replications per line.

Seedlings were removed from the roll and separated into root and shoot. Intact root systems were scanned (as above) and assessed with *WinRhizo* to obtain: total root length, root surface area, root volume, average diameter, and percentage of root length in diameter classes 0-0.5 mm and 0.5-1 mm. Additionally, the number of seminal axes was counted. Roots and shoots were dried at 80°C for 48 hours and then weighed so root:shoot ratio could be calculated.



Image 2.5. Seedling screen experimental set up. A) One block of germination paper rolls after 11 days of growth in the controlled environment. B) Four blocks of germination paper rolls containing pre-germinated seed in nutrient solution before going into the controlled environment. Each roll contains three seeds of a doubled haploid line with each block containing a roll for each of the 89 doubled haploid lines.

#### 2.2.4. Rhizotrons

Nineteen lines were selected based on total root length, average diameter and root dry weight measured in the seedling experiment, to capture observed root trait variation in glaucous and non-glaucous lines, including parents Shamrock and Shango (Table 2.1). A wild emmer accession, obtained from the John Innes Centre Germplasm Resource Unit, was also included to give a comparison of rooting traits in a wild relative.

Table 2.1. Selected doubled haploid lines in the rhizotron experiment and their respective root length (TRL), average diameter (AvgDiam) and root dry weight (RDW) in the seedling screen.

DH Line	Glaucous	Seed TRL (cm)	Seed AvgDiam (mm)	Seed RDW (mg)
6	No	50.99	0.476	0.0188
93c	No	55.06	0.417	0.0205
25	No	55.58	0.464	0.0165
10	No	55.67	0.452	0.0142
Shamrock	No	57.60	0.432	0.0168
86	No	65.59	0.433	0.0165
93b	No	66.20	0.441	0.0159
39	No	66.45	0.436	0.0151
58	No	68.79	0.425	0.0156
56	No	72.27	0.434	0.0147
35	Yes	55.51	0.458	0.0178
21	Yes	56.54	0.452	0.0186
8	Yes	57.95	0.447	0.0173
Shango	Yes	62.13	0.448	0.0169
3	Yes	67.36	0.448	0.0145
64	Yes	67.75	0.444	0.0141
73	Yes	68.04	0.450	0.0164
55	Yes	68.66	0.427	0.0173
76	Yes	69.21	0.435	0.0159

The lines were grown in 1 m tall x 0.3 m wide x 0.05 m deep root observation chambers (rhizotrons) constructed from PVC sheets with a clear acrylic sheet bolted onto the front of the box (adapted from Liao et al. (2006)). Four 8 mm diameter holes were drilled into the base and a thin layer of 10 mm gravel was placed at the bottom of each

rhizotron to aid drainage. A loamy sand (Sporting Surface Supplies Ltd., Smallfield, UK) of composition 40% sand, 40% silt and 20% clay was sieved to 6 mm and packed to a bulk density of 1.2 g/cm<sup>3</sup>; the soil had 20% moisture when packed. This was done using a flat ended rod to pack pre-weighed soil into the boxes with the acrylic front attached. Rhizotrons were individually wrapped in thermawrap silver foil to insulate them and to ensure the soil was not exposed to light. Seeds of uniform mass (0.05 g  $\pm$ 0.005 g) were pre-germinated as for the seedling experiment. Four germinated seeds were sown in a row close to the clear acrylic front of each rhizotron and thinned to two plants per rhizotron after eight days. The equivalent of 50 kg N/ha as urea and 8 kg P/ha as superphosphate was applied on the soil surface in a solid form. The rhizotrons were placed at a 30° angle from vertical against a steel frame, to allow the roots to grow against the clear acrylic front, and spaced 0.10 m apart. Rhizotrons of each genotype were replicated in three randomised blocks in a naturally lit glasshouse in the spring of 2015 at the University of Reading, UK (Image 2.6). Wheat plants were grown to GS 29 (Zadoks et al., 1974) when roots had reached the 1 m deep base in 50% of rhizotrons, 45 days after sowing (DAS). Average daily temperatures during the growth period ranged from a minimum of 11°C to a maximum of 25°C, with a natural photoperiod of about 12 h. Adequate moisture was provided by watering every three days.

From 10 DAS, roots were manually traced twice a week on to acetate sheets taped to the front of each rhizotron (Image 2.7). On 45 DAS (GS 29) the shoots were removed from the crown and dried at 80°C for 48 h. The soil and roots in each rhizotron were collected and stored in 0.2 m depths at 2-5°C until the roots were sieved from the soil using a 4 mm mesh sieve. Sieved root samples and acetate sheets were scanned as described above with *WinRhizo*. Finally, root samples were weighed after drying at 80°C for 48 h.

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Image 2.6. Rhizotron experimental set up at tillering. Glasshouse containing 20 genotypes, each replicated three times in a three randomised block design.



Image 2.7. Rhizotrons. A) Tracing roots on acetate taped to the front of rhizotron. B) Rhizotron with acrylic cover removed at the end of six weeks of growth.

#### 2.2.5. Statistical analysis

For the field experiment an attempt was made to control error variation within the blocks by including row and column of the plot positions as incomplete blocks within an analysis of residual maximum likelihood (REML; Genstat v15 (VSN International)) i.e. the random model was Block/(Row + Column) for roots collected in 2014 and

Block/(Row+Column)/Plot/Core/Depth for roots collected in 2015. The fixed model was Line and adjusted 'means' were calculated (best linear unbiased predictors). The interaction of RLD with depth was assessed using Depth\*Line as the fixed model. For assessing effect of core position within the plot the fixed model was Line/Depth/Position and the random model the same as above for 2014 and 2015 data. For the seedling experiment Line means and errors were calculated within an analysis of variance (ANOVA) combining both replicate experiments (block structure: Replicate experiment/Block). The correlation matrix of the seedling variate x Line means was used in a Principal Component Analysis (PCA). For the rhizotron experiment, two ANOVAs were conducted; the first with a treatment structure of Line and block structure of Block and the second ANOVA with glaucousness as a fixed effect within a nested treatment structure of Species/Glaucousness where Species is either wild emmer or bread wheat.

The line means from seedling and field experiments were used in a QTL analysis with composite interval mapping (CIM) in Windows QTL Cartographer version 2.5 (S. Wang et al., 2010). Standard model 6 with the forward and backward regression method was used. Ten control markers were automatically selected; window size was set at 10 cM and a walk speed setting of 1 cM was used for the analysis. LOD threshold values (P<0.05) were set by running 1000 permutations to identify significant QTL. Due to the glaucous trait being binary Multiple Interval Mapping (MIM) was used to identify QTL for this trait with the same thresholds (Li et al., 2006).

#### **2.3. Results**

#### 2.3.1. Influence of soil core position on root length density

In 2014 there were no significant differences (P>0.05) between Shamrock and Shango for RLDs within or between rows at any of the depths analysed. The range of data for RLD values within and between rows was assessed as an average per plot due to only the parents being analysed in the first year (Fig. 2.3). The average of RLDs within the two core positions were similar (line plot in Fig. 2.3) but the interquartile range of values was higher for within row soil cores, in addition to the 95<sup>th</sup> percentiles (box plots, Fig. 2.3). Standard error of the mean for between and within row cores was 0.120 and 0.171 respectively.

In 2015 there was a significant effect of core position on RLD in the DH population (P<0.001). A box plot produced for RLD values, averaged for each DH line, within and between rows shows the larger range of values found in within row soil cores (Fig. 2.4). With greater interquartile ranges for each depth and higher 95<sup>th</sup> percentile values, similar to that seen in 2014. The standard error of the mean for between and within row RLDs were 0.0085 and 0.0134. Shamrock consistently had higher RLDs within the row compared to Shango, significantly so at 60-70 cm depth (Table 2.2).



Figure 2.3. Box plot for RLDs averaged per plot for A) between, n = 40 and B) within row, n = 24, soil cores. Box plots show lower quartile, median and upper quartile, whiskers are 5<sup>th</sup> and 95<sup>th</sup> percentiles. Line plot is the average value for RLDs in all within and between soil cores.



Figure 2.4. Box plot for RLDs averaged per doubled haploid line A) between and B) within row soil cores, n = 89. Box plots show lower quartile, median and upper quartile, whiskers are 5<sup>th</sup> and 95<sup>th</sup> percentiles. Line plot is the average value for RLDs in the whole doubled haploid population for within and between row soil cores.

Table 2.2. Average RLDs for Shamrock and Shango in 2015, for within and between row soil cores, n = 12. SED compares the same core position between the two cultivars, \* represents P<0.05.

Depth	Shamrock	$(cm/cm^3)$	Shango	SED	
(cm)	Between	Within	Between	Within	_
50-60	0.405	0.440	0.530	0.307	0.0910
60-70	0.488	0.504*	0.323	0.247*	0.0910
70-80	0.254	0.292	0.124	0.180	0.0910

## 2.3.2. Genetic diversity in the doubled haploid population for rooting at depth in the field

The decline in root length density with depth observed in 2014 (Fig. 2.5) is typical for the site (Ford et al., 2006), with the plough depth presenting a notable demarcation. Shamrock had consistently higher RLD than Shango below the plough layer; significantly so (P<0.05) in the 50-60 and 60-70 cm layers. Shamrock also had significantly greater root dry weight (RDW) in the 60-70 cm layer (Shamrock = 0.0063 mg/cm<sup>3</sup>, Shango = 0.0044 mg/cm<sup>3</sup>, SED 0.00051).



Figure 2.5. Root length density (RLD) of field-grown Shamrock and Shango winter wheat at anthesis in 2014. Average of 32 cores per genotype. Error bars are + and - SED, \* P<0.05. Dotted line indicates the plough layer.

In 2015 significant differences (P<0.001) were found between DH lines for RLD and RDW in the 50-80 cm layer at anthesis (Fig. 2.6 and 2.7). Root length density values averaged in the 50-80 cm layer ranged from 0.116 to 0.660 cm/cm<sup>3</sup>, with no line exceeding 1 cm/cm<sup>3</sup> (SED 0.1401). The population exhibited transgressive segregation for this trait which is important for QTL identification, doubled haploid lines which consistently performed well within the 10 cm soil layers for RLD between 50-80 cm were 23, 52, 58, 119c, 74, 14 and 20. Consistently low performing lines were 9, 1, 7, 56, 43b, 62 and 94a. RLD and RDW were positively correlated (r = 0.82, P<0.001). Root dry weight values ranged from 0.004 to 0.045 mg/cm<sup>3</sup> (SED 0.0099). Lines differed significantly (P<0.02) for mean root diameter in the 50-80 cm layer (Fig. 2.8), ranging from 0.202 to 0.252 mm (SED 0.0141) but no significant differences were found for root diameter within the different soil depths. Shamrock had significantly

(P<0.02) finer roots than Shango within the 50-80 cm soil core (0.216 and 0.238 mm respectively, SED 0.0099). Diameter correlated negatively with RLD (r = -0.33, P<0.01).



Figure 2.6. Root length densities (RLD) of a Shamrock x Shango doubled haploid (DH) population in field grown plots at anthesis. A) 50-60 cm depth B) 60-70 cm depth C) 70-80 cm depth. Black bars denote non-glaucous lines and grey bars glaucous lines. Shamrock and Shango means are labelled. Circle points represent means of glaucous (blank circles) and non-glaucous (filled circles) lines. Error bar is 1 SED for line means and glaucous and non-glaucous average; a) SED for Shamrock x Shango, n = 12 and b) SED for DH line, n = 6.



Figure 2.7. Root dry weights (RDW) of a Shamrock x Shango doubled haploid (DH) population in field grown plots at anthesis. A) 50-60 cm depth B) 60-70 cm depth C) 70-80 cm depth. Black bars denote non-glaucous lines and grey bars glaucous lines. Shamrock and Shango means are labelled. Circle points represent means of glaucous (blank circles) and non-glaucous (filled circles) lines. Error bar is 1 SED for line means and glaucous and non-glaucous average; a) SED for Shamrock x Shango, n = 12 and b) SED for DH line, n = 6.


Figure 2.8. Root diameter of a Shamrock x Shango doubled haploid (DH) population in field grown plots at anthesis, averaged over the 50-80 cm soil core. Black bars denote non-glaucous lines and grey bars glaucous lines. Shamrock and Shango means are labelled. Circle points represent means of glaucous (blank circles) and non-glaucous (filled circles) lines. Error bar is 1 SED for line means and glaucous and non-glaucous average; a) SED for Shamrock x Shango, n = 12 and b) SED for DH line, n = 6.

Root length density and RDW of DH lines also differed significantly with depth in the 50-80 cm soil core (P<0.001) (Fig. 2.6 and 2.7). The location of the non-glaucous gene was confirmed on the short arm of 2B (Table 2.3, (Simmonds et al., 2008)). Glaucousness did not show an association with average RLD, RDW or root diameter in the field over the whole 50-80 cm core in the DH population. However, the interaction of RLD with soil depth and glaucousness was significant (P = 0.01), because the mean 'effect' of glaucousness at 50-60 cm depth contrasted with that at 60-70 cm depth (Fig. 2.6, detailed in Fig 2.9). Consequently, a QTL was identified on 2BS, in close proximity to the glaucous QTL, explaining variation in RLD within the 50-60 cm soil layer, with Shango contributing the high value allele (Table 2.3). Non-glaucousness associated with higher grain yields (P = 0.02, effect = +0.43 t/ha, SED 0.186), when averaged over the two field seasons (data presented in chapter 3).



Figure 2.9. RLDs in field grown plots at anthesis for A) Shamrock and Shango in 2014, n = 32 B) Shamrock and Shango in 2015, n = 12 and C) Glaucous and Non-glaucous doubled haploid lines in 2015, n = 282 and 264 respectively. Error bars are 2 x SED and \* P<0.05.

Three QTL associated with average RLD between 50-80 cm were identified on chromosomes 5D, 6B, and 7B (Table 2.3). In terms of variation explained, the QTL with the greatest positive effect on RLD was on 6B where the Shamrock allele had a positive effect. Shamrock was also the high value allele for the QTL on 5D, whereas Shango contributed the high value allele for the QTL identified on 7B, in addition to a more significant QTL further down the linkage group for RDW. A second QTL for RDW was identified on chromosome 2A, with Shango also contributing the high value allele.

Due to the significant interacting effect on RLD and RDW of doubled haploid lines with depth, QTL analysis was also undertaken for the rooting traits within each 10 cm soil layer between 50 and 80 cm (Table 2.3). QTL that explained variation in the population for average RLD were also present in the individual 10 cm depths. This included the QTL on the short arm of 5D, contributed by a high value allele from Shamrock, for both RLD and RDW within the 50-60 cm layer. Additionally, the QTL on 7B, contributed by a high value allele from Shango was identified for RLD within the 60-70 cm layer (Table 2.3); the peak markers for both these QTL differed but the confidence intervals overlapped.

Trait	Chromosome	Position	Confidence	LOD	Peak Marker	Additive	High value	Variation
		(cM)	Interval (cM)			Effect	allele	explained (%)
Glaucousness	2B	1.0	0-2.1	58.5	BS00084668	0.489	Shamrock	95.6
RLD	5D	31.3	18.5-39.5	3.1	BS00158384	0.0355	Shamrock	8.4
RLD	6B	73.1	66.4-81.9	4.3	AX-94475756	0.0431	Shamrock	13.9
RLD	7B	12.8	11.1-22.8	3.4	AX-94826552	-0.0342	Shango	8.8
RDW	2A	41.5	38.5-45.1	3.3	AX-94604266	-0.0026	Shango	8.7
RDW	7B	50.9	47.2-54.1	6.1	AX-95194687	-0.0058	Shango	17.3
RLD								
50-60 cm	1B	132.0	118.1-137.2	3.1	BS00022609	0.0705	Shamrock	8.7
50-60 cm	2B	5.8	2.1-7.5	3.1	AX-94505732	-0.0452	Shango	7.9
50-60 cm	2D	72.5	67.4-77.5	3.8	AX-94485593	-0.0566	Shango	12.4
50-60 cm	5D	35.5	29.9-40.8	3.1	AX-94774616	0.0483	Shamrock	7.9
60-70 cm	6A	30.9	30.3-33.4	3.2	AX-94579171	0.0633	Shamrock	9.4
60-70 cm	7B	11.7	4.0-21.0	5.1	AX-95652919	-0.0625	Shango	15.7
70-80 cm	5D	264.1	256.8-268.8	3.7	AX-94916991	-0.0287	Shango	9.7
RDW								
50-60 cm	1A	96.5	94.2-99.6	3.3	AX-94826839	0.0043	Shamrock	9.0
50-60 cm	1B	16.5	6.1-26.6	3.9	AX-94790297	-0.0045	Shango	14.2
50-60 cm	2D	70.5	60.8-78.6	3.2	AX-94485593	-0.0037	Shango	9.3
50-60 cm	5D	35.5	28.8-40.6	3.6	AX-94774616	0.0040	Shamrock	9.8
60-70 cm	7A	0.0	0.0-5.9	3.0	AX-94603119	-0.0039	Shango	10.8
70-80 cm	5D	267.1	263.4-269.2	4.2	AX-94916991	-0.0036	Shango	12.0
Diam								
50-60 cm	2D	25.9	22.7-39.6	5.2	AX-95102138	0.0058	Shamrock	14.8
50-60 cm	5A	196.2	185.6-201.3	3.1	AX-95235821	-0.0044	Shango	8.3

Table 2.3. Quantitative trait loci (QTL) from a Shamrock x Shango doubled haploid population for glaucousness and root length density (RLD, cm/cm<sup>3</sup>), root dry weight (RDW, mg/cm<sup>3</sup>) and root diameter (Diam, mm).

Co-locating QTL were found for both RLD and RDW at multiple depths with a QTL on 2D which explained variation in DH lines for RLD and RDW within the 50-60 cm layer, with Shango contributing the high value allele. Additionally, within the 70-80 cm layer a QTL was identified on the long arm of 5D that explained variation in both RLD and RDW, with the high value allele contributed from Shango. Further QTL were identified for RLD within the 50-60 cm layer on the long arm of 1B, with a high value allele coming from Shamrock and the QTL on 2BS mentioned previously. Two QTL were found to explain variation within the DH lines for RLD in the 60-70 cm layer on 6A and 7B, with high value alleles contributed from Shamrock and Shango respectively.

For RDW in the 50-60 cm layer additional QTL were identified on 1A and the long arm of 1B, with high value alleles being contributed from both Shamrock and Shango respectively. A single QTL explained variation in the population for RDW within the 60-70 cm layer on the short arm of 7A, contributed by a high value allele from Shango. Only QTL within the 50-60 cm layer were identified for variation in root diameter; these comprised a QTL on the short arm of 2D, contributed by a high value allele from Shamrock, and the long arm of 5A, contributed by a high value allele from Shango.

# 2.3.3. Non-glaucous doubled haploid lines had greater rooting at depth in the rhizotrons at tillering

Root length density (cm/cm<sup>3</sup>) of roots separated from the soil correlated significantly (r =  $0.82 \ P < 0.001$ ) with RLD (cm/cm<sup>2</sup>) measured on the acetate sheets. Consequently, only root length results from the acetate tracings are reported hereafter. Selected DH lines differed significantly (P < 0.05) for RLD in the 40-60 cm profiles (Fig. 2.10). As in the field, Shamrock had higher root length densities at depth compared to Shango, significantly so (P < 0.05) at 40-60 cm (0.66 and 0.63 cm/cm<sup>2</sup> respectively SED 0.069;

Fig. 2.10 and 2.11). The larger rooting system of Shamrock was further confirmed in the dry weight distributions (Table 2.4), with Shamrock having significantly greater RDW compared to Shango in the 40-60 and 60-80 cm layers, as well as the average in the whole profile. Shamrock had a greater shoot dry weight compared to Shango but this was not significant (P>0.05); no significant differences in root:shoot ratios were found between the DH lines.



Figure 2.10. Root length density (RLD) of selected doubled haploid lines from Shamrock (filled circles) x Shango (blank circles) and a wild emmer accession (filled square symbol). Average of three replicates per genotype. Glaucous doubled haploid lines are blank triangles and non-glaucous doubled haploid lines are filled triangles. Values derived from acetate tracings for the root profile of lines grown in rhizotrons. Error bar is 1 SED.

Doubled haploid lines that exhibited the non-glaucous trait after flag leaf emergence in the field had, on average, increased rooting at depth, both for RLD and RDW in the rhizotrons before stem extension (Fig. 2.12; Table 2.4). Mean RLD of non-glaucous lines was 0.649 and 0.458 cm/cm<sup>2</sup> in the 40-60 and 60-80 cm soil layers respectively; significantly (P<0.05) higher than glaucous lines where average RLDs were 0.563 and 0.405 cm/cm<sup>2</sup> (SED 0.0282 and 0.0262 for the 40-60 and 60-80 cm layers respectively). The single accession of wild emmer used had particularly high RLDs between 20 and 60 cm (0.84 and 0.77 cm/cm<sup>2</sup> 20-40 and 40-60 cm respectively), and differed significantly (P<0.02) from the mean of the DH lines at these soil depths (0.66 and 0.61 cm/cm<sup>2</sup> SED 0.0624 and 0.0647 for the 20-40 and 40-60 cm respectively; Fig. 2.10; 2.11 and 2.12). Wild emmer also produced finer roots so the increased RLD was therefore not reflected in greater root mass (Table 2.4).

Table 2.4. The effect of different genotypes and genotype groups on shoot and root characteristics in different depth ranges in the rhizotrons. SED *
P<0.05.

Lines	Shoot dry	Root	dry weight (mg/cm <sup>2</sup>	3)	Mean r	(mm)	
	matter (g)	0-96 cm	40-60 cm	60-80 cm	20-40 cm	40-60 cm	60-80 cm
Shamrock	3.21	0.084	0.078	0.038	0.39	0.36	0.36
Shango	2.66	0.049	0.046	0.021	0.36	0.69	0.41
SED	0.346	0.0171*	0.0157*	0.0074*	0.057	0.115*	0.036
Non-glaucous	3.06	0.072	0.062	0.034	0.39	0.38	0.38
Glaucous	2.95	0.064	0.060	0.028	0.40	0.44	0.40
SED	0.134	0.0061	0.0060	0.0026*	0.018	0.036	0.011
Wild emmer	2.69	0.055	0.053	0.021	0.30	0.29	0.32
Doubled haploid mean	3.01	0.068	0.061	0.031	0.39	0.41	0.39
SED	0.306	0.0139	0.0014*	0.0059	0.041*	0.082	0.026*



Figure 2.11. Full profile acetate tracings from a replicate rhizotron of each cv. Shango, cv. Shamrock and a wild emmer accession, 45 days after sowing. Dashed lines denote separate rhizotron profiles.



Figure 2.12. Root length density (RLD) of A) Glaucous and non-glaucous selected DH lines, n = 27 and 30 respectively and B) averaged selected DH lines, n = 57 (Bread) compared against a wild emmer accession, n = 3 (Emmer). Values derived from acetate tracings for the root profile of lines grown in rhizotrons. Error bar is 1 SED, \* P<0.05.

For selected doubled haploid lines RLD varied between 0.444 and 0.765 cm/cm<sup>2</sup> in the 40-60 cm layer and 0.326 and 0.562 cm/cm<sup>2</sup> in the 60-80 cm layer (Fig. 2.10). Root length densities of DH lines at the late tillering growth stage did not show a strong relationship with RLDs in the field at anthesis. Genotypes 56 and 93c were high

performing lines in the 40-80 cm layer in the rhizotrons (Fig. 2.10) but only ranked 85<sup>th</sup> and 80<sup>th</sup> respectively, in the 50-80 cm soil layer in the field. Comparing average RLDs from the acetate tracings for the A and B allele calls in the average field RLD SNPs (Table 2.3) also did not show a significant difference in rooting ability within the different layers of the rhizotrons (Table 2.5). However, the QTL identified on 2BS, in close proximity to that for glaucousness, which explained RLD in the 50-60 cm soil layer in the field, showed a significant association at 40-60 and 60-80 cm depths in the rhizotrons. Doubled haploid lines exhibiting the A allele from Shamrock had significantly greater root length densities at these depths, in contrast to that in the field where lines exhibiting the B allele from Shango had greater RLDs in the 50-60 cm layer post anthesis.

Table 2.5. Average root length density (RLD,  $cm/cm^2$ ) from the acetate tracings at each depth for the A (Shamrock) and B (Shango) alleles present in the selected lines of QTL for average RLD in the field (5D; 6B; 7B, Table 2.3) and allocation of RLD in the 50-60 cm depth in the field (2BS, Table 2.3). SED \* P<0.05.

Depth (cm)	5D (RLD cm/cm <sup>2</sup> )	6B (RLD cm/cm <sup>2</sup> )	7B (RLD cm/cm <sup>2</sup> )	2BS (RLD cm/cm <sup>2</sup> )
0-20	A: 0.459 B: 0.474	A: 0.447 B: 0.475	A: 0.448 B: 0.477	A: 0.471 B: 0.458
SED	0.0195	0.0200	0.0203	0.0192
20-40	A: 0.645 B: 0.684	A: 0.661 B: 0.662	A: 0.668 B: 0.657	A: 0.669 B: 0.653
SED	0.0290	0.0297	0.0302	0.0290
40-60	A: 0.627 B: 0.582	A: 0.605 B: 0.610	A: 0.622 B: 0.598	A: 0.649 B: 0.563
SED	0.0292	0.0299	0.0304	0.0289*
60-80	A: 0.447 B: 0.414	A: 0.433 B: 0.420	A: 0.451 B: 0.420	A: 0.458 B: 0.405
SED	0.0268	0.0275	0.0280	0.0265*
80-100	A: 0.190 B: 0.185	A: 0.198 B: 0.182	A: 0.195 B: 0.182	A: 0.177 B: 0.200
SED	0.0238	0.0243	0.0248	0.0235

# 2.3.4. Non-glaucous doubled haploid lines were associated with smaller seedling root systems

Significant (P = 0.001) differences occurred within the DH population for the root and shoot traits observed in the individual lines at seedling stage (summarised in Table 2.6; detailed in supplementary Table A1, refer to appendices). Shamrock tended to have a smaller seedling root system, in terms of length, surface area and volume than Shango, contradictory to root length measurements seen in the rhizotrons and in deeper soil in the field. However, as in the field and rhizotrons, Shamrock produced finer roots, having a significantly higher percentage of roots within the lower root diameter class 0-0.5 mm (Table 2.6).

The population showed a normal distribution for the majority of traits measured (Fig. 2.13). The continuous variation within the population for these traits did show some skew with a high number of lines exhibiting a root:shoot ratio below the population mean. This was also the case when looking at root diameter traits as more lines had a higher percentage of total root length in the lower root diameter class (0-0.5 mm) compared to the upper class (0.5-1 mm), resulting in more genotypes exhibiting low average root diameter compared to the mean.



Figure 2.13. Histograms showing frequency distribution of seedling rooting traits for the DH population. Data are the means of 24 plants for each line. Sm, Shamrock; Sg, Shango parent means within the distribution. Arrows show population mean.

Table 2.6. The effect of line from a doubled haploid progeny of Shamrock x Shango winter wheat on root and shoot characteristics when grown as seedlings in germination paper rolls. SED \* P<0.05 between Shamrock and Shango, n = 24. Glaucousness QTL on 2BS, Shamrock is A allele and Shango is B.

		Tot	al root size			]	Root diamete	er	Shoot	Root: Shoot (ratio)
-	Length (cm)	Surface area (cm <sup>2</sup> )	Volume (cm <sup>3</sup> )	Dry matter (mg)	Seminal Axes (no.)	Mean (mm)	0-0.5 mm (%)	0.5-1 mm (%)	dry matter (mg)	
Parental lines										
Shamrock	57.6	7.82	0.085*	16.8	3.96	0.432	0.91*	0.08*	12.7	1.37
Shango	62.1	8.67	0.097*	16.9	4.00	0.448	0.87*	0.12*	13.2	1.34
Minimum value a	nd responsibl	e line amor	ngst populat	ion						
Minimum	51.0	7.18	0.075	13.6	3.75	0.417	0.77	0.05	10.7	0.88
Line (Glaucous allele A/B)	6(A)	93c(B)	93c(B)	10(A)	18(A)	93c(B)	6(A)	93c(B)	93c(B)	64(A)
Maximum value a	nd responsib	le line amoi	ngst popula	tion						
Maximum	72.3	9.86	0.110	19.6	4.72	0.476	0.94	0.23	16.3	1.94
Line (Glaucous	56(A)	56(A)	72(B)	93c(B)	64(B)	6(A)	93c(B)	6(A)	64(B)	93c(B)
allele A/B)										
SED n=712	3.64	0.506	0.0062	1.19	0.206	0.0093	0.020	0.019	1.00	0.177
Pop. mean	62.7	8.73	0.097	16.2	4.27	0.444	0.89	0.11	14	1.21

The principal component analysis captured 90% of the variation within the Line x ten variate table (Table 2.7) by three principal components (Fig. 2.14). Principal component (PC) 1 accounted for variation mostly in 'size' of the root system as measured by *WinRhizo* i.e. number of root axes, root length, and root surface area. Principal component 1 was also positively associated with shoot dry weight (and hence negatively associated with root: shoot ratio) but negatively associated with root dry weight. Principal component 2 accounted for measures of root diameter, high values representing the finest roots. Principal component 3 mostly accounted for additional variation in root dry weight, not already accounted for by PC1 and showing less of an association with shoot dry weight. This method of analysis summarises where the variation in the population is occurring rather than analysing single traits.

Doubled haploid lines with the Shamrock allele for the glaucousness SNP marker (Table 2.3) had a negative association (P<0.05) with total root length and root surface area (r = -0.23 and -0.26 respectively), the opposite association seen in non-glaucous lines in the rhizotrons. Doubled haploid lines 93c and 6 had particularly short seedling roots (Table 2.6, Fig. 2.14) and fine and coarse roots respectively. Both these lines, in contrast, performed well in the rhizotron experiment (Fig. 2.10), the relationships seen in the principal component analysis and the negative relationship with the non-glaucous allele are still significant without these outliers. However, doubled haploid line 56 achieved the highest seedling root length and surface area and this line did perform well in the rhizotrons (Table 2.6, Fig. 2.10).

Variate	Mean root diameter	Roots 0-0.5 mm diameter (%)	Roots 0.5-1 mm diameter (%)	Total root length	Total root dry matter	Root: shoot ratio	Total root surface area	Total shoot dry matter	Number of seminal axes
Roots 0-0.5 mm diameter (%)	-0.91								
Roots 0.5-1 mm diameter (%)	0.91	-0.99							
Total root length	-0.31	0.39	-0.38						
Total root dry matter	0.00	-0.07	0.06	-0.37					
Root: shoot ratio	0.01	-0.11	0.10	-0.54	0.85				
Total root surface area	0.10	0.01	-0.01	0.91	-0.39	-0.56			
Total shoot dry matter	-0.06	0.15	-0.13	0.63	-0.50	-0.80	0.63		
Number of seminal axes	-0.21	0.27	-0.26	0.76	-0.39	-0.45	0.71	0.49	
Total root volume	0.47	-0.34	0.34	0.68	-0.34	-0.48	0.92	0.53	0.55

Table 2.7. Correlations (r, d.f. = 88; critical values for P = 0.05 and 0.01 are 0.21 and 0.28 respectively, highlighted) between seedling variates using means from 89 lines of a doubled haploid population from Shamrock x Shango winter wheat.



Figure 2.14. Line (Shamrock x Shango doubled haploid) scores (A,C) and vector loadings (B,D) from a principal components (PC) analysis of seedling root traits. Area = root surface area, Axes = number of seminal axes, Diam = mean root diameter, Length = total root length, RWt = total root dry mass (DM), R:S = root:shoot mass ratio, SWt = total shoot mass (DM), Vol = total root volume, 0-0.5 mm = proportion (%) of roots with a diameter of 0-0.5 mm, 0.5-1 mm = proportion (%) of roots with a diameter of 0.5-1 mm. Point labels in A are the selected lines used in the rhizotron experiment. Filled circles are non-glaucous lines and blank circles are glaucous lines.

Trait	Chromosome	Position (cM)	Confidence Interval (cM)	LOD	Peak Marker	Additive Effect	High value allele	Variation explained (%)
TRL	2A	221.8	217.8-226.6	4.2	BS00081630	-1.397	Shango	11.8
SA	2A	223.0	217.8-227.0	3.1	BS00021693	-0.152	Shango	7.6
SA	2B	111.9	103.8-113.0	3.0	BS00022950	-0.164	Shango	8.7
Vol	1A	78.3	76.2-85.0	7.1	AX-94831368	-0.0032	Shango	20.1
AvgDiam	1A	64.3	62.0-64.6	5.0	BS00064197	-0.0035	Shango	7.9
AvgDiam	2D	51.1	47.9-60.4	5.4	BS00009606	0.0037	Shamrock	8.6
AvgDiam	5A	159.6	151.1-162.7	7.1	AX-94694244	-0.0045	Shango	12.2
AvgDiam	5B	131.0	124.5-139.9	4.7	BS00034333	-0.0036	Shango	8.0
AvgDiam	6A	30.9	29.1-32.5	7.4	AX-94579171	0.0045	Shamrock	12.4
AvgDiam	7A	90.4	86.5-94.5	6.5	BS00077445	0.0043	Shamrock	10.9
% TRL 0-0.5 mm	1A	76.0	73.8-81.8	4.1	AX-95683697	0.0087	Shamrock	7.8
% TRL 0-0.5 mm	5A	128.0	116.2-131.5	4.3	AX-95657946	0.0093	Shamrock	8.1
% TRL 0-0.5 mm	5B	128.0	120.2-137.5	3.2	BS00034333	0.0081	Shamrock	6.2
%TRL 0-0.5 mm	7A	103.3	101.6-106.7	4.4	AX-94498468	-0.0095	Shango	8.2
%TRL 0.5-1 mm	1A	78.3	74.0-81.3	4.0	AX-94831368	-0.0080	Shango	7.0
%TRL 0.5-1 mm	1D	49.1	40.9-50.1	3.8	AX-94413085	-0.0078	Shango	6.6
%TRL 0.5-1 mm	2D	30.4	26.9-36.2	5.4	BS00065456	0.0104	Shamrock	9.8
%TRL 0.5-1 mm	5A	123.5	113.3-131.7	5.6	AX-94536022	-0.0110	Shango	12.2
%TRL 0.5-1 mm	5B	124.0	117.8-132.4	6.1	BS00034333	-0.0107	Shango	11.4
%TRL 0.5-1 mm	7A	103.3	100.6-105.6	5.7	AX-94498468	0.0105	Shamrock	10.5
RDW	5A	165.6	160.1-175.1	3.8	AX-94663230	-0.3506	Shango	8.4
RDW	6A	92.9	89.5-102.9	5.1	AX-94893553	0.4179	Shamrock	11.9
SDW	2B	17.3	16.3-19.9	3.7	AX-94529633	-0.7352	Shango	12.5
SDW	6A	92.9	90.4-101.0	3.4	AX-94893553	-0.3439	Shango	9.6

Table 2.8. Quantitative trait loci (QTL) from a Shamrock x Shango doubled haploid population for seedling root traits.

R:S	6A	92.9	90.7-103.4	3.2	AX-94893553	0.0594	Shamrock	10.1
No Axes	2B	111.9	104.3-113.0	3.7	BS00022950	-0.0855	Shango	10.8
No Axes	6B	57.7	48.4-65.8	3.1	AX-95082971	-0.0848	Shango	11.0
No Axes	7A	162.8	155.1-168.8	3.6	BS00022169	0.0918	Shamrock	10.4

TRL; total root length, SA; surface area, Vol; root volume, AvgDiam; average diameter, %TRL 0-0.5 mm; percentage of root length in the 0-0.5 mm diameter class, %TRL 0.5-1 mm; percentage of root length in the 0.5-1 mm diameter class, RDW; root dry weight, SDW; shoot dry weight, R:S; root shoot ratio, No Axes; number of seminal axes.

Table 2.9. Quantitative trait loci (QTL) from a Shamrock x Shango doubled haploid population for principal component (PC) scores derived from seedling root traits.

Chromosome	Position (cM)	Confidence Interval (cM)	LOD	Peak Marker	Additive Effect	Positive allele	Variation explained (%)
PC1 'Root system size'							
2B	111.9	104.0-113.0	3.8	BS00022950	-0.772	Shango	12.2
4A	18.8	9.8-27.2	3.8	BS00065863	0.837	Shamrock	11.4
6A	81.0	73.1-87.4	4.5	BS00021965	-0.949	Shango	12.4
PC2 'Root diameter'							
1A	76.0	73.5-81.8	3.3	AX-95683697	0.459	Shamrock	8.5
1D	49.1	39.0-50.1	3.5	AX-94413085	0.469	Shamrock	9.1
5B	128.0	122.4-139.1	3.0	BS00034333	0.442	Shamrock	8.0
7A	89.4	86.2-91.4	7.0	BS00077445	-0.630	Shango	13.7
PC3 'Root dry weight and size'							
1B	144.8	138.4-150.4	4.0	BS00071895	-0.360	Shango	9.9
3A	44.8	38.0-56.1	3.6	AX-94591588	0.347	Shamrock	7.9
5A	144.6	132.1-153.9	4.0	BS00069414	-0.374	Shango	9.6

QTL analysis was completed for all the seedling root traits studied (Table 2.8). In total 28 QTL were identified on 10 linkage groups, explaining between 6.2 and 20.1% of the phenotypic variation, with 16 of these QTL being associated with root diameter. Shango was the high value allele associated with root length, surface area, volume and shoot dry weight. A QTL identified on 6A was associated with shoot and root dry weight, explaining variation in root:shoot ratio (Table 2.8). The QTL identified from the PC scores collate these traits and explain overall variation in the root systems of the DH population and will therefore be focused on in this study. Three QTL were evident for seedling root size (PC1, Table 2.9) with Shango contributing the positive allele for QTL on 2B and 6A and Shamrock contributing a positive allele for the QTL identified on 4A. Shamrock contributed the majority of high value alleles for finer root diameter (PC2) with QTL on 1A, 1D and 5B and Shango contributed a high value allele on 7A. Three QTL were identified for PC3, with Shango contributing positive alleles for QTL on 1B and 5A and Shamrock contributed a positive allele for QTL identified on 3A. These OTL, as expected, also explained phenotypic variation in the individual root traits, specifically 2B for root surface area and number of axes, 1A for root volume and proportion of roots in the two diameter classes and 5B and 7A for average root diameter (Fig. 2.15, Table 2.8).

There were very few common QTL explaining variation in both field and seedling root traits. However, there was some overlap of QTL confidence intervals and co-located QTL identified for root traits within the 10 cm soil layers in the field and seedling root traits (Fig. 2.15, Table 2.3 and 2.8). The confidence intervals for the QTL identified on 2D for proportion of root length in the higher diameter class of 0.5-1 mm and the QTL explaining variation in field root diameter in the 50-60 cm layer did overlap and the peak markers for the traits were neighbouring. QTL identified on 6A for average

seedling root diameter and field RLD in the 60-70 cm layer co-located on the short arm of 6A. Shamrock contributed the high value alleles for these related QTL.



Figure 2.15. Diagram of six linkage groups (1A, 5A, 2B, 6A, 2D) of Shamrock x Shango with QTL locations. SNP marker names are labelled on the right of linkage groups and distance in cM is stated on the left of linkage group. Only linkage groups with co-locating QTL are shown. QTL locations and confidence intervals are given on bars to the right of linkage groups. QTL are labelled and black and grey bars represent field and seedling data respectively, + and - signs represent high value allele from Shamrock/Shango respectively.

#### 2.4. Discussion

### 2.4.1. Phenotypic and genetic diversity of rooting in Shamrock x Shango

The experiments described here were primarily designed to investigate the association between the non-glaucous trait exhibited by the cv. Shamrock and root length densities at depth in the field, at mature growth stages susceptible to drought. Existence of such an association would indicate that the wild emmer introgression in the breeding of Shamrock contributes to the cultivar's greater RLDs at depth, and would aid future incorporation of root architecture into breeding programmes. We have confirmed that RLDs at depth in commercially relevant field conditions in the UK (Ford et al., 2006) were considerably smaller post anthesis than reported in previous studies (Gregory et al., 1978; Barraclough and Leigh, 1984). This could be due to a multitude of reasons including lower nitrogen applications, as Gregory et al. (1978) applied 97 kg N/ha and Barraclough and Leigh (1984) applied between 105 and 220 kg N/ha within a growing season, compared to 200-235 kg N/ha in this study. High soil nitrogen levels can suppress root elongation in cereal crops (Shen et al., 2013). Additionally, in these earlier rooting studies cores were taken both within and between plant rows in equal number whereas within row cores have been reported to overestimate root length densities, particularly in more shallow soil depths (Buczko et al., 2009).

Analysing differences in root length density of cores taken within and between rows showed more variable data occurred in soil cores taken within the rows both in 2014 and 2015 (Fig 2.3 and 2.4), although the average for RLD at each depth for within and between cores is similar. Therefore, statistical differences are more likely to be identified between genotypes with a weighted sampling scheme for between row cores (Van Noordwijk et al., 1985). In terms of root allocation within and between rows, this differed significantly between DH lines indicating different rooting behaviours within the population. Shamrock had higher RLD within the row compared to Shango and significantly so in the 60-70 cm soil depth (P<0.001;Table 2.2), which could indicate straighter axes and more compact rooting as observed within the rhizotrons (Fig 2.11).

Increasing rooting at depth is important to improve the acquisition of solutes and subsoil water to increase resource use efficiency in future wheat cultivars (Atta et al., 2013; Foulkes et al., 2009). Multiple studies have identified the inadequacy of rooting at depth in wheat cultivars (Hoad et al., 2004; White et al., 2015), below the defined RLD of 1 cm/cm<sup>3</sup>, thought to be sufficient for water uptake (Barraclough et al., 1989; King et al., 2003; Van Noordwijk, 1983). RLDs in this study varied between 0.12 and 0.66 cm/cm<sup>3</sup> below 50 cm in the DH population in 2014/15 (Fig 2.6). These values are particularly low and could be a result of underestimation due to taking just three cores per plot whereas in 2013/14 eight cores per plot were sampled. Additionally more rainfall in the first field season may have contributed to Shamrock and Shango achieving average RLDs of 1 and 0.7 cm/cm<sup>3</sup> below 50 cm (Fig. 2.5). These values are similar to those collected in recent UK studies of winter wheat cultivars, where average RLDs were found to be 0.74 and 0.52 cm/cm<sup>3</sup> in the 40-60 and 60-80 cm soil horizons respectively (White et al., 2015) and generally less than 1 cm/cm<sup>3</sup> below 40 cm (Hoad et al., 2004). We have further added to the previously available evidence that Shamrock can have greater RLDs in deeper soil horizons compared to other UK-adapted wheats at critical periods of development (Ford et al., 2006; Gregory et al., 2005). We have also demonstrated that a significant (P < 0.001) variation in RLD and RDW at depth exists in field-grown doubled haploid progeny of Shamrock x Shango. Additionally, selected lines grown within rhizotrons exhibited different rooting patterns down the 1 m soil profile, with some lines decreasing RLD to a greater extent down the profile with other

lines' RLD values peaking within the 20-40 or 40-60 cm layers (Fig. 2.10). QTL were identified on 5D and 6B explaining variation in RLD within the 50-80 cm field soil layer at anthesis, contributed by Shamrock alleles. Additionally, Shango contributed alleles for RLD on 7B and for RDW on 2A and 7B; with these QTL explaining between 8.4 and 17.3% of the phenotypic variation occurring in the population (Table 2.3).

The relevance of QTL studies for future marker assisted selection of new crop cultivars was demonstrated by Merchuk-Ovnat et al. (2016). Backcrossing of drought resistant QTL, associated with agronomic traits, into durum and bread wheat cultivars was achieved using a previous study of a durum wheat and wild emmer RIL population (Merchuk-Ovnat et al., 2016; Peleg et al., 2009). QTL on chromosomes 2BS and 7AS improved productivity in water limited and well-watered conditions. Specifically, introgressed markers at 24.8, 64.6 and 78.9 cM on 2B gave significantly higher grain yields than the parent cultivar in both water treatments (Merchuk-Ovnat et al., 2016). This study indicates the potential of the identified root QTL in this investigation in improving future allocation of wheat biomass to the root system, succeeding the confirmation of these QTL in additional field seasons.

Through assessing root phenotypic traits at mature growth stages in the field, we found an association between the non-glaucous trait mapped to the short arm of 2B (Simmonds et al., 2008) and allocation of root length at different depths within the 50-80 cm soil cores. Shango achieved a higher RLD in the 50-60 cm layer and was the contributing high value allele identified on 2BS for root allocation in this soil layer. Shamrock and non-glaucous DH lines had an increase in RLD between the 50-60 and 60-70 cm depths and slower decrease of rooting density down the soil core (Fig. 2.9). This is a similar pattern seen when comparing glaucous and non-glaucous selected DH lines in the rhizotron experiment (Fig. 2.10). However, the differences seen in the field

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were small and not significant (P>0.05) and any improvement to resource uptake is questionable. Larger differences between rooting in glaucous and non-glaucous lines were seen in the rhizotrons below 40 cm at the end of tillering (GS 29). Selected DH lines with the Shamrock allele at the identified 2BS QTL in the field had significantly (P<0.05) higher RLD at the 40-60 and 60-80 cm depths (Table 2.5), contrary to the field where Shango was the high value parent. This was in a glasshouse environment where water supply was controlled and the soil profile was not allowed to dry out. Additionally, although non-glaucous DH lines were associated with higher yields (P=0.02) over the two field seasons, when broken down the non-glaucous trait was associated with significantly (P<0.001) greater grain mass in the 2013/14 season and a non-significant trend in the 2014/15 season. The influence of the non-glaucous trait may then vary seasonally for its effects on rooting and associated yield traits, being influenced by the genotype x environment interaction (Acuna and Wade, 2012). There was no significant (P>0.05) relationship found between rooting at depth in the two field seasons and yield of the DH population.

#### 2.4.2. Seedling root size and associated quantitative trait loci

The high number of QTL identified in seedling root screens indicates the multiple genetic influences effecting root architecture (Atkinson et al., 2015; Bai et al., 2013; Maccaferri et al., 2014; Petrarulo et al., 2015). The PCA used in this study was a useful method to identify genetic controls on seedling root characteristics which influence multiple related features such as length and surface area (Fig. 2.14). Shango had greater seedling root length and surface area in the seedling screen compared to Shamrock (Fig. 2.14; Table 2.6), contributing a high value allele for a QTL identified on the long arm of 2B for root system size (PC1; Table 2.9), which also explained variation in the single root traits of surface area and number of seminal axes (Table 2.8; Fig. 2.15). The

negative association of DH lines, carrying the Shamrock allele at the glaucousness QTL on 2BS, with root length and surface area, is likely to be an association of the QTL on 2BS and 2BL as they coincide on the same linkage group. This conclusion was drawn because the relationship between glaucous DH lines and larger seedling root size becomes insignificant (*P*>0.05) when the additive effect of the 2BL QTL is included, as singularly 2BL has a larger association with these traits. Other wheat seedling root screens have also identified QTL explaining root size phenotypic traits on the long arm of 2B. QTL which explain variation in wheat seedling total root length in both DH and RIL populations of durum and bread wheat were identified on the long arm of 2B by Kabir et al. (2015) and Maccaferri et al. (2014). Horn et al. (2016) also found a QTL on the long arm of 2B explaining variation in root hair length in a DH population of Charger x Badger. In addition to root length, QTL for root number were also identified in multiple studies on the long arm of 2B (Liu et al., 2013; Maccaferri et al., 2016; Zhang et al., 2013). This consensus with the literature indicates the importance of the genetic influence of 2BL on seedling root size.

An unexpected negative relationship occurred between root dry weight and total root length/root system size, which has not been reported in previous seedling screens. Shoot dry weight was positively correlated with root system size (PC1), which were both negatively related to root dry weight. PC3 explains the variation between the DH lines for total root length that is not associated with shoot dry weight. Subsequently, root dry weight and root length both show a positive relationship with PC3. These relationships could be indicating a carbon balance between the root and shoots (Sheng and Hunt, 1991) which is also indicated by a QTL identified on 6A explaining the phenotypic variation in seedling root:shoot ratio, with Shamrock contributing a high value allele for root dry weight and Shango contributing a high value allele for shoot dry weight (Table 2.8). Alternatively, this may be a result of seedling root age as DH lines with more seminal axes had a higher root length, this could have caused a lower root dry weight due to more recent emergence of some axes, and if measured, fresh weight may have had a positive relationship with root length.

To our knowledge no other study has identified QTL for wheat root length at mature growth stages in the field and therefore we cannot directly compare QTL found in this study to the literature. However, Ehdaie et al. (2016) studied a wheat RIL population in 80 cm tall sand filled columns to maturity. QTL were identified on the short arm of 7B for the longest root, close to the RLD QTL in this study on 7B and a QTL for root dry weight in the top 30 cm on the short arm of 2A. Bharti et al. (2014) also studied wheat roots to maturity but in 20 x 30 cm polythene bags. In this study a QTL was identified in a RIL population on 1B explaining variation in root volume, in a similar location to the QTL in this study on 1B for RLD in the 50-60 cm soil layer.

#### 2.4.3. Rooting characteristics in different growth environments

Phenotypic root traits, specifically root length and dry mass, of field-grown mature Shamrock x Shango doubled haploid lines did not show any correlation to observations made within the rhizotrons or the seedling screen. Lines which produced the least root length in the seedling screen were amongst those with the greatest root lengths in the rhizotrons and selected lines which performed best in the rhizotrons had the lowest average RLD below 50 cm in the field. Highly significant effects of the interaction between genotype and growing conditions on cereal root system size and morphology were found in other studies. For example, root length was found to be significantly greater in gel media compared to soil possibly due to reduced penetration resistance and nutrient concentration (Hargreaves et al., 2009; Wojciechowski et al., 2009). In a study comparing Indian and Australian wheat cultivars in different regions in the two countries, over multiple years, root length traits differed significantly between sites and seasons. Between seven and 90 genotypes were studied over a three year period at three sites in India and two sites in western Australia, ranging between two and eight replicates of each genotype. This indicates the large influence of soil heterogeneity, weather patterns and management on rooting traits down the soil profile (Rich et al., 2016). Specifically, this particular study compared lines grown in two different controlled environments in addition to two field seasons with varying weather patterns, with the second growing season receiving half the amount of rainfall during stem extension and 235 mm less rainfall in total.

Further, genotype effects also seem to interact with growth stage of wheat plants. In a seedling screen of a spring wheat recombinant inbred line (RIL) population, root lengths were shown to correlate with field root length at the two and five leaf stages, but not at anthesis (Watt et al., 2013). Motzo et al. (1993) studied genotypic differences in rooting traits in durum wheat at stem elongation and heading. Genotypic ranking of root number and dry weight changed depending on soil nutrient concentration and growth stage, due to differences in growth rate and tillering (Motzo et al., 1993). Additionally, Ehdaie et al. (2016) studied rooting ability in wheat in 80 cm tall cylinders and found weak correlations within genotypes for root dry weight at mid-tillering and maturity.

Nonetheless, in the rhizotron experiment presented here, Shamrock did have greater RLD at depth compared to Shango (Fig. 2.10 and 2.11), an observation consistent with that found in the field in the 60-80 cm soil layers (Fig. 2.6 and 2.7). Root length of Shamrock was distributed more evenly throughout the profile, possibly associated with straighter seminal axes and more uniform branching in each soil layer. Shango had

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more tortuous seminal axes with longer root branches, which may have caused less proliferation below 60 cm depth. Narrow seminal axes in wheat have been identified as a trait which increases rooting at depth due to reduced horizontal root growth and a more compact root system (Manske and Vlek, 2013). Shamrock also consistently had finer roots than Shango in the seedling, rhizotron and field experiments, with Shamrock alleles having mainly positive effects on QTL for smaller root diameter in the seedling screen (Table 2.9). Root diameter was negatively associated with root length in the DH lines at the different growth stages. Wild emmer had finer roots in the rhizotrons and greater root length in the top 60 cm. Producing roots of a smaller diameter reduces the metabolic cost of the roots, thereby allowing more root biomass production and proliferation (Lynch, 2013). This aids resource uptake which is more closely related to root length and surface area than root mass (Eissenstat, 1992).

The co-location of QTL for root traits in the seedling stage and mature roots in the field were associated with root diameter. A peak marker on the short arm of 6A explained variation in average diameter of seedling roots and RLD of field roots in the 60-70 cm soil layer. The relationship between these traits indicates the influence root diameter has on total root length due to the lower metabolic cost of fine roots (Lynch, 2013). Additionally, overlap of confidence intervals was seen on 2D for QTL explaining both percentage of total root length in the higher diameter class 0.5-1 mm in the seedlings and average diameter in field roots within the 50-60 cm soil layer (Fig. 2.15). This suggests that diameter is a more transmissible trait than others related to length due to its influence at both early and mature growth stages and suggested effect on root length at depth in the field at anthesis. Root diameter is one of the few traits which have been bred for in wheat roots with Richards and Passioura (1989) reducing xylem diameter in

Australian cultivars to increase axial resistance of water flow to the roots to ensure water is not used up before critical growth periods.

#### 2.5. Conclusions

Due to the lack of information for the specific location of the wild emmer introgression into Shamrock, the positive effect of the identified QTL on average RLD at depth in the field cannot be reliably attributed to a contribution from wild emmer. There is potential to study the emmer introgression further, either by investigating potential segregation distortion within Shamrock x Shango which can be indicative of alien introgression (Niu et al., 2011) or comparing Shamrock DNA sequences with emmer genes when the T. dicoccoides' genome has been sequenced. It has not been proven with confidence that the known emmer introgression on 2BS is responsible for Shamrock's improved rooting at depth below the plough layer, due to non-glaucous lines having smaller seedling root size and Shango contributing the high value allele for a QTL on 2BS explaining higher rooting in the field at 50-60 cm. The genetic diversity of rooting traits within the Shamrock x Shango population and Shamrock's consistently higher RLDs, in different environments and growth stages is important for the further study of genetic controls on root architecture traits which improve rooting at depth in the field at principal growth stages (Wasson et al., 2012). Therefore, continued study of this population and the association of the QTL on 2BS would be worthwhile in additional seasons and in environments where drought during grain-filling can be controlled. The integration of increased rooting at depth into breeding programmes is beneficial to provide stability to current wheat yields and has the potential to be implemented using more significant and repeatable QTL which relate to high rooting densities improving resource uptake (de Dorlodot et al., 2007).

## Chapter 3. Associating canopy and rooting characteristics in a wheat doubled haploid population in a UK field environment

### **3.1. Introduction**

Phenotyping crop rooting for integration into breeding programmes is currently laborious and time consuming, especially for mature plants grown in the field. Improving the ease and repeatability of these measurements is necessary for the inclusion of resource uptake and abiotic stress adaptation traits in new varieties to be realistic (Pinto et al., 2010). The assessment of crop water status can be indicative of deep rooting and the ability to extract soil water. Crop water status can be indicated by transpiration rate, stomatal conductance, carbon isotope discrimination or canopy temperature (Pask and Reynolds, 2013). Research on the ability of wheat genotypes to adapt to drought, focused mostly in the dry wheat growing regions of Mexico and Australia, has used these measurements to identify superior genotypes and correlate them with root mass and water uptake at depth (Pask and Reynolds, 2013; Pinto and Reynolds, 2015).

Improved drought tolerance is associated with a more hydrated canopy under waterlimited conditions. Canopy hydration can be expressed through a lower canopy temperature, more open stomata and higher carbon isotope discrimination (Olivares-Villegas et al., 2007). Carbon isotope discrimination is the ratio of stable isotopes of carbon (<sup>13</sup>C/<sup>12</sup>C) in plant material relative to the atmosphere. Discrimination of <sup>13</sup>C decreases with water stress because of lowered stomatal conductance (Farquhar et al., 1989). Canopy temperature has been used as a proxy to identify better yielding genotypes under abiotic stress due to its relatively high heritability and ease of measurement (Olivares-Villegas et al., 2007). Low canopy temperature has been indicative of genotypes with increased water uptake through better rooting (Lopes and Reynolds, 2010). Lower canopy temperatures during grain filling in wheat genotypes were associated with an increase of 7.8 g/m<sup>2</sup> of increased root mass at 60-120 cm depth and 80 g/m<sup>2</sup> for yield (Lopes and Reynolds, 2010). Canopy temperature was also shown to track changes in water uptake from 60-120 cm soil depths in CIMMYT (International Maize and Wheat Improvement Centre) spring wheat cultivars under different irrigation regimes, with a -1°C change during grain filling associated with 42 mm of water taken up from the soil (Pask and Reynolds, 2013).

An additional high throughput measurement to assess crop water status is the use of spectral reflectance indices. Reflectance of light by vegetative material depends on wavelength. The photosynthetically active region of the electromagnetic spectrum spans wavelengths from 400 to 700 nm, also known as the visible spectrum. Reflectance is controlled by leaf pigments and is low in the red and blue light wavelengths and higher in the green wavelengths (Pfitzner et al., 2011). Light reflectance increases significantly in the near infrared (NIR) region where values are commonly 40-50% or more between 700 and 1300 nm, primarily controlled by cell structure (Pfitzner et al., 2011). The normalised difference vegetation index (NDVI) is the ratio of visible and NIR wavelengths and has been widely used for remote sensing of vegetation (Gao, 1996). NDVI is a measure of photosynthetically active vegetative tissue and has been used to segregate superior yielding wheat genotypes through delayed senescence, as values decrease as green tissue is lost (Babar et al., 2006; Lopes and Reynolds, 2012).

Water-sensitive wavelengths have been used to assess plant water status, specifically at 970 nm. Peñuelas et al. (1993a) introduced a remote sensing index of the water absorption band and a reference wavelength, producing the ratio R970/900, which

identified changes in the relative water content of pepper (*Capsicum annuum*) and bean (Phaseolus vulgaris) plants subjected to drought stress. Normalised water indices (NWI) using the water absorption band and different reference wavelengths have since been developed as a proxy to select for high yielding wheat cultivars. Spectral reflectance indices measured at booting, heading and grain fill correlated with yield related traits, in terms of biomass, chlorophyll content and grain yield, allowing for their use in selecting higher yielding genotypes (Babar et al., 2006; Prasad et al., 2007). High NWI values are associated with reduced canopy water contents. Gutierrez et al. (2010) studied the relationship between NWI and plant and soil water status to determine its suitability as a proxy for these traits. A variety of wheat germplasm was assessed in different water-deficit conditions and a particular NWI [NWI3: (R970- $R_{880}/(R_{970}+R_{880})$ ] was found to associate best with plant water status measurements of leaf water potential, canopy temperature and available soil water (Gutierrez et al., 2010). NWI3 was positively associated with canopy temperature when measurements from the different growth stages were combined ( $r^2 = 0.64$  to 0.81) and negatively associated with available soil moisture at deeper soil layers ( $r^2 = 0.40$  to 0.72). The relationship with available soil water indicates the potential of this spectral reflectance index to screen for wheat genotypes with improved access to subsoil water (Gutierrez et al., 2010).

This study seeks to assess the capacity of canopy trait measurements to act as proxies for rooting status in a wheat doubled haploid population of Shamrock x Shango, grown in a UK field experiment. As shown in Chapter 2 and elsewhere (Ford et al., 2006), Shamrock has significantly greater root length densities below 40 cm compared to other UK elite wheat cultivars. Shamrock is a winter wheat with recent introgression from wild emmer (*Triticum dicoccoides*), from which it has inherited a non-glaucous trait,
because of reduced epicuticular wax on the leaves and stem. This trait gives Shamrock a characteristic green colour and is associated with a stay green effect. Consequently, the Shamrock x Shango doubled haploid population differs for glaucousness and green leaf area duration (Simmonds et al., 2008).

The glaucousness trait is thought to be an adaptation to drought environments as the wax layer on the leaves reduces water loss from the plant cuticle, which can improve water use efficiency. The canopy is protected against high radiation by increased light reflectance, which reduces canopy temperatures (Bennett et al., 2012; Febrero, 1998; Ryo Nishijima et al., 2014). Multiple studies have identified a yield advantage of glaucous over non-glaucous genotypes. Durum wheat exhibiting glaucousness, grown in a Mediterranean environment, had significantly higher flag leaf carbon isotope discrimination values which were strongly associated with higher yield, compared to non-glaucous wheats (Merah et al., 2000). The same relationship was found in bread wheat cultivars differing in glaucousness. Higher carbon isotope discrimination values correlated with a higher transpiration rate and lower transpiration efficiency, which was associated with yield caused by significantly higher grain weights (Monneveux et al., 2004). An additional study of durum wheat near isogenic lines (NILs) grown in varying soil water conditions, found the glaucous genotypes yielded significantly higher grain and dry matter but only in the higher yielding environments, no difference was found in the drier environment (Johnson et al., 1983).

Non-glaucousness is controlled by wax inhibition genes Iw1 on chromosome 2B and Iw2 on chromosome 2D, inherited by *T. dicoccoides* and *Aegilops tauschii* respectively (Wu et al., 2013). Iw1 and Iw2 inhibit the wax production genes W1 and W2 located on the same respective chromosomes. Only one Iw gene needs to be present to prevent glaucousness (Adamski et al., 2013). The major components of the epicuticular waxes 129

are  $\beta$ -diketones, C29 and C31 hydrocarbons, primary alcohols, ketones and esters (Adamski et al., 2013; Xu et al., 2015). *Iw1* specifically inhibits the production of the  $\beta$ -diketones in the peduncle and flag leaf cuticles and has been shown to be a dominant gene as the non-glaucousness trait persisted when Shamrock was crossed with six different hexaploid wheats, i.e. *Iw1* is independent of genetic background (Adamski et al., 2013).

The delayed senescence trait, present in Shamrock, is associated with non-glaucousness inherited from wild emmer (Simmonds et al., 2008). The extension of green leaf area duration post-anthesis increases the grain filling period and is thought to be a trait indicative of drought tolerance (Foulkes et al., 2007; Naruoka et al., 2012). Stay green in wheat genotypes has been associated with rooting at depth in multiple studies. Hebbar et al. (2014) found that early senescing wheat lines had 42% less root biomass at booting and subsequently total plant nitrogen was less than half that in later senescing lines; suggesting that early senescence was caused by lower root biomass reducing access to soil nitrogen and water resources. In RIL populations of wheat green leaf area duration after heading was significantly associated with grain weight under late season heat and drought stress conditions, but not in cool non water-limited conditions (Naruoka et al., 2012). Xylem exudate was positively correlated with green leaf area duration after heading, indicating the role of root activity and water uptake in this trait (Naruoka et al., 2012). Conversely, Borrill et al. (2015) studied transgenic lines of wheat with reduced expression of the homologues of the NAM-B1 transcription factor, causing a photosynthetically active canopy 10 days later than a control. Although NAM RNAi plants achieved 40% more photosynthesis post-anthesis the rate and duration of starch accumulation during grain filling and final grain weight did not differ to control plants. This was due to NAM RNAi plants storing additional photosynthate as fructans

in the stem, requiring the need for the delayed senescence trait to be combined with an increase in grain fill capacity (Borrill et al., 2015).

The aim of this study is to identify potential high-throughput proxy measurements for rooting at depth in a doubled haploid wheat population of Shamrock x Shango which differs for root length at depth (Chapter 2; Ford et al., 2006). Multiple canopy measurements involving spectral indices, photosynthetic ability and canopy temperature were taken during two seasons in field experiments at the University of Reading Crops Research Unit in Sonning, UK. The influence of glaucousness, which differs within the population, on canopy measurements and the relationship of the root and shoot is assessed.

# **3.2. Methodology**

## 3.2.1. Plant material

This study used a doubled haploid (DH) wheat population of 87 lines of a Shamrock x Shango cross, together with the two parents. The population segregates for a nonglaucous trait, (non-glaucous; NG and glaucous; G) which Shamrock inherited from recent introgression from wild emmer (*Triticum dicoccoides*). Shamrock is a group 1 bread making wheat derived from a cross between NW Europe germplasm (CWW 4899/25 - Moulin x Monopol) and a *T. dicoccoides* derivative (Comp Tig 323-1-3 M). Shango is a group 1-2 winter wheat variety suitable for growing in northern France (Simmonds et al., 2008).

## 3.2.2. Field experiment

The DH population was grown at the Reading University Crops Research Unit, Sonning, UK ( $0^{\circ}54$ ' W,  $51^{\circ}29$ ' N) in each of the 2013/14 and 2014/15 seasons in a randomised block design replicated twice; the parents were sown twice in each block. The field was power harrowed and seed drilled in 2 x 5 m<sup>2</sup> plots at 300 seeds/m<sup>2</sup> on a free draining sandy loam overlying coarse red-brown sand (Sonning series (Jarvis, 1968)). In the first year, the wheat was the third cereal after a grass ley; in the second year the wheat was the first cereal after a 3-year grass plus clover ley. The experiment received 16 kg of S/ha in both years, and 200 kg/N/ha in 2013/14 and 235 kg/N/ha in 2014/15 as granular fertiliser. Plots were maintained free of weeds and disease with the appropriate herbicides and fungicides. Weather data was recorded in both seasons using a weather station on site. Yield was assessed through harvesting of the whole plot with a combine harvester. In the 2014/15 field experiment destructive sample collection meant the plots at maturity were reduced to 1 x 1 m<sup>2</sup> areas.

## **3.2.3.** Photosynthetic active radiation and thermal time to senescence

Photosynthetic active radiation (PAR) interception was measured throughout the growing season. A ceptometer (AccuPAR LP-80; Decagon Devices Inc, Pullman Washington) was used to measure PAR above and below the canopy. A logistic curve, a+c/(1+exp(-b(t-m))), was fitted to percentage interception over time (t) to provide daily estimates of PAR interception. This was combined with daily radiation receipts to calculate PAR interception during the crop growth cycle for each plot, as described in Gooding et al. (2002). The end of canopy photosynthetic function was determined as the onset of rapid senescence which coincides with the point of 80% maximum green cover. Green cover was assessed by measuring the far red (730 nm), red (660 nm) reflectance wavelengths with sensors (SKR 1800, Skye Instruments Ltd, Llandrindod Wells, UK), also throughout crop growth. The far red: red ratio was calculated as a percentage of that measured for bare ground at the same time point (-1). 'Greenness' of each plot was determined as a percentage of the maximum value of that plot from anthesis onwards, in order to remove differences of genotype colour and ground cover.

This calculation over time (t) was used to fit a modified Gompertz curve (100\*exp(-exp(-b(t-m))) to allow the point of 80% maximum green cover to be interpolated (Addisu et al., 2010). The mean of three readings per plot on each assessment date were used for both of these canopy assessments (refer to appendices supplementary Fig. A1 and A2).

#### **3.2.4.** Canopy temperature

A handheld, thermal imaging camera (FLIR model T335, FLIR Systems, Oregon USA) was used to measure canopy temperature weekly from anthesis to the onset of senescence (9<sup>th</sup> and 25<sup>th</sup> June and 2<sup>nd</sup>, 10<sup>th</sup> and 16<sup>th</sup> July) in 2014 and at ear emergence and anthesis (21<sup>st</sup> May and 4<sup>th</sup> June) in 2015. Measurements were taken in the afternoon on cloud-free days between 12.00 and 15.00, when crop transpiration rates were expected to be at their highest. Average canopy temperature was taken from three readings per plot.

### **3.2.5. Spectral reflectance**

Spectral reflectance of the canopy was measured in the 325 to 1075 nm range at 1 nm intervals using the FieldSpec HandHeld 2 Spectroradiometer (Analytik, Cambridge UK). Measurements were taken on cloud-free days between 10.30 and 15.00 after the instrument was calibrated with a white reference panel, which reflects 100% of the incident light throughout the spectral range. This was also repeated every 10 minutes to account for solar and atmospheric changes during the measurement period, or additionally when light levels changed noticeably. The average reflectance of three measurements per plot were taken from an oblique angle at a measurement height of 0.5 m and field of view of 25° (Gutierrez et al., 2010). In 2014 measurements were taken weekly between anthesis and the onset of senescence (11<sup>th</sup>, 20<sup>th</sup> and 25<sup>th</sup> June, and 2<sup>nd</sup>,

10<sup>th</sup> and 16<sup>th</sup> July) and in 2015 measurements were taken at ear emergence and anthesis (21<sup>st</sup> May and 4<sup>th</sup> June).

A Normalised Water Index (NWI3) was calculated to estimate the crop water status of the canopy, which has previously been shown to identify genotypic differences in terms of drought tolerance in wheat (Gutierrez et al., 2010). This was calculated using the equation  $[R_{970}-R_{880}] / [R_{970}+R_{880}]$  where R is the reflectance of the NIR wavelength measured in nm (Prasad et al., 2007). Normalised difference vegetation index (NDVI) was also calculated from spectral wavelengths to assess the amount of photosynthetic vegetative tissue in the canopy, using the ratio  $[R_{770}-R_{660}] / [R_{770}+R_{660}]$  (Gao, 1996).

### **3.2.6.** Statistical analysis

GenStat v15 (VSN International) was used to analyse experimental data. Means and standard errors were calculated using ANOVA to identify differences between Lines in terms of PAR interception and far red: red reflectance over the two growing seasons, reflectance in the visible (VIS) and NIR spectrum in 2014 and canopy temperature in 2014 and 2015. Within the ANOVA the fixed model was the DH Line or glaucousness and the random model was Block. The first field experiment (2013/14) was affected by flooding and take-all disease which had a significant effect on some of the plots. To remove these variables from phenotypic plot differences the number of days flooded and the percentage of the plot affected by take all were used as covariates in the statistical models. An analysis of residual maximum likelihood (REML) was used to statistically analyse reflectance in the (VIS) and NIR spectrum in 2015, including spectral reflectance indices for NDVI and NWI3. This was in order to control error variation within the blocks by including row and column of the plot positions as incomplete blocks. The random model was Block/(Row + Column) and the fixed model

was Line, adjusted 'means' were calculated (best linear unbiased predictors). Statistical assessment of rooting characteristics measured from the field experiments around anthesis in both 2014 and 2015 is discussed in chapter 2.

Pearson correlation coefficients and regression analyses were calculated. The correlation matrix between the canopy trait Line means was used in a Principal Component Analysis (PCA). The production of a genetic linkage map using single nucleotide polymorphism (SNPs) markers and quantitative trait loci (QTL) analysis is discussed in chapter 2.

# **3.3. Results**

#### 3.3.1. Glaucosity

The non-glaucous trait in DH lines, which Shamrock inherited from wild emmer, was associated with delayed senescence (P<0.001) and increased PAR intercepted over the season (P<0.001). Thermal time to senescence did not differ significantly between Shamrock and Shango when measurements were averaged over the two field seasons (A, Fig. 3.1) but Shamrock intercepted significantly more PAR than Shango (B, Fig. 3.1). Two QTL for increased total PAR interception were identified on chromosomes 2B and 4A. Shamrock contributed the positive allele for both these QTL, with the QTL on 2B explaining nearly 20% of the phenotypic variation and neighbouring the glaucousness QTL on 2BS (Table 3.1). Three QTL were identified for thermal time to senescence on chromosomes 1B, 2B and 5A. The QTL on 2B coincided with the glaucousness and total PAR interception QTL although the SNP markers differed for these traits. Shamrock also contributed the QTL on 5A for thermal time to senescence and Shango was the contributing parent for the QTL on 1B, explaining 9.9 and 11.1% of the phenotypic variation respectively (Table 3.1).

Yield did not differ significantly within the DH population in either of the 2013/14 and 2014/15 field seasons when Line was included as the fixed effect. However, when glaucousness was the fixed effect, non-glaucous lines had significantly higher grain yield than glaucous lines when averaged over the two years (P = 0.02) (effect = +0.43 t/ha, S.E.D. = 0.186). When assessed in a single field season, only yield data in 2013/14 showed a significant (P=0.001) difference between glaucous and non-glaucous doubled haploid lines (A, Fig. 3.2). Total PAR interception and thermal time to senescence had a significant (P<0.001) positive association with yield ( $r^2 = 0.15$ , y = 0.3+0.0104x and  $r^2 = 0.28$ , y = -21.03+0.0117x respectively), in the 2013/14 field season, but there was no relationship with PAR interception and time to senescence in the 2014/15 field season.



Figure 3.1. A) Thermal time to senescence averaged over 2013/14 and 2014/15 seasons. B) Total PAR intercepted over the season, averaged over 2013/14 and 2014/15 seasons. Error bars are + and – S.E.D, \* P<0.05. Sham/Shang n = 8, glaucous/non-glaucous n = 192/176.



Figure 3.2. Yield data for the A) 2013/14 and B) 2014/15 field seasons. Error bars are + and - S.E.D, \* P<0.05. Sham/Shang n = 4, glaucous/non-glaucous n = 96/88.

	Chromosome	Position (cM)	Confidence Interval (cM)	LOD	Peak Marker	Additive Effect	High value allele	Variation explained (%)
Glaucousness	2B	1.0	0-2.1	58.5	BS00084668	0.489	Shamrock	95.6
TotalPAR	2B	4.7	2.1-6.9	6.5	AX-94939920	10.31	Shamrock	19.6
TotalPAR	4A	5.8	0.8-11.7	3.1	BS00065863	6.55	Shamrock	7.9
ttSen	1B	85.6	84.1-90.8	5.2	AX-94981461	-9.81	Shango	11.1
ttSen	2B	3.5	0.0-6.2	8.4	AX-94777767	12.60	Shamrock	19.4
ttSen	5A	89.4	88.9-90.5	4.7	AX-94472861	14.67	Shamrock	9.9

Table 3.1. Quantitative trait loci from a Shamrock x Shango doubled haploid mapping population for glaucousness, total PAR interception (PAR; MJ/m2) and thermal time to senescence (ttSen; °Cd) averaged over the 2013/14 and 2014/15 field seasons.

Presence or absence of epicuticular wax within the DH population resulted in the glaucous lines reflecting significantly (P<0.001) more light. This was seen in the visible PAR wavelength regions, averaged over 400 and 650 nm, on each measurement date (Table 3.2) and at 50 nm intervals within the PAR region (Fig. 3.3). At anthesis, glaucous lines reflected 38% more light in the 400 to 650 nm range than non-glaucous lines, averaged over the two seasons.

Table 3.2. Average PAR reflectance in the 400 - 650 nm wavelengths on each measurement date in the 2013/14 and 2014/15 seasons (NG; non-glaucous and G; glaucous). \* P<0.05 comparing NG/G; Shamrock and Shango and defining significant differences between DH lines. NG/G n = 88/96, Shamrock/Shango n= 4, DH mean n = 184.

Average PAR reflectance (%)							
2014	NG	G	SED	Shamrock	Shango	SED	DH mean
11/6/14	8.68	13.08	0.533*	8.44	16.28	2.774*	10.92*
25/6/14	9.08	14.32	0.785*	8.06	11.48	4.815	11.80
2/7/14	9.03	10.80	0.657*	7.31	11.99	3.806	9.90
10/7/14	8.37	9.69	0.385*	9.24	10.05	2.194	9.13*
16/7/14	10.84	14.58	1.324*	7.82	9.43	8.284	12.80
2015	NG	G	SED	Shamrock	Shango	SED	DH mean
21/5/15	4.02	4.611	0.1306*	3.514	4.11	0.742	4.33
4/6/15	4.10	6.50	0.412*	2.56	5.14	2.336	5.35*
3/6/15	12.58	15.54	0.958*	8.18	12.60	4.961	14.12*



Figure 3.3. Canopy reflectance in the PAR region at anthesis, averaged over the 2013/14 and 2014/15 field seasons. Error bars are + and - S.E.D \* P<0.05, Non-glaucous/glaucous n = 88/96.

The correlation matrix amongst the DH lines for reflectance from the 325 to 1075 nm wavelengths at one nm intervals was subject to principal component analysis, in order to describe variation in light reflectance between DH lines within the visible and NIR spectrums. Increased light reflectance, measured at an oblique angle, in glaucous DH lines was associated with reduced canopy temperature (Fig. 3.4). Principle component (PC) 1 for each measurement date in 2014, which explained between 69 and 89% of the variation between DH lines for light reflectance, had a stronger relationship with canopy temperature than the other principle components. PC2, which explained 14% of the variation between DH lines in 2015 at the different measurement dates, had a stronger relationship with canopy temperature than the other principle components in this analysis. A significant (P<0.05) negative correlation between canopy temperature and reflectance occurred at grain fill in 2014 (C, Fig. 3.4) and a significant positive

association occurred at ear emergence in 2015 (low values of PC2 were associated with increased reflectance in D, Fig. 3.4). However, the relationship at grain fill 2014 was not significant when the high PC1 outlier was removed (correlation coefficient reduced from -22 to -18). No significant differences were found between glaucous and non-glaucous lines or the DH population for canopy temperature in 2014 but non-glaucous lines had significantly higher canopy temperature than glaucous lines at ear emergence and anthesis (P<0.01 and P<0.05 respectively) in 2015 (Table 3.3).



Figure 3.4. Relationship between principal component scores derived from canopy reflectance between 325 and 1075 nm at 1 nm intervals in the DH population and average canopy temperature between anthesis and grain fill in 2014 and ear emergence and anthesis in 2015. Measurement date and correlation coefficient for each graph is A) 11/6/14 r = -0.06 (without outlier 74 PC1: r = -0.04) B) 25/6/14 r = -12 C) 2/7/14 r = -0.22\* (without outlier 104 PC1: r=-0.18) D) 21/5/15 r = 0.26\* E) 4/6/15 r = -0.13. \* = p<0.05. Clear dots are non-glaucous lines and filled dots are glaucous lines, n = 89.

Table 3.3. Average canopy temperature at each measurement date for the 2013/14 and 2014/15 seasons (NG; non-glaucous and G; glaucous). \* P<0.05 comparing NG/G; Shamrock and Shango and defining significant differences between DH lines. NG/G n = 88/96, Shamrock/Shango n= 4, DH mean n = 184.

	Average canopy temperature (°C)						
2014	NG	G	SED	Shamrock	Shango	SED	DH mean
9/6/14	22.38	22.36	0.251	21.64	20.95	1.462	22.36
25/6/14	20.39	20.42	0.291	20.92	18.98	1.702	20.39
2/7/14	24.36	24.22	0.106	24.36	23.96	0.589	24.29
10/7/14	23.48	23.4	0.219	23.83	22.76	1.262	23.48
16/7/14	27.48	27.41	0.240	27.26	27.79	1.402	27.47
2015	NG	G	SED	Shamrock	Shango	SED	DH mean
21/5/15	19.17	18.65	0.181*	19.20	18.96	1.084	18.91
4/6/15	24.25	24.01	0.114*	24.19	24.38	0.718	24.12

#### 3.3.2. Shamrock Shango rooting vs canopy traits 2013/14

Shamrock and Shango plots were replicated four times in each of the 2013/14 and 2014/15 field experiments. Root cores were taken in the DH parent plots at anthesis in 2014 but not for the rest of the DH population (see chapter 2 for methodology). Therefore, root measurements from 50-70 cm depth for the first year were summed for each plot to compare with canopy traits. Canopy measurements at anthesis and the beginning of grain fill (9<sup>th</sup> June and 20<sup>th</sup> June) were compared with root traits.

There was an apparent but non-significant negative association between canopy temperature and root length density (RLD) and root dry weight (RDW), at both the start of anthesis and grain fill in 2014 (P>0.05; Fig. 3.5). However, without the extreme RLD outlier there was a significant (P<0.05) positive correlation between canopy temperature and RLD at anthesis (A; Fig. 3.5). RLD and RDW had a more significant relationship with canopy temperature when field plots for Shamrock and Shango were

grouped and therefore glaucous and non-glaucous genotypes were separated. The relationship between RDW and canopy temperature at anthesis differed significantly between Shamrock and Shango (P = 0.02). Shamrock had a significant negative relationship with canopy temperature (P<0.01) and Shango had a significant positive relationship with canopy temperature (P<0.01) (A; Fig. 3.6). However, without the extreme non-glaucous outlier P = 0.06 for canopy temperature vs. RDW when assessing Shamrock. At early grain fill the two DH parents did not differ significantly for the effect of RDW on canopy temperature.



Figure 3.5. Relationships between A) Canopy temperature and RLD. Filled dots and full trendline show canopy temperature at anthesis 2014 (r = -0.25; without outlier 1.4 cm/cm<sup>3</sup>:  $r = 0.48^*$ ). White dots and dashed trendline show canopy temperature at start of grain fill 2014 (r = -0.33; without outlier 1.4 cm/cm<sup>3</sup>: r = 0.1) B) Canopy temperature and RDW. Filled dots correspond to full trendline, canopy temperature at anthesis 2014 (r = -0.36; without outlier 0.0084 mg/cm<sup>3</sup>: no change). White dots correspond to dashed trendline, canopy temperature taken at start of grain fill 2014 (r = -0.46; without outlier 0.0084 mg/cm<sup>3</sup>: r = -0.15). RLD and RDW are taken at anthesis, averaged per plot (n=8) for Shamrock and Shango at anthesis 2014, \* P<0.05.



Figure 3.6. Relationship between canopy temperature and RDW in 2014. Regression grouped for glaucousness. Percentage variance accounted (v.a.f) for in regression model stated. A) Filled dots and full regression line correspond to glaucous lines while white dots and dashed regression line correspond to non-glaucous lines, at anthesis. NG = 32.01+(-1588\*RDW) s.e 305\*. G = (32.01-23.9)+((-1588+3858)\*RDW) s.e 763\*. Relationship does not change without outlier 0.0084 mg/cm3 but NG vs RDW P<0.1 B) White dots correspond to non-glaucous lines and filled dots correspond to glaucous lines, at start of grain fill. No effect of glaucousness as a group, no change without 0.0084 mg/cm3 outlier. Slope is not significant (P>0.05). RDW taken at anthesis 2014 is average of separate Shamrock and Shango field plots (n=4); \* P<0.05.

RDW had a stronger association with NWI3  $[R_{970}-R_{880}] / [R_{970}+R_{880}]$  than RLD with average RDW per plot apparently having a negative relationship with NWI3 at anthesis  $(r^2 = 0.17, y = 0.087-20.8x)$  but not to a significant extent (*P*>0.05). No relationship was found between RDW and NWI3 at the start of grain fill. RDW appeared to be more related to PAR interception than RLD. At anthesis there was a suggestion of a positive association with PAR interception and RDW but not to a significant extent (*P* = 0.068) and this relationship was not seen without the extreme RDW outlier (0.0084 mg/cm<sup>3</sup>). There was also an apparent positive association between RDW and PAR intercepted at early grain fill but this was also not significant (Fig. 3.7).



Figure 3.7. Relationship between RDW and PAR intercepted, taken from ceptometer readings. RDW is averaged per plot (n=8) for Shamrock and Shango field plots at anthesis 2014; \* P<0.05. Filled dots correspond to full trendline for PAR readings at anthesis 2014 (r2 = 0.36, y = 82.27+1412x); no relationship without outlier 0.0084 mg/cm3. White dots correspond to dashed trendline for PAR readings at early grain fill 2014 (r2 not significant); no change without outlier 0.0084 mg/cm3.

# **3.3.3.** Associating canopy traits with rooting at depth in the DH population

Root cores taken for the whole DH population in the 2014/15 field experiment were collected 0-3 weeks after anthesis. Canopy measurements taken at ear emergence (21/5/15) and anthesis (4/6/15) were compared with root traits for the DH population. Regression analyses of DH lines were grouped by glaucousness due to the effect of this phenotype on canopy traits.

The association between canopy temperature and RLD and RDW was stronger at anthesis than ear emergence. There was a significant (P = 0.03) negative relationship between canopy temperature and RLD for non-glaucous and glaucous lines at anthesis (A; Fig. 3.8). RDW and canopy temperature for glaucous and non-glaucous DH lines had a suggested negative relationship (P = 0.075) but this was not significant (B; Fig. 3.8). Diameter was not significantly related (P>0.05) with canopy temperature at anthesis (C; Fig. 3.8). The regression line for glaucous genotypes sat significantly below non-glaucous genotypes, due to their lower canopy temperatures, but the angle of the slopes did not differ significantly (Fig. 3.8).

The relationship between NWI3  $[R_{970}-R_{880}]/[R_{970}+R_{880}]$  and rooting in the DH population differed depending on growth stage and glaucosity. Non-glaucous lines had lower NWI3 values on all measurement dates in both the 2013/14 and 2014/15 seasons. At early grain fill (25/6 and 2/7) in 2014 and anthesis (4/6) in 2015 non-glaucous lines had significantly (P<0.05) lower NWI3 values than glaucous lines but there was no significant difference between DH lines in the population (Table 3.4). Grouped regression was assessed without the extreme NWI3 outliers of -0.0096 and -0.088 at ear emergence. RLD of non-glaucous lines had a significant negative relationship with NWI3 (P=0.01) and RLD of glaucous lines had a positive, but not significant (P=0.07) relationship with NWI3 (percent variance accounted for 4.8), at ear emergence. RDW had a more significant relationship with NWI3, with non-glaucous lines having a significant (P=0.001) negative relationship with NWI3 and glaucous lines a significant (P=0.005) positive relationship (percentage variance accounted for 12.9). There was no relationship between NWI3 and root diameter and rooting did not significantly associate with NWI3 at anthesis.



Figure 3.8. Effect of rooting ability on canopy temperature in the DH population at anthesis in the 2014/15 field season. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Line equation and s.e for each graph: A) NG = 24.595+(-1.02\*RLD) s.e 0.473\*, G = (24.595-0.251)+(-1.02\*RLD) s.e 0.103\* B) NG = 24.49+(-11.5\*RDW) s.e 6.38, G = (24.49-0.248)+(-11.5\*RDW) s.e 0.104 C) NG = 23.31+(4.18\*Diam) s.e 4.73, G = (23.31-0.265)+(4.18\*Diam)), s.e 0.106. Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.

Table 3.4. NWI3 at each measurement date for the 2013/14 and 2014/15 seasons (NG; nonglaucous and G; glaucous). \* P<0.05 comparing NG/G; Shamrock and Shango and defining significant differences between DH lines. NG/G n = 88/96, Shamrock/Shango n= 4, DH mean n = 184.

	NWI3						
2014	NG	G	SED	Shamrock	Shango	SED	DH mean
11/6/14	-0.058	-0.051	0.0056	-0.048	-0.020	0.0318	-0.054
25/6/14	-0.102	-0.089	0.0035*	-0.092	-0.105	0.0310	-0.095
2/7/14	-0.094	-0.086	0.0019*	-0.080	-0.088	0.0117	-0.090
10/7/14	-0.059	-0.057	0.0175	-0.049	-0.054	0.0029	-0.058
16/7/14	-0.016	-0.015	0.0228	-0.013	-0.036	0.0038	-0.015
2015	NG	G	SED	Shamrock	Shango	SED	DH mean
21/5/15	-0.048	-0.045	0.0018	-0.050	-0.046	0.0119	-0.046
4/6/15	-0.062	-0.058	0.0018*	-0.064	-0.073	0.0129	-0.060
3/6/15	-0.081	-0.078	0.0229	-0.078	-0.068	0.0039	-0.079

The relationship between rooting traits and NDVI was the opposite for non-glaucous and glaucous DH lines at ear emergence for RLD and RDW. A higher NDVI value was associated with greater rooting in non-glaucous lines; but not to a significant extent for RLD and RDW (P=0.1 and 0.3 respectively) (A and B; Fig. 3.9). In contrast, rooting of glaucous lines had a significant negative relationship with NDVI at ear emergence for both RLD and RDW (P<0.05) (A and B; Fig. 3.9). The relationship of non-glaucous and glaucous DH lines with diameter did not differ significantly however the regression line for the glaucous genotypes was significantly below that for non-glaucous genotypes (P<0.001). There was a significant negative relationship between NDVI and diameter for DH lines (P = 0.5) (C; Fig. 3.9). There was no significant relationship found for rooting traits and NDVI for the DH lines at anthesis.



Figure.3.9. Relationship between rooting traits at anthesis and NDVI measured at ear emergence for DH lines in the 2014/15 field trial. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Line equations and s.e for each graph: A) NG = 0.9433+(0.0111\*RLD), s.e 0.0073, G = (0.9433+0.0029)+((0.0111-0.0331)\*RLD), s.e 0.0105\*B) NG = 0.945+(0.099\*RDW), s.e 0.104, G = (0.945-0.0018)+((0.099-0.309)\*RDW), s.e 0.144\* C) NG = 0.9718+(-0.11\*Diam), s.e 0.053\*. G = (0.9718-0.0081)+(-0.11\*Diam) s.e 0.00119\*. Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.

The correlation matrix for reflectance in the 325-1075 nm wavelength region, at one nm intervals, was subject to principal component analysis (PCA) for the DH lines at ear emergence and anthesis in 2015, in order to summarise the variation in light reflectance between DH lines within the visible and NIR spectrums. At ear emergence, three principal components (PC) explained a total of 99% of the variation in reflectance between 325 and 1075 nm. Variation in spectral reflectance at ear emergence explained by PCA showed significant relationships with RLD and RDW (Fig. 3.10). Nonglaucous and glaucous lines had a significantly (P < 0.01) opposing relationship with PC1 for both RLD and RDW with non-glaucous lines being negatively associated with PC1 and glaucous lines positively associated with PC1 (A and B; Fig. 3.10). Nonglaucous and glaucous lines had the same negative relationship with PC2 for RLD (P<0.01) and RDW (P=0.02) (C and D; Fig. 3.10). PC3 exhibited the alternate relationship to PC1 for RLD with non-glaucous lines associated positively with PC3, but not to a significant extent (P>0.05) and glaucous lines having a significantly negative association with PC3 (P<0.05). The relationship of RDW and PC3 did not differ between glaucous and non-glaucous lines and the relationship was not significant (P>0.05). Line equations are given in Table 3.5.



Figure 3.10. Field root traits of the DH population at anthesis with principal components from a correlation matrix of reflectance in the 325-1075 nm region at ear emergence. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Regression coefficient and (standard error) for lines are: A) NG: -95.1 (29.8\*) G: 150.5 (42.8\*) B) NG: -1549.0 (400.0\*) G: 2408.0 (552.0\*) C) -19.38 (7.08\*) D) -231.1 (95.3\*) E) NG: 4.05 (5.83) G: -19.28 (8.37\*) F) -44.9 (57.5). Glaucous/non-glaucous n = 46/43; s.e\* P<0.05. Line equations in table 3.5.

Canopy measurements also showed significant associations with variation in spectral reflectance at ear emergence, explained by PCA (Fig. 3.11). The intercept of nonglaucous and glaucous DH lines differed significantly (P < 0.05) but the slope of regression lines for PC1, 2 and 3 was not significant against canopy temperature (A, B and C; Fig. 3.11). NWI3 for both glaucous and non-glaucous DH lines associated positively with PC1 to a significant extent (P < 0.001) (D; Fig. 3.11). However, NWI3 of glaucous and non-glaucous lines showed the opposite relationships with PC2: nonglaucous lines had a negative relationship with PC2 (P=0.01) but glaucous lines had a positive relationship with PC2 (P < 0.001), P = 0.06 when the outlier -0.0096 was removed (E; Fig.3.11). NWI3 of non-glaucous lines did not show a relationship with PC3 but glaucous lines had a significant (P=0.03) negative association; however this was not significant (P>0.05) when the outlier -0.0096 was removed (F; Fig. 3.11). For NDVI, non-glaucous and glaucous lines both associated negatively (P<0.001) with PC1 and there was no effect of glaucousness (G; Fig. 3.11). For PC2 and PC3 (H and I; Fig. 11) the line intercepts of glaucous and non-glaucous DH lines differed significantly (P < 0.001) but the slopes were the same with DH lines associating positively with PC2 and PC3 (P<0.001). Total PAR absorbed and thermal time to senescence did not show any significant relationship with the principal components (Fig. 3.12).



Figure 3.11. Canopy traits of the DH population with principal components from a correlation matrix of reflectance in the 325-1075 nm region at ear emergence. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Regression coefficient and (standard error) for lines are: A) 1.08 (2.96) B) 0.584 (0.954) C) -0.465 (0.558) D) 1384 (204\*) same relationship without outliers NWI3 -0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G: P<0.1 F) NG: -8.2 (55.4) G: -219.6 (99.2\*) P>0.05 for NG and G without outliers NWI3 - 0.088 and -0.0096 G) -2071 (306\*) H) 670 (125\*) I) 408.7 (72\*). Glaucous/non-glaucous n = 46/43; s.e\* P<0.05. Line equations in table 3.5.



Figure 3.12. Regressions of total PAR intercepted and thermal time to senescence in the 2014/15 seasons against principal components from a correlation matrix analysis of reflectance in the 325-1075 nm region at ear emergence. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Regression coefficient and (standard error) for lines are: A) 0.0397 (0.0969) B) 0.0197 (0.0312) C) -0.0194 (0.0182) D) -0.0164 (0.0674) E) 0.0321 (0.0215) F) 0.0059 (0.0127). Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.

For spectral reflectance within the 325-1075 nm range measured at anthesis, three principal components (PC) explained 100% of the variation in reflectance for both nonglaucous and glaucous DH lines. Variation in spectral reflectance at anthesis showed no significant relationships with rooting traits of RLD, RDW and diameter (Fig. 3.13). For canopy traits PC1 and PC2 did not show an association with canopy temperature (A and B; Fig. 3.14) in glaucous and non-glaucous lines; however grouped DH lines for PC3 were negatively associated (P = 0.02) with canopy temperature (C; Fig. 3.14). NWI3 did not show any significant associations with the principal components but the intercept of glaucous and non-glaucous DH lines differed significantly (P<0.01) (D, E and F; Fig. 3.14). Both non-glaucous and glaucous lines had a negative relationship with NDVI for PC1 and PC2 (P<0.001) (G and H; Fig. 3.14), there was no effect of glaucousness on the relationship. The intercept of the regression line differed significantly (P<0.001) for glaucous and non-glaucous lines but both showed a positive relationship with PC3 (P<0.001) (I; Fig. 3.14). Total PAR absorbed and thermal time to senescence did not show any significant relationship with the PCs (Fig. 3.15).



Figure 3.13. Field root traits of the DH population with principal components from a correlation matrix of reflectance in the 325-1075 nm region at anthesis. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Regression coefficient and (standard error) for lines are: A) -25.8 (23.1) B) 3.82 (6.97) C) -198 (309) D) 44.2 (93.0) E) 378 (225) F) 38.9 (68.5). Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.



Figure 3.14. Canopy traits of the DH population with principal components from a correlation matrix of reflectance in the 325-1075 nm region at anthesis. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f) in regression model stated. Regression coefficient and (standard error) for lines are: A) 3.48(5.17) B) 1.17(1.55) C) -1.335(0.54\*) D) 349(304) E) -62.4(91.9) F) 14(30.7) same relationship for D, E, F without outlier NWI3 -0.099 G) -620(126\*) H) -414.2(37.7\*) I) 98.7(26.1\*) same relationship for G, H I without outlier NDVI 0.85. Line equations in table 3.5. Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.

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Figure 3.15. Regressions of total PAR intercepted and thermal time to senescence in the 2014/15 seasons against principal components from a correlation matrix analysis of reflectance in the 325-1075 nm region at anthesis. White dots are non-glaucous lines and correspond to the dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f,) in regression model stated. Regression coefficient and (standard error) for lines are: A) 0.0501 (0.0983) B) 0.0062 (0.0296) C) -0.0463 (0.0683) D) 0.0122 (0.0205). Glaucous/non-glaucous n = 46/43; s.e\* P<0.05.

The correlation matrix for the canopy measurements and rooting traits was subjected to Principal Component Analysis to assess the relationship between them. Figures 3.16 and 3.17 show the PC scores and loadings derived from the correlation matrices. For non-glaucous lines PC1 and PC2 for canopy traits at ear emergence and roots measured at anthesis explained a total of 54% of the variation between the lines for these traits. NDVI, RLD and RDW were all associated with negative values of PC1 whereas NWI3 and reflectance in the PAR region were associated with positive values of PC1. PC2 explains some variation in thermal time to senescence and total PAR interception in the non-glaucous lines but this does not appear to show an association with rooting or spectral reflectance indices (A and C; Fig. 3.16). Vector loadings for principal components of glaucous lines (D; Fig. 3.16) for PC1 and PC2 at ear emergence, explained a total of 51% of the variation. In these PCs NDVI showed the opposite association with PC1 and PC2 compared to NWI3 and PAR reflectance, as with the non-glaucous lines. RLD and RDW were associated with positive values of PC1 whereas NDVI was associated with negative values, with both these root and canopy traits being associated with positive values of PC2. Root diameter was associated with negative values of PC2 whereas canopy temperature, PAR intercepted and thermal time to senescence were associated with positive values of PC2, to different extents (B and D; Fig. 3.16).

At anthesis PAR interception, thermal time to senescence and canopy temperature were associated with positive values of PC1 and PC2 for non-glaucous lines. RLD and RDW are associated with negative values of PC1 whereas NWI3 was associated with positive values of PC1. Here PC1 and PC2 explained 45% of the variation between the DH lines for these traits (A and C; Fig. 3.17). At anthesis for glaucous lines root diameter, canopy temperature, NWI3 and reflectance in the PAR region were associated with

negative values of PC1. RLD, RDW, thermal time to senescence and PAR interception were all associated with positive values of PC1. PC1 and PC2 explained 47% of the variation in glaucous DH lines.



Figure 3.16. Non-glaucous (blank circles; A, C) and glaucous (filled circles; B, D) line scores (A, B) and vector loadings (C, D) from a principal components (PC) analysis of canopy characteristics, at ear emergence (B, D) and rooting traits at anthesis in the 2014/15 field trial. Scores are labelled with DH line name in A and B. Glaucous/non-glaucous n = 46/43.



Figure 3.17. Non-glaucous (blank circles; A, C) and glaucous (filled circles; B, D) line scores (A, B) and vector loadings (C, D) from a principal components (PC) analysis of canopy characteristics, at anthesis (B, D) and rooting traits at anthesis in the 2014/15 field trial. Scores are labelled with DH line name in A and B. Glaucous/non-glaucous n = 46/43.

Associations of NWI3 and canopy temperature did not appear until later on in the growth season as in 2014 NWI3 and canopy temperature were significantly positively associated on the 10<sup>th</sup> and 16<sup>th</sup> July ( $r^2 = 0.04 P = 0.03$ ;  $r^2 0.21 P < 0.001$ ).

RLD and RDW of DH lines were not associated with yield but when grouped for glaucousness, RLD of non-glaucous lines did not show an association with yield but glaucous lines had a suggestive positive association with yield (P = 0.075; Fig. 3.18).



Figure 3.18. RLD of DH lines at anthesis with yield of DH lines for the 2014/15 field season. White dots are non-glaucous lines and correspond to dashed regression line. Filled dots are glaucous lines and correspond to the full regression line. Regression analysis grouped for glaucousness. Percentage variance accounted for (v.a.f,) in regression model stated. NG =8.66+(-1.41\*RLD) s.e 2.29 P>0.05 G = (8.66-2.47)+(8.66+5.91)\*RLD)) s.e 3.29 P<0.1. Average for each line; glaucous/non-glaucous n = 46/43.

Table 3.5. Regression line equations for slopes significantly different from zero (P<0.05) for root and canopy traits against principal components from a PCA correlation matrix analysis of wavelength reflectance between 325 and 1075 nm at 1 nm intervals, from the 2014/15 season. NG is line equation for non-glaucous lines and G is line equation for glaucous lines. SxS is line equation when all data points taken into account.

	Ear emergence							
	PC1 (82%)	PC 2 (14%)	PC 3 (3%)					
RLD (cm/cm <sup>3</sup> )	PC1NG = 23.5+(-95.1*RLD)	PC2NG = 13.75+(-19.38*RLD)	PC3NG = slope not significant ( $P$ >0.05)					
	PC1G = (23.5-34.3)+((-95.1+150.5)*RLD)	PC2G = (13.75-13.85)+(-19.38*RLD)	PC3G = (-2.73+9.22)+((4.05-19.28)*RLD)					
RDW (mg/cm <sup>3</sup> )	PC1NG = 23.72+(-1549*RDW)	PC2NG = 12.03+(-231.1*RDW)	PC3 = slope not significant for NG and G lines					
	PC1G = (23.72-34.1)+((-1549+2408)*RDW)	PC2G = (12.03-13.77)+(-231.1*RDW)	( <i>P</i> >0.05)					
NDVI	PC1SxS = 1953+(-2071*NDVI)	PC2NG = -627+(670*NDVI)	PC3NG = -388.5+(408.7*NDVI)					
		PC2G = (-627-8.3)+(670*NDVI)	PC3G = (-388.5+6.07)+(408.7*NDVI)					
NWI3	PC1NG = 58.1+(1384*NWI3)	PC2NG = 24.75+(363.9*NWI3)	PC3NG = slope not significant ( $P$ >0.05)					
	PC2G = (58.1+12.89)+(1384*NWI3)	PC2G = (24.75-33.59)+((363.9-410*NWI3)	PC3G = (-1.76-7.31)+(-8.2-219.6*NWI3)					
	Anthesis							
	PC1 (85%)	PC 2 (14%)	PC3 (1%)					
Canopy Temp (°C)	PC1 = slope not significant for NG and G lines $(P>0.05)$	PC2 = slope not significant for NG and G lines ( $P$ >0.05)	PC3SxS = 32.2+(-1.335*Temp)					
NDVI	PC1SxS = 564+(-620*NDVI)	PC2SxS = 376.7+(-414.2*NDVI)	PC3NG = -92.7+(98.7*NDVI)					
			PC3G = (-92.7+5.614)+(98.7*NDVI)					
#### **3.4.** Discussion

#### **3.4.1.** The effect of glaucousness on canopy characteristics

The non-glaucous trait in DH lines was associated with delayed senescence and increased seasonal PAR interception in both field seasons (Fig. 3.1). QTL for these characteristics found on 2B co-located with the glaucousness allele mapped in this study and in previous studies (Table 3.1; Simmonds et al., 2008; Frizell-Armitage, 2016). Simmonds et al. (2008) associated delayed senescence directly to the glaucousness allele which was also found in this study. Additionally, this trait influences PAR interception, with canopy duration being more important than canopy size in this population. Additional QTL on 4A for PAR interception and 1B and 5A for thermal time to senescence indicate the multiple other factors influencing these traits (Table 3.1).

The effect of delayed senescence, and therefore PAR interception, on yield of the DH population depended on the year. Averaged over the two seasons non-glaucous lines had significantly higher yields but only in the 2013/14 season was this significant (Fig. 3.2), and delayed senescence and PAR interception was positively associated with yield. Seasonal differences in the effect of delayed senescence on yield in non-glaucous Shamrock x Shango lines were also seen by Simmonds et al. (2008) where no significant yield effect was found when abnormally hot weather was experienced during grain ripening. Temperatures during grain filling and ripening (July to harvest) averaged 16.6°C in 2014 and 18.4°C in 2015. Rainfall in this period was 61 mm in 2014 and 30.6 mm in 2015, indicating a potentially drought-prone environment in 2015; this may have had a greater influence on senescence than the non-glaucous trait. Christopher et al. (2008) found that a stay green wheat genotype yielded significantly higher than an earlier senescing genotype but only in environments where access to

deep soil moisture was present. The stay green genotype exhibited a narrower root system which was able to root deeper and access subsoil water which delayed senescence and increased yields (Christopher et al., 2008).

The increased reflectance of glaucous cereal genotypes seen in this study (Table 3.2; Fig. 3.3) has been reported in previous studies. A NIL population of durum wheat differing for glaucousness had 8-15% higher reflectance in glaucous types (Johnson et al., 1983) and a 20% increase in reflectance was measured in glaucous barley genotypes (Febrero, 1998). The effect of reflectance on PAR absorbance is complicated. In this study, there was no clear trend of PAR interception and PAR reflectance; canopy size will influence measurements of interception, coupled with the measurement of reflectance taken at an oblique angle. Richards et al. (1986) reported photosynthesis of the wheat ear in irrigated conditions to be 0.28 mg  $CO_2/m^2/s$  in glaucous genotypes and 0.36 mg  $CO_2/m^2/s$  in non-glaucous genotypes. However, in a study of NILs created by crossing Shamrock with six UK winter wheat varieties, no difference in PAR absorbance of the flag leaves with wax content was found, despite differences in light reflectance (Frizell-Armitage, 2016). This suggests that more light travels through the canopy in non-glaucous DH lines rather than being reflected.

The association between higher light reflectance in glaucous lines and lower canopy temperatures was weak in this study (Fig. 3.4). However, the significant relationship between variation in light reflection at anthesis, explained by principal component 3, and canopy temperature (Fig. 3.14, C) suggests that reflectance of certain wavelengths is influencing canopy temperature more than others. Additionally, glaucous DH lines did have significantly lower canopy temperatures compared to non-glaucous lines at both ear emergence and anthesis (Table 3.3). Previous studies of populations differing in glaucousness reported a 0.3°C and 0.7°C difference in canopy temperature under 166

irrigated and droughted conditions, with glaucous lines having the cooler canopy (Richards et al., 1986). In a separate study, glaucous wheat genotypes had average leaf and spike temperature depressions of 5.6 and 4.5°C respectively, compared to 4.3 and 2.9°C in non-glaucous wheat under heat stress conditions. The difference in leaf temperature depression had a significant positive correlation with wax content, but this was weak ( $r^2 = 0.14$ ) (Mondal et al., 2015). Both these studies were performed under glasshouse conditions as leaf temperatures are influenced by changes in radiation, wind speed, air temperature and vapour pressure deficit (Takai et al., 2010). Reduced canopy temperatures can improve tolerance to heat and drought stress conditions and has been shown to provide a yield benefit in stress conditions (Mason and Singh, 2014). Therefore, increased reflectance in glaucous wheats may protect yields under more extreme weather patterns in the UK.

#### 3.4.2. Rooting vs canopy traits in 2013/14 for Shamrock and Shango

Root length densities in the 50-60 and 60-70 cm soil layers and root dry weight in the 60-70 cm soil layer were significantly higher in Shamrock than Shango field plots (see chapter 2) which may explain the more significant negative relationship with canopy temperature in Shamrock compared to Shango (Fig. 3.6). The small number of data points from the 2013/14 field experiment reduces the significance of the relationship of rooting with canopy temperature but the trend is as expected when assessing average RLD and RDW per plot, with greater rooting being associated with cooler canopies (Fig. 3.5). However, for RLD (A; Fig. 3.5) this relationship is reliant on an extreme rooting average in a Shamrock field plot whereas the relationship is more stable but not significant for RDW against canopy temperature (B; Fig. 3.5). The negative relationship with NWI3 and positive relationship with PAR interception on a per plot basis was promising as a means to identify rooting mass at depth through measuring canopy traits

(Fig. 3.7). However, without the extreme RDW outlier there is not a relationship of RDW and PAR interception. The association of rooting with canopy temperature, NWI3 and PAR interception was more significant at anthesis compared to early grain fill. This was most likely because the canopy is still productive at anthesis and using soil resources, compared to later growth stages when the plant is senescing and diverting assimilates to the grain (Wasson et al., 2012).

## **3.4.3.** Associating canopy traits with rooting at depth in the DH population

The intercept of glaucous and non-glaucous regression lines differed significantly (P < 0.05) when assessing the relationship between canopy temperature and rooting, due to glaucous lines having significantly lower temperatures in the 2014/15 field season (Table 3.3). However, the slope of the lines did not differ depending on glaucousness (Fig. 3.8). RLD was negatively (P < 0.05) associated with canopy temperature, indicating the potential of canopy temperature at anthesis to identify better rooting wheat genotypes in a UK field environment. RDW and diameter of DH lines did not show a significant association with canopy temperature, this may be a result of root length being better associated with water uptake than root mass. Additionally, the unstressed environment may have resulted in the potential differences in the water status of the canopy being small. Despite measurements being made on clear and still days, there was still variable wind conditions and occasional cloud cover which affected the uniformity of these measurements throughout the field plots (Rebetzke et al., 2012). Root diameter of DH lines showed a suggested positive relationship with canopy temperature at anthesis (Fig. 3.8). Small diameter roots have previously been shown to be important for drought tolerance in synthetic hexaploid wheats, due to their association with greater rooting densities and surface area (Becker et al., 2016).

Root traits of DH lines showed a significant relationship with NWI3 at ear emergence but this changed based on glaucousness, with RLD and RDW of non-glaucous lines showing a negative relationship with NWI3 and glaucous lines showing a positive relationship. The low variance explained by the regressions (4.8-12.9 percent) may again be due to the unstressed environment not resulting in a large variability in the water status of the canopy. Non-glaucous DH lines had significantly lower NWI3 values, associated with a higher canopy water content, at later time points in both 2014 and 2015 (Table 3.4). This may have been influenced by delayed senescence in nonglaucous lines, with greener canopies containing more water. NWI3 was successfully correlated to leaf water potential and available soil water in the dry environment of CIMMYT's experimental station in Mexico (Gutierrez et al., 2010); whereas in this study within a UK field site, canopy temperature was better associated with rooting. Weak relationships between shoot phenotypes as indicators of root phenotypes was found by Wasson et al. (2014) who studied different wheat genotypes in 'hill plots'; densely sown areas of seed. Maximum rooting depth did correlate with canopy temperature (r = 0.45) and chlorophyll reflectance (r = 0.32) but no association with other root traits were found (Wasson et al., 2014).

The glaucous trait also interfered with the measurement of the root shoot relationship at ear emergence for NDVI, with RLD and RDW being positively associated with NDVI in non-glaucous lines but negatively associated in glaucous lines (Fig. 3.9). The lack of any relationship found for NDVI at anthesis could be down to greater variability of the data as green leaf area started to decline in some lines more than others. The standard error of the mean for NDVI at ear emergence was 0.0057 compared to 0.0182 at anthesis. Kindred et al. (2016) studied the relationship between spectral indices and crop biomass and canopy N in wheat and found relationships deteriorated after ear emergence due to varietal differences affecting relationships.

NDVI is associated with the photosynthetic capacity of the canopy (Sellers, 1985), indicating that reduced photosynthetic tissue in glaucous DH lines at ear emergence is associated with higher RLDs and RDWs. The significant relationship at ear emergence suggests the importance of canopy productivity pre-flowering in determining root biomass at later reproductive growth stages. A potential explanation for lower NDVI values being associated with higher RLD and RDWs could be a difference in trade-off effects in glaucous and non-glaucous lines (Grime, 1981). In non-glaucous lines the positive relationship of NDVI with RLD and RDW suggests the more productive canopy of non-glaucous genotypes is associated with greater rooting at depth (Bingham et al., 2002). Alternatively, increased productive green vegetation in glaucous lines was associated with reduced RLDs and RDWs with lower productivity potentially being caused by increased growth of the root system. Hoad et al. (2004) reported a trade-off between root and shoot growth, specifically in higher yielding UK environments (8-10 t/ha), associated with green area index and total root length, where yield increases could be achieved by either additional growth in the root or shoot.

Another explanation for the opposite relationship between NDVI and rooting, in addition to NWI3, in glaucous and non-glaucous lines is the influence of increased reflectance on spectral indices. Sims and Gamon (2002) studied the use of spectral indices to measure leaf pigment content across species. Weak correlations were found between spectral indices and chlorophyll content when applied to multiple species, with leaf surface reflectance being the most influential factor causing this variation (Sims and Gamon, 2002). This relationship is more significant between NDVI and RLD indicating photosynthetic capacity could be more related to root length than root mass. This could be indicative of greater root proliferation and resource uptake, supported by

the significant (P<0.05) negative relationship between NDVI and root diameter in glaucous and non-glaucous DH lines (Grossman and Rice, 2012). The differing relationship of NDVI and rooting in glaucous and non-glaucous lines has not been reported previously and demonstrates the complicated relationship between glaucousness and spectral reflectance indices, which represent active green vegetation.

The PCA describing the variation in spectral reflectance indices between 325 and 1075 nm indicates a more complicated relationship of the glaucous trait and reflectance of light (Table 3.6). The principal components pick up variation between DH lines for different wavelength reflectance responses. Rooting relates differently to these variations and is opposing within glaucous and non-glaucous lines for different principal components (PC1 and PC3; Fig. 3.10). PC2 at ear emergence shows that at certain wavelengths the DH lines had a similar response, showing the same relationships with RLD and RDW (C and D; Fig. 3.10). This indicates that reflectance of certain wavelengths can identify greater rooting genotypes within the DH population that are not associated with glaucousness. This may be a result of PC2 explaining variation in the population which is related to glaucousness as the relationship between glaucousness and the principal components was more significant for PC2 compared to PC1 and PC3 (r = -0.68 for PC2 compared to 0.34 and 0.29 for PC1 and PC3; P<0.01). The relationship between these principal components and canopy measurements was most associated with NDVI at both ear emergence and anthesis (Fig. 3.11 and Fig. 3.14; Table 3.6), indicating that the majority of variation in light reflectance in the DH population is occurring within the visible spectrum (325-770 nm). The variation in light reflectance represented by the principal components did not associate with thermal time to senescence or total PAR absorbed over the season (Fig. 3.12 and Fig. 3.15). This may be because light reflectance measured at ear emergence and anthesis gave snap shots of canopy productivity whereas light absorbed over the season and time to senescence measure canopy productivity over a longer time period and the differences seen between DH lines are occurring at the end of the season. Table 3.6. Summary table of significant (P<0.05) relationships of root and shoot traits of glaucous and non-glaucous DH lines with principal components (PC) at ear emergence and anthesis. / means no significant relationship was found, variance accounted for (v.a.f) in regression model stated.

Trait	Ear emergence									
		PC1 (82%)		PC2 (14%)		PC3 (3%)				
RLD	NG	positive	v.a.f = 21	negative	v.a.f = 50	/	v.a.f = 12			
	G	negative		negative		negative				
RDW	NG	positive	v.a.f = 26	negative	v.a.f = 49	/	v.a.f = 8			
	G	negative		negative		/	v.a.1 – 0			
NWI3	NG	positive	v.a.f = 41	positive	v.a.f = 53	/	v.a.f = 13			
	G	positive	v.a.1 - 41	negative		Negative	v.a.1 – 15			
NDVI	NG	negative	v.a.f = 34	positive	v.a.f = 59	Positive	v.a.f = 32			
	G	negative	v.a.1 – 54	positive		Positive	v.u.1 – <i>52</i>			
Trait	Anthesis									
		PC1 (85%)		PC2 (14%)		PC3 (1%)				
Canopy Temp	NG	/	v.a.f = 10	/	v.a.f = 52	Negative	v.a.f = 6			
	G									
NDVI	NG	negative	v.a.f = 21	negative	v.a.f = 58	Positive	v.a.f = 30			
	G	negative		negative						

The principal component analysis explaining the variation in canopy characteristics with rooting traits (Fig. 3.16 and Fig. 3.17) showed the different relationships glaucous and non-glaucous DH lines had with shoot and root interactions. Specifically, at ear emergence, variation in RLD and RDW of non-glaucous lines was related to NDVI and negatively related to diameter, NWI3 and reflectance in the PAR region (A and C; Fig. 3.16). Rooting showed the opposite association to NDVI, explained by principal component 2, in glaucous lines at ear emergence (B and D; Fig. 3.16). Average temperature in glaucous lines at anthesis (B and D; Fig. 3.17) showed an association with PAR interception and thermal time to senescence, demonstrating the effect senescence has on canopy temperature due to leaf matter losing water content (Lopes and Reynolds, 2012). This is reiterated by the opposite association of NDVI and NWI3 with the principal components as green canopies have higher water contents which is why rooting at depth has been associated with stay green (Christopher et al., 2008). Diameter of glaucous lines is better associated with average temperature and NWI3 at anthesis compared to non-glaucous lines (Fig. 3.17).

The significant positive association of canopy temperature and NWI3 seen at measurements taken in July 2014 indicates that in a UK environment NWI3 may be a more suitable method of measuring canopy water content late in the growing season, when water is more limiting and genotypic differences are more significant. In glaucous lines variation in root diameter and average temperature showed the opposite association with PCs at ear emergence (D; Fig. 3.16). This relationship, mentioned previously, indicates that finer roots are important for water uptake and could be contributing to these cooler canopies at anthesis (King et al., 2003). This may also be influencing the stronger positive relationship RLD of glaucous lines had with yield, compared to non-glaucous lines (Fig. 3.18).

#### 3.5. Conclusions

The results from this study relating root phenotypes with shoot phenotypes in the Shamrock x Shango DH population indicates a complicated interplay between these factors. The 'stay green' trait in non-glaucous lines, associated with the wild emmer introgression, appears to be more significant in environments with better water availability. Therefore, this trait specific to Shamrock, is possibly an extension of photosynthetic capacity when conditions allow, rather than a drought tolerant trait. Improved RLDs above 1 cm/cm<sup>3</sup> were seen in the 2013/14 season, which may have contributed to the more significant delayed senescence. Therefore, improving rooting in drier seasons such as 2014/15 could help increase PAR interception post-anthesis.

The relationships between crop water status and rooting in the field in this study were too weak for these measurements to predict rooting extent of a wheat crop in a UK field environment, although higher RLDs of DH lines were significantly associated with lower canopy temperatures at anthesis. Additionally, the relationship between root diameter and NDVI indicated the importance of finer root diameters for resource uptake and productivity. These relationships may potentially become more significant with the addition of multiple years of data, but the unstressed environment does not allow for large differences in water uptake or water use efficiency between genotypes. Subsequently, the analysis of biomass or photosynthetic capacity of the crop may be a more suitable measure to indicate root system size and activity in an environment where water is not limited.

The difference in glaucosity in this DH population was associated with differences in light reflectance which also influenced canopy temperatures. The significant relationships between rooting and principle components explaining variation in canopy

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light reflectance indicates that spectral reflectance has potential to become a high throughput method of identifying better rooting genotypes in a UK environment. Glaucosity was associated with differences in the root shoot relationships. Glaucous genotypes had an apparent stronger association of rooting and canopy traits, a potential indicator of a more active root system or indicative of differences in water use regimes, such as glaucous lines reducing transpiration and water loss from the canopy. The different relationship between light reflectance/absorbance and rooting between nonglaucous and glaucous phenotypes needs further study but demonstrates the need to assess wheat populations with the same wax profile when assessing root:shoot relationships and spectral indices.

### **Chapter 4. Final discussion**

#### 4.1 Thesis Objectives

The aim of this research project was to study the diversity of rooting at depth in the wheat doubled haploid population of Shamrock x Shango from both a phenotypic and genetic perspective. The objectives of this study were to determine whether Shamrock has greater RLDs at depth in the field compared to Shango, at mature growth stages and subsequently explore whether this deeper rooting is associated with the wild emmer (*Triticum dicoccoides*) introgression that causes the non-glaucous trait in Shamrock. The doubled haploid (DH) population of Shamrock x Shango, consisting of 89 lines including the two parents, was studied at different growth stages in controlled environments to identify root architectural trait differences at early growth stages; these were then compared with root systems of stands in the field at anthesis. Finally, assessment of photosynthetic capacity and the water content of the canopy of DH lines in the field was used to identify potential proxy traits which correlate with increased rooting below 50 cm depth at mature growth stages. This discussion will focus around the hypotheses stated at the beginning of this thesis, which are:

1. Shamrock has greater root length densities at depth in the field, at anthesis, compared to Shango

2. The non-glaucous trait, inherited from wild emmer, is associated with increased rooting at depth

3. Root architecture traits studied in controlled environments at the seedling and late tillering stage relate to improved rooting in the field at anthesis

4. Photosynthetic capacity and water content of the canopy can act as proxies for increased rooting at depth in the field at anthesis

Trait	Chromos- ome	Position (cM)	Confidence Interval (cM)	LOD	Peak Marker	Additive Effect	High value allele	Variation explained (%)
Field								
Glaucousness	2B	1.0	0-2.1	58.5	BS00084668	0.489	Shamrock	95.6
RLD	5D	31.3	18.5-39.5	3.1	BS00158384	0.0355	Shamrock	8.4
RLD	6B	73.1	66.4-81.9	4.3	AX-94475756	0.0431	Shamrock	13.9
RLD	7B	12.8	11.1-22.8	3.4	AX-94826552	-0.0342	Shango	8.8
RDW	2A	41.5	38.5-45.1	3.3	AX-94604266	-0.0026	Shango	8.7
RDW	7B	50.9	47.2-54.1	6.1	AX-95194687	-0.0058	Shango	17.3
RLD 50-60 cm	2B	5.8	2.1-7.5	3.1	AX-94505732	-0.0452	Shango	7.9
RLD 60-70 cm	6A	30.9	30.3-33.4	3.2	AX-94579171	0.0633	Shamrock	9.4
Diam 50-60 cm	2D	25.9	22.7-39.6	5.2	AX-95102138	0.0058	Shamrock	14.8
Total PAR	2B	4.7	2.1-6.9	6.5	AX-94939920	10.31	Shamrock	19.6
Total PAR	4A	5.8	0.8-11.7	3.1	BS00065863	6.55	Shamrock	7.9
ttSen	1B	85.6	84.1-90.8	5.2	AX-94981461	-9.81	Shango	11.1
ttSen	2B	3.5	0.0-6.2	8.4	AX-94777767	12.60	Shamrock	19.4
ttSen	5A	89.4	88.9-90.5	4.7	AX-94472861	14.67	Shamrock	9.9
Seedling								
PC1 'Root system size'	2B	111.9	104.0-113.0	3.8	BS00022950	-0.772	Shango	12.2
SA	2B	111.9	103.8-113.0	3.0	BS00022950	-0.164	Shango	8.7
No Axes	2B	111.9	104.3-113.0	3.7	BS00022950	-0.0855	Shango	10.8
RDW	6A	92.9	89.5-102.9	5.1	AX-94893553	0.4179	Shamrock	11.9
SDW	6A	92.9	90.4-101.0	3.4	AX-94893553	-0.3439	Shango	9.6
R:S	6A	92.9	90.7-103.4	3.2	AX-94893553	0.0594	Shamrock	10.1
AvgDiam	6A	30.9	29.1-32.5	7.4	AX-94579171	0.0045	Shamrock	12.4
%TRL 0.5-1 mm	2D	30.4	26.9-36.2	5.4	BS00065456	0.0104	Shamrock	9.8

Table 4.1. QTL table for the main QTL effects in the Shamrock x Shango DH population for root and shoot traits identified at the seedling stage and in the

#### 4.2 Increased rooting at depth

In the 2013/14 field season Shamrock had significantly greater RLD at 60 and 70 cm depths (Fig. 2.5) and significantly greater RDW at 70 cm depth compared to Shango, in soil cores collected at anthesis. This agreed with previous data collected by Ford et al. (2006) that found Shamrock had significantly greater rooting below 40 cm depth compared to six other elite, UK grown wheat cultivars (Fig. 1.17). This finding supports the first hypothesis of this study 'Shamrock has greater RLDs at depth in the field, at anthesis, compared to Shango'.

In the 2014/15 field season the DH population differed significantly for RLD and RDW between 50 and 80 cm depth in the field at and shortly after anthesis (Fig. 2.6; 2.7). No DH lines had a RLD at or above the determined critical value of 1 cm/cm<sup>3</sup>, which has been calculated to allow sufficient uptake of soil water (Barraclough et al., 1989; Van Noordwijk, 1983). Shamrock had a greater RLD than Shango at 60-70 and 70-80 cm depths in the second growing season but this was not significant, perhaps reflecting variation in weather patterns. Several studies have reported significant annual effects on rooting traits of wheat genotypes. For example, Thorup-Kristensen et al. (2009) and Svoboda and Haberle (2006) reported differences in rooting depth and root length, explained by variations in accumulated temperatures over the growing season and soil inorganic nitrogen accumulation, thought to be a consequence of changes in rainfall pattern. Differences in RLD at depth between years were also reported by Rich et al. (2016) for wheat genotypes. These were ascribed to the two-fold difference in rainfall during the growing seasons studied. In the present study, rainfall was 657 mm in 2013/14 and 408 mm in 2014/15, which may have influenced root growth and hence affected the previously measured differences between Shamrock and Shango.

Significant genotypic differences within the DH population were also identified for rooting within and between rows. Shamrock had higher RLDs within the row compared to Shango for each depth within the 50-80 cm soil core, with significantly (*P*<0.001) more RLD within the row at 60-70 cm depth (Table 2.2). A more compact root system increases root length directly under the crop and has been associated with narrower seminal axes and improved rooting at depth (Hammer et al., 2009; Manschadi et al., 2008). This growth characteristic was implicated in Shamrock within the rhizotrons (Fig. 2.11). Assessing RLD in soil cores taken within and between crop rows separately, indicated the high variability of RLD data from within row cores (Fig. 2.3; 2.4) and confirms the importance of using a weighted scheme to assess field crop rooting to be representable and to identify genotypic differences (Van Noordwijk et al., 1985).

The significant difference in root system traits within the DH population in the field at mature growth stages makes these genotypes important in the study of rooting at depth. The continuous distribution of field root traits indicated the degree of genetic variability in the population, suitable for linking these phenotypic traits to genomic regions and developing markers for marker assisted selection (Wasson et al., 2012). This study, to the author's knowledge, is unique in the research of rooting at depth in the field, at mature growth stages, in a wheat doubled haploid population and the identification of quantitative trait loci (QTL) to explain phenotypic variation. Bharti et al. (2014) studied a recombinant inbred line (RIL) wheat population and identified QTL for root length, root dry weight and root volume of RILs grown in soil bags 20 x 30 cm at maturity. This study is among the few which have assessed mature root traits in a mapping population but only measured roots restricted to a depth of 30 cm. A significant QTL was found on 4A which explained 30% of the phenotypic variation in root volume. An

additional QTL of lower effect (LOD 2.8) located on chromosome 1B, explaining variation in root volume, was identified in a similar location to the QTL in this study for RLD in the 50-60 cm soil layer (Table 2.3), with the Shamrock allele having a positive effect. Root volume in the RIL population did significantly correlate with root length (r=0.96) and therefore comparisons can be made (Bharti et al., 2014). Endaie et al. (2016) also studied mature root systems in a wheat RIL population, within 80 cm tall sand filled columns. QTL were identified on the short arm of 7B for the longest root, close to the RLD QTL in this study on 7B and a QTL for root dry weight in the top 30 cm on the short arm of 2A, in a similar location to the RDW QTL in this study (Table 4.1).

#### 4.3 The association of the non-glaucous trait and rooting

The second hypothesis of this study was that the non-glaucous trait, mapped to the short arm of 2B, is associated with greater root length at depth in the field in Shamrock x Shango DH lines. In the seedling screen non-glaucous lines were negatively associated with root length and root surface area and Shango contributed a positive allele for a QTL explaining variation in root system size (root length, root surface area and root volume) which mapped to 2B, but did not co-locate with the glaucous QTL (Table 4.1). This negative relationship between non-glaucous DH lines and seedling root size was concluded to be an association of the glaucous QTL on 2BS with the seedling QTL on 2BL, as they coincide on the same linkage group. The relationship between glaucous DH lines and larger seedling root size becomes insignificant (P>0.05) when the additive effect of the 2BL QTL is included, as singularly 2BL has a larger association with these traits. Other wheat seedling screens have identified 2BL as being significant in explaining differences in traits related to root size, such as total root length, root hair length and root number (Horn et al., 2016; Kabir et al., 2015; Liu et al., 2013; Maccaferri et al., 2014; Zhang et al., 2013). The significant differences in the Shamrock x Shango population for seedling root and shoot traits can be beneficial for identifying genotypes with better early vigour. Numerous studies have reported genotypic differences in seedling vigour of wheat, which mostly depend on seed size and shoot length (Kakhki et al., 2008; Maydup et al., 2012). As the seed size was uniform in this study differences may have occurred due to growth rate or small differences in embryo size, which have been shown to correlate with shoot length (Botwright et al., 2002; Nik et al., 2011). Shoot dry weight correlated with root length and surface area in the DH seedlings and therefore differences in seedling vigour of 6A (Table 4.1) explained variation in the population for root and shoot dry weight and root shoot ratio, which could be indicative of the variation in vigour in the population.

This negative effect of non-glaucous lines associated with early growth did not translate to the rhizotrons assessed at the late tillering stage (GS 29). Selected non-glaucous lines had significantly greater RLD at 40 - 80 cm depths (Fig. 2.12). Previous studies have measured the relationship of rooting and nitrogen uptake in wheat genotypes grown to stem extension in rhizotrons of similar design (Liao et al., 2006; Palta et al., 2007). These rhizotron studies identified genotypic differences in RLDs of wheat genotypes differing for tillering ability and vigour, with increased rooting being associated with greater proliferation and branching of roots rather than speed of vertical growth or deep rooting (Palta et al., 2007). In a separate study of wheat genotypes differing for vigour, more vigorous growth due to earlier branching and horizontal growth in the top 70 cm caused a two-fold increase in nitrogen fertiliser uptake between 20-70 cm depths than non-vigorous genotypes (Liao et al., 2006). Consequently, the increased RLD in selected non-glaucous lines at growth stage 29 (Zadoks et al., 1974) may be important for soil water and nutrient capture in the pre-stem extension period. This study of selected Shamrock x Shango DH lines could be repeated with the manipulation of soil conditions to examine the difference in soil water and nutrient uptake related to greater RLDs.

In the field, rooting traits measured at 50-80 cm depth, at and shortly after anthesis in the DH population, did not associate with glaucousness exhibited in the field at the flag leaf stage or with the genomic region identified on the short arm of 2B. However, RLD also differed with depth within the 50-80 cm soil core and there was a significant (P=0.01) interaction of RLD with soil depth and glaucousness, because the mean 'effect' of glaucousness at 50-60 cm depth contrasted with that at 60-70 cm depth (Fig. 2.9). Consequently, a QTL was identified on 2BS, in close proximity to the glaucous QTL, explaining variation in RLD within the 50-60 cm soil layer (Table 4.1). Shango contributed a high value allele for this QTL as it achieved higher RLDs in the 50-60 cm soil depth and decreased thereafter, whereas Shamrock and non-glaucous lines had an increase in RLD between 50-60 and 60-70 cm depths.

QTL identified for RLD and RDW were found on 5D, 6B, 7B and 2A with additional QTL identified for RLD, RDW and root diameter within the different soil depths (50-60; 60-70 and 70-80 cm). The strongest QTL was identified on 7B, explaining 17% of the phenotypic variation in RDW, with Shango contributing the positive allele. A second QTL on 6B explained 14% of the phenotypic variation in average RLD, with Shamrock contributing the positive allele (Table 4.1). There is no information on the specific location of the wild emmer introgression in Shamrock and therefore it cannot be stated unequivocally whether QTL contributed by a Shamrock allele are, or are not, associated with previously introgressed material. There are no earlier studies which report specific genomic regions in hexaploid wheat that explain variation in rooting

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ability and are associated with wild emmer introgression. However, the location of rooting QTL contributed by Shamrock on 1B, 6A and 6B are mentioned in the literature for providing disease resistance or quality benefits to cultivated wheat through wild emmer introgression. A powdery mildew resistant gene was identified on the long arm of 6B within a durum wheat cultivar Langdon, with a Triticum dicoccoides 6B chromosome substitution line. The addition from the wild emmer accession gave resistance to 47 powdery mildew isolates collected from wheat species within Israel and Switzerland (Xie et al., 2012). An additional study involving a cross between a durum wheat cultivar and a T. dicoccoides accession identified a QTL of the short arm of 6A which explained a significant percentage of variation in grain protein content (Blanco et al., 2006). Cakmak et al. (2004) also identified 6AS as being important for seed quality as T. dicoccoides substitution lines in Chinese Spring and durum wheat cv. Langdon exhibited increased zinc and iron concentrations in the seed, compared to their recipient parents. A QTL identified in wild emmer on 1BL was successfully introgressed into a durum wheat cultivar and provided improved grain yield and total dry matter under drought conditions as well as increased yield stability across drought and well-watered environments (Merchuk-Ovnat et al., 2016).

The effect of the QTL identified in this study on rooting at depth at mature growth stages in the DH lines are important in the future breeding of wheat cultivars with improved rooting at depth. This has been demonstrated in rice with the successful introgression of the deep rooting QTL DRO1 into shallow rooting genotypes, increasing rooting at depth and improving yields by 10% (Arai-Sanoh et al., 2014). Previously identified drought resistant QTL identified in a durum wheat and wild emmer RIL population were successfully backcrossed into durum and bread wheat cultivars to improve agronomic traits in well-watered and water limited conditions (Merchuk-Ovnat

et al., 2016; Peleg et al., 2009). These studies indicate the potential of marker assisted selection of these rooting QTL to improve future cultivars.

The strong association of the non-glaucous trait and greater RLDs in deeper soil layers in the rhizotrons may be due to the controlled water supply in the rhizotrons influencing the association of non-glaucousness and greater rooting at depth. The field results suggest that the effect of non-glaucousness on the stay green trait changes depending on seasonal soil water differences. Delayed senescence and increased photosynthetic active radiation (PAR) interception significantly associated with yield in 2013/14 but not 2014/15. This indicates a yearly variation effect on physiological traits associated with glaucosity which may have also influenced rooting.

# **4.4** Associating seedling root traits with deep rooting at anthesis in the field

Numerous QTL were identified for the different root and shoot phenotypic traits studied in the seedling screen (Table 2.8) and very few overlapped with root QTL identified in the field at anthesis. However, there was some overlap of QTL explaining variation in seedling and field root traits which related to root length and diameter traits. The confidence intervals for QTL explaining field root diameter in the 50-60 cm depth and percentage of total root length within the higher diameter class of 0.5-1 mm overlapped on the short arm of 2D, with the positive allele being contributed by Shamrock for both these QTL (Fig. 2.15). Additionally, the same peak marker was identified on 6A for QTL explaining variation in field RLD in the 60-70 cm depth and average seedling root diameter. These common QTL indicate the influence root diameter can have on root length due to finer roots allowing greater root proliferation because of the reduced metabolic cost (Lynch, 2013). Mean root diameter was negatively correlated with root length at the different growth stages and environments studied in this thesis. The colocated QTL for diameter traits in DH lines at the seedling stage and mature growth stages in the field suggest that diameter is a more heritable trait than other root phenotypic traits. Root diameter is one of the few traits which have been bred for in wheat roots; Richards and Passioura (1989) reduced xylem diameter in Australian cultivars to increase axial resistance of water flow to the roots to ensure water was not used up before critical growth periods.

Despite these co-located QTL phenotypic root traits, specifically root length and dry mass, of field-grown mature Shamrock x Shango DH lines did not show any correlation to observations made within the seedling screen. This finding is confirmed by Watt et al. (2013) who also found root length in seedlings did not correlate to root length in the field at anthesis. Additionally, Cane et al. (2014) compared seedling traits with agronomic performance in the field but found poor associations between seedling traits such as root length and root number with agronomic traits including yield, thousand grain weight and ear peduncle length. Consequently, the use of seedling screens to identify genetic control of rooting traits to improve rooting at depth at mature growth stages is questionable especially for root traits which are largely influenced by environment or which cannot be assessed in seedlings, such as root branching and nodal root architecture. Use of seedling screens may be more suitable to identify early vigour and establishment traits (Rebetzke et al., 2014). Additionally, selection of more heritable root traits, such as root angle, have proved more successful in seedlings which correlate with rooting at depth in later growth stages (Christopher et al., 2013; Manschadi et al., 2008). Root diameter was consistent at different growth stages in this population, identifying it as a potential trait which can be studied in seedlings. Shango had coarser roots in the seedling screen, with a significantly higher proportion of root length in the larger diameter class (0.5-1 mm) compared to Shamrock (Table 2.6).

These findings were confirmed by the QTL identified for the PCA analyses with QTL associated with fine roots being contributed by Shamrock (Table 2.9).

#### 4.5 Canopy traits related to rooting at depth in the field

Non-glaucous DH lines had significantly greater delayed senescence and subsequently intercepted significantly more PAR averaged over the 2013/14 and 2014/15 field seasons (Fig. 3.1). QTL identified for these traits were found to associate with the glaucousness QTL on the short arm of 2B, with Shamrock contributing the positive allele (Table 4.1), this indicates the influence of the wild emmer introgression on these traits. However, there was no association with delayed senescence or increased PAR interception over the growing season and greater rooting at depth in DH lines, because of the lack of a strong association of non-glaucousness and rooting in the field. RLD of DH lines was negatively associated (P < 0.05) with canopy temperature (Fig. 3.8) at anthesis in 2014/15 whereas RDW had a weaker negative association (P=0.075). Canopy temperature is known to associate with greater rooting ability of wheat in the field (Lopes and Reynolds, 2010; Pinto and Reynolds, 2015) and these results indicate the significance of root length in resource uptake compared to root mass. Spectral reflectance indices measured at ear emergence were significantly associated with rooting at depth in doubled haploid lines, although the relationship differed based on glaucosity. NDVI measures live green vegetation and has been shown to associate with yield and track changes in leaf area index and biomass in wheat crops (Serrano et al., 2000; Sultana et al., 2014). RLD and RDW of glaucous DH lines associated negatively with NDVI measured at ear emergence and non-glaucous lines associated positively with NDVI (Fig. 3.9). Additionally, contrasting relationships between rooting and NWI3 were seen at ear emergence. NWI3 has been found to associate negatively with moisture in deeper soil layers (Gutierrez et al., 2010). RLD and RDW of non-glaucous

lines associated negatively with NWI3 but glaucous lines associated positively with NWI3

Differences in reflectance have been previously reported between genotypes and growth stages due to different pigment concentrations, specifically chlorophyll a, chlorophyll b and carotenoids (Feng et al., 2008). To identify where the differences between the glaucous and non-glaucous genotypes are occurring reflectance parameters need to be controlled. Sims and Gamon (2002) reported that spectral indices did not translate across species and leaf structures for determining leaf chlorophyll content, mostly due to differences in leaf reflectance. The use of a constant, as a measure of reflectance, within spectral indices was found to correlate with chlorophyll content to a higher significance within and across species; as the differences in reflectance was removed from spectral indices determining chlorophyll contents. This measure of reflectance was assessed at 445 nm where reflectance is minimal due to combined chlorophyll and carotenoid absorbance (Sims and Gamon, 2002). The opposing relationships between rooting at depth and NDVI/spectral reflectance indices in this study has not been previously reported and raises questions related to the comparison of spectral indices, used as proxies for rooting in wheat, in both glaucous and non-glaucous phenotypes.

The Principle Component Analysis (PCA) completed for the correlation matrix of reflectance in the 325 – 1075 nm regions also exhibited significantly different relationships between RLD and RDW of glaucous/non-glaucous genotypes with spectral reflectance. The principal components (PC) describe the variation between the genotypes for different reflectance properties which can partially be interpreted by the relationship of the PCs with other canopy spectral indices (Fig. 3.11), specifically NDVI and NWI3; although, at anthesis NWI3 showed weaker relationships with the PCs (Fig. 3.14). However, RLD and RDW of glaucous and non-glaucous DH lines

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showed the same relationship with some PCs associated with the variation in wavelength reflectance, indicating that the two phenotypes only differ for reflectance in some of the spectrum. It is thought that reflectance in green bands (550 nm) and red edge bands (715 nm) are sensitive to leaf chlorophyll content and near infrared (NIR) wavelength bands associate with canopy structure (Feng et al., 2008). Differences in these parameters may be being picked up by the different PCs. These relationships indicate the importance of taking varietal differences into account, such as colour and wax content of the leaves, when measuring spectral reflectance indices between cultivars (Samborski et al., 2015; Wiltshire et al., 2002). This last hypothesis cannot be proven or disproven due to the significant effect of glaucousness on the spectral reflectance indices, although there was promising correlations with rooting at depth in addition to the negative relationship seen between RLD and canopy temperature. The difference in glaucosity needs to be studied further to identify where the main differences in reflectance are occurring and for what reason. Research by Frizell-Armitage (2016) did confirm that the increased reflectance in glaucous lines does not cause a difference in light absorption compared to non-glaucous lines. Therefore, the contradictory relationship between rooting and spectral indices is likely due to reflectance differences between DH lines.

#### 4.6 Problems encountered and limitations of this study

#### 4.6.1 Experimental limitations

In the first year field trial, the crop was affected by take-all disease due to the fact it was a third wheat. This significantly affected canopy measurements and introduced an additional variant into root shoot relationships. Moreover, the crop also experienced flooding due to high rainfall in the early part of 2014 resulting in eight plots being removed from the experiment and several others experiencing significant damage. This meant that the identification of canopy temperature differences and measurement of canopy water status within the DH lines was compromised.

Root observations studied throughout the project, particularly within the field and rhizotrons were very labour and time intensive. In the second year time was constrained due to the rhizotron experiment taking place during the spring and early summer when observations and measurements needed to be taken in the field. The rhizotron experiment was concluded after 7 weeks to ensure time could be given to soil coring in the field trial at anthesis. If more time were given for the growth of plants within the rhizotrons, differences in rooting may have been seen in the bottom soil layer (below 80 cm), in addition to root: shoot relationships occurring within the lines, particularly within the wild emmer accession.

The laborious process of soil coring in the field, washing soil cores and finally analysing roots for mass and density constrained the number of possible cores collected per plot for the DH population to three. The large amount of time needed for this sampling and analysis also meant that some field measurements were limited as a result, such as canopy measurements taken between ear emergence and grain fill in the second year field trial. Additionally, if time allowed, other measurements could have been taken such as leaf water potentials and harvest index measurements. Having two years of field root data for the DH population at anthesis would better substantiate the conclusions of this project, help test the hypotheses in more detail, and potentially strengthen the QTL data.

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## 4.6.2 Assumptions of root function and microbial interaction with the rhizosphere

The rhizosphere is defined as the soil surrounding the root surface that is influenced by root secretions and colonized by soil microbiota (Pinton et al., 2001). Plant-microbe interactions within the rhizosphere vary depending on plant species, cultivar, plant age/developmental stage and soil factors such as temperature and pH (Donn et al., 2015; Huang et al., 2014). Plant root exudates influence plant-microbe interactions by attracting certain microbial species as well as providing an energy source to microbes. An estimated 5-21% of photoassimilated carbon is given as soluble sugars or secondary metabolites to plant growth promoting rhizobacteria, epiphytes and mycorrhizal fungi (Huang et al., 2014; Kaiser et al., 2014). The multiple benefits these interactions can provide plants include nutrient availability, resilience to abiotic and biotic stresses and disease suppression through inhibition of pathogen growth (Huang et al., 2014; Kaiser et al., 2014; Pérez-Montaño et al., 2014; Rascovan et al., 2016). The control of plant stress responses by soil microbes involves the modulation of plant ethylene levels through the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase; an enzyme which reduces the ethylene precursor ACC and subsequently reduces levels of the plant hormone (Glick, 2005; Rascovan et al., 2016). High levels of ethylene production in the response to stress can lead to cell damage and affect plant development, therefore its reduction helps facilitate plant growth and resistance to stress conditions (Pérez-Montaño et al., 2014).

In a study analysing the root-associated microbiomes of soybean and wheat, within agricultural fields in Argentina, bacterial isolates were collected from the rhizosphere (Rascovan et al., 2016). In a study of the function of over 700 bacterial isolates 37% were able to fix nitrogen, 38% could solubilise inorganic phosphorus, 14% showed

ACC deaminase activity and 38% were able to produce indole-acetic acid (IAA), a plant growth promotor (Rascovan et al., 2016). Donn et al. (2015) studied the evolution of these microbial communities within the rhizosphere in an intensive wheat cropping system over two years. The simplest soil bacterial populations were associated with young crop plants and diversity increased with plant age. Bacterial diversity was also greater within the second year wheat with decomposing residues from the previous crop proving to be an important source of microbes for newly developing rhizospheres (Donn et al., 2015). Watt et al. (2005) confirmed the importance of remnant roots as a source of soil bacteria in field-grown wheat as compared to current roots, remnant roots contained 1.8 times more bacteria. Studying wheat roots in situ, bacteria were found clustered on 40% of roots within 11 mm of the root surface, with an increase in filamentous bacteria where roots were in contact with remnants (Watt et al., 2005). However, remnant roots can also cause the spread of soil microbes that have negative effects on the crop such as the fungus *Gaeumannomyces graminis*, which causes take all disease seen in the first year of this study due to the crop being a third year wheat.

Arbuscular mycorrhizal fungi are important rhizosphere microbes and form symbiotic relationships with plant roots, improving nutrient availability in return for plant carbon (Huang et al., 2014). Kaiser et al. (2014) specifically showed how mycorrhizal fungi can extend the access of root systems to a bigger area of soil and subsequently increase access to water and nutrients. Wheat plants directed photoassimilated carbon directly to associated mycorrhizal fungi which in turn take up ammonium, nitrate, amino acids and phosphate but also translocate plant carbon to other soil microbes (Kaiser et al., 2014). The significance of these interactions occurring within the rhizosphere and their effect on plant function and root architecture was not considered in this study due to the focus on root density at depth. As the DH lines were grown in the same environment in all

studies the effect of soil environment on microbial interactions should be minimal. However, it cannot be assumed that genotype did not influence plant-microbe interactions with potential differences in root exudate and plant carbon assimilation to the rhizosphere.

Root anatomical features are an additional factor not considered within the Shamrock x Shango DH population. This encompasses multiple traits such as root hairs, metaxylem number and diameter, aerenchyma and root cross-sectional area. An increase in root hair number can improve root surface area and specifically aids root soil contact when roots grow through existing soil pores, consequently increasing water uptake (Passioura, 1988). Root hair length has been shown to vary between different wheat genotypes and significantly correlates with rhizosheath size: soil that adheres to plant roots (Delhaize et al., 2015). Kadam et al. (2015) showed that anatomical plasticity in wheat roots improved tolerance to drought stress through conservation of water in deeper soil layers at the vegetative stage. Specifically, two drought tolerant wheat cultivars exhibited increased metaxylem diameter and lower metaxylem number near the root tip, lowering axial conductance to conserve subsoil water. At the root shoot junction wheat plants had decreased metaxylem diameter and higher metaxylem number, thought to support water transport and increase water uptake by lateral roots in the top soil layers (Kadam et al., 2015). The diversity of anatomical traits within wheat was shown using 10 durum wheat cultivars whose roots were collected at stem elongation in field-grown plots (Nazemi et al., 2016). Significant differences were identified between cultivars for aerenchyma area and number, cortex cell wall area, root cross section area and total cortex area. Heritability was highest for aerenchyma related root anatomical traits (67-72%) and intermediate for traits related to cross sectional area (46-69%) (Nazemi et al., 2016).

It is assumed that anatomical differences within the DH genotypes within this study are minimal and that it did not significantly affect root water uptake or transport mechanisms which could have caused differences in the canopy measurements studied in chapter 3.

#### 4.7 Future Work

### 4.7.1 Improving introgression of rooting at depth traits into wheat cultivars

The limited research on genetic diversity in deep rooting of wheat at mature growth stages in the field is because of labour and time constraints. Additionally, the plasticity of roots and the large influence of the soil environment require data from multiple years and multiple sites in order to test hypotheses rigorously. Wasson et al. (2012) discuss the value of mapping populations which show diversity of a particular trait for breeding programmes. The Shamrock x Shango DH population provides a promising genetic resource to investigate this diversity further within multiple years and sites. Study of this population in a stressed environment would also give an indication of the benefits greater RLDs have on resource uptake and whether this trait is enhanced under reduced soil water and nutrient supply. An increase in studies at mature growth stages in the field in wheat mapping populations for deep rooting is needed. Although soil coring and washing of cores to collect roots is the most reliable method of sampling, a more highthroughput method which involves counting roots in a cross section of a soil core was first introduced by Van Noordwijk et al. (2000) and successfully implemented by Wasson et al. (2014). This technique has potential for use in a study such as this one but requires careful site specific calibration.

#### 4.7.2 Root architectural traits influencing rooting at depth

More detailed study of root architectural differences within this DH population would help to understand why the differences in RLD are occurring. Specifically, in the rhizotron study, measuring root growth rate over time could have given an indication as to why non-glaucous lines had greater root proliferation at deeper layers and may have confirmed the slow establishment of seedlings seen in the seedling screen. Root penetration rate, the degree of downward growth in the vegetative stage, has been identified as a trait that promotes rooting at depth (Kirkegaard and Lilley, 2007). Additionally, Eissenstat (1991) associated faster root growth rate with finer diameters and therefore this could be the case in non-glaucous lines as their root diameters tended to be smaller than glaucous lines.

### 4.7.3 The association of the wild emmer introgression and deeper rooting

The genetic control of greater RLD at depth in DH lines in this study could be further investigated by researching the specific location/s of the wild emmer introgression into Shamrock and correlate this with QTL identified in the field. If association was found between identified QTL and wild emmer introgression, the identified QTL would be very valuable in marker assisted selection and candidate gene identification for the breeding of cultivars with improved rooting. With limited information about the breeding of Shamrock its genome could be studied in more detail to identify specific segregation distortion, which is indicative of alien introgression (Niu et al., 2011). Identifying *Triticum dicoccoides* introgression based on segregation distortion is being focused on at NIAB, with the multi-parental population MAGIC and other bi-parental populations, with Shamrock x Shango included in this. Additionally, with the

sequencing of the *T. dicoccoides* genome near completion Shamrock DNA sequences can be compared with emmer genes through exome capture to identify similarities.

## 4.7.4 Relationship between rooting in the field and canopy spectral reflectance indices

Lastly, the different root and shoot relationships for spectral reflectance indices in glaucous and non-glaucous lines needs further study. This can be analysed in more detail by normalising the reflectance differences between the DH lines to identify any differences in light reflectance occurring due to pigmentation or leaf structure. Assessing physical traits such as leaf water potential and biomass of DH lines in the field might give a better insight into the factors affecting these spectral indices measurements. By studying the reflectance spectrum within the DH lines, specific wavelengths can be identified where the biggest differences are occurring and the importance of these wavelengths in canopy function. Additionally, the significant correlations of RLD and RDW with spectral reflectance indices may reveal co-locating QTL, which indicate rooting traits that are influencing canopy productivity and function.

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## Appendices

Appendix 1. 'Establishment and root development in wheat crops', chapter for the proposed book 'Achieving sustainable wheat cultivation' edited by Professor Peter Langridge. ı

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## Appendix 2.Shamrock x Shango genotyping and linkage map production methodology

In 2014 DNA was extracted from seedling leaves of doubled haploid (DH) lines and sent to Bristol University for genotyping using Kompetitive Allele Specific PCR (KASP $\underline{TM}$ ) chemistry. 169 successful single nucleotide polymorphism (SNP) markers were genotyped in the population, assessed by using cluster plots in the programme SNPViewer (LGC Ltd., Teddington UK).

In 2016 the DNA of the population was extracted from seedling leaves of all 89 lines of the DH population and sent to Bristol University. The Axiom© Wheat Breeder's 35k Genotyping Array, (Affymetrix Inc., Santa Clara, CA; Allen et al., 2016) was used. Post-processing the quality of the 35,000 SNP calls vs the background for each DH line was assessed using DishQC (Axiom©) using a threshold of 0.8. One DH line of the 89 failed (line 37) and therefore was not used in the linkage map production or quantitative trait loci (QTL) analyses. SNPolisher (Affymetrix, Thermo Fischer Scientific) was then used to process the genotyping results which were filtered based on: call rate >97%; Fischer's Linear Discriminant >3.6; Heterozygous Strength Offset (HetSO) >-0.1, the number of no calls and segregation distortion. SNP calls from CerealsDB were used for the DH parents Shamrock and Shango due to a high number of monomorphic calls within the seedling DNA genotyping, thought be caused by outcrossing in the field. A total of 4369 SNPs were used for linkage map production.

MapDisto version 2.0 (Lorieux, 2012) was used to produce a linkage map of the Shamrock x Shango population. An LOD threshold of 3 and a recombination fraction of 0.3 was used to find linkage groups using the SNP calls. These linkage groups were then broken down using higher LOD thresholds if genetic distances were more than 50 cM and placed back together after ordering. Genetic distances were computed using the Kosambi (1943) mapping function and markers with significant segregation distortion values were removed from the map, assessed using the chi-squared test. SNPs were anchored to chromosomes using the International Wheat Genome Sequencing Consortium (IWGSC) chromosomal survey sequence (Marcussen et al., 2014). The ripple and inversion functions were used to find the best order of SNPs on linkage groups. Duplicated markers were removed from the map based on agreement with the survey sequence and previously genetically mapped SNPs from cerealsDB (Wilkinson et al., 2012). If a marker matched the position in the survey sequence it was retained but if no previous position was defined it was removed. Duplicates were removed at random if both agreed with the survey sequence position. The final linkage map contained 21 linkage groups and 729 SNP markers of unique location, spanning a distance of 3126 cM. Refer to chapter 2 for full linkage map.

Supplementary Table A1. The effect of line from a doubled haploid progeny of Shamrock x Shango winter wheat on root and shoot characteristics when grown as seedlings in germination paper rolls.

Line	Viride-		Total root size					Root diameter			Root:
	scent	Length	Surface	Volume	Dry	Seminal	Mean	0-0.5	0.5-1	dry	Shoot
		(cm)	area	(cm³)	matter	Axes	(mm)	mm	mm	matter	(ratio)
			(cm²)		(mg)	(no.)	-	(%)	(%)	(mg)	
Shm*	Yes	57.6	7.82	0.085	16.8	3.96	0.432	0.911	0.084	12.7	1.37
Shng*	No	62.1	8.67	0.097	16.9	4.00	0.448	0.872	0.120	13.2	1.34
1	No	63.2	8.86	0.099	15.8	4.17	0.452	0.872	0.123	14.8	1.13
2	No	67.7	9.17	0.099	14.9	4.67	0.431	0.904	0.094	15.7	0.95
3*	No	67.4	9.46	0.106	14.5	4.58	0.448	0.865	0.131	15.0	0.99
4	No	61.9	8.26	0.089	18.2	4.08	0.421	0.915	0.083	13.2	1.48
5	No	67.2	9.35	0.104	16.1	4.67	0.444	0.901	0.096	14.4	1.13
6*	Yes	51.0	7.57	0.090	18.8	4.00	0.476	0.770	0.226	11.8	1.77
7	Yes	61.8	8.30	0.089	16.6	4.25	0.428	0.925	0.071	13.3	1.30
8*	No	58.0	8.13	0.091	17.3	4.29	0.447	0.884	0.112	13.6	1.36
9	No	60.5	8.42	0.093	14.4	4.38	0.445	0.869	0.128	13.1	1.00
10*	Yes	55.7	7.89	0.095	13.6	4.00	0.445	0.868	0.128	13.6	1.00
10	Yes	56.1	8.24	0.089	16.1	4.00	0.432	0.808	0.129	13.5	1.23
11	Yes	60.6	8.69	0.097	14.6	4.00	0.467	0.852	0.102	13.3 14.2	1.25
12	No	60.6 66.1	8.69 9.30	0.099 0.105	14.6 16.0	4.27 4.38	0.457	0.857	0.141	14.2 14.4	1.09
									0.096		
14 15	No No	63.8 59.5	8.85 8.15	0.098 0.089	15.5 15.1	4.33 4.08	0.443 0.435	0.899 0.883	0.096	15.9 15.3	1.01 1.00
16	No	60.1	8.54	0.097	15.9	3.92	0.453	0.858	0.137	13.4	1.24
17	No	60.8	8.40	0.093	15.2	4.29	0.440	0.898	0.100	14.1	1.12
18	Yes	57.2	7.90	0.087	17.2	3.75	0.440	0.891	0.106	13.2	1.34
20	No	66.9	9.01	0.097	16.6	4.42	0.430	0.907	0.091	14.0	1.27
21*	No	56.5	7.97	0.090	18.0	3.98	0.452	0.861	0.136	10.9	1.83
22	Yes	65.4	9.06	0.100	16.4	4.33	0.443	0.888	0.111	14.1	1.23
23	No	65.6	9.16	0.102	17.1	4.50	0.443	0.894	0.102	13.1	1.49
24	No	60.8	8.88	0.104	16.1	4.33	0.465	0.830	0.166	14.6	1.15
25*	Yes	55.6	8.13	0.095	16.5	4.04	0.464	0.840	0.156	13.6	1.26
26	No	63.5	8.75	0.096	14.5	4.42	0.439	0.915	0.084	14.8	0.99
27	No	60.7	8.14	0.087	15.6	4.13	0.431	0.915	0.079	12.1	1.37
28	No	65.8	8.92	0.097	17.5	4.17	0.433	0.884	0.114	14.5	1.25
29	No	62.7	8.58	0.094	14.4	4.46	0.436	0.920	0.078	13.2	1.12
30	No	65.3	9.09	0.101	16.7	4.67	0.442	0.894	0.104	13.3	1.34
35*	No	55.5	7.91	0.090	17.8	3.83	0.458	0.865	0.128	13.3	1.48
37	No	66.4	9.10	0.100	15.8	4.25	0.434	0.890	0.108	14.5	1.15
39*	Yes	66.5	9.08	0.099	15.1	4.67	0.436	0.898	0.100	15.4	1.00
41	Yes	62.9	8.97	0.102	15.1	4.33	0.456	0.855	0.141	14.6	1.05
42	Yes	60.3	8.66	0.099	16.7	4.29	0.459	0.848	0.148	13.5	1.26
43a	Yes	61.2	8.87	0.103	16.6	3.88	0.462	0.834	0.161	13.2	1.31
43b	Yes	64.1	9.06	0.102	16.8	4.29	0.451	0.869	0.127	14.5	1.18
43c	No	62.8	8.96	0.102	15.9	4.31	0.455	0.869	0.124	13.4	1.20
43d	Yes	65.0	8.71	0.093	17.6	4.29	0.427	0.925	0.073	14.7	1.27
45	Yes	65.5	8.93	0.097	15.6	4.58	0.436	0.910	0.086	15.0	1.05
45b	Yes	60.3	8.36	0.093	17.0	4.33	0.442	0.881	0.115	13.6	1.44
47	Yes	63.3	8.55	0.092	15.4	4.29	0.431	0.910	0.089	14.8	1.08
51	Yes	63.9	8.68	0.094	16.2	4.17	0.434	0.918	0.080	14.7	1.14
52	Yes	59.9	8.40	0.094	15.7	4.08	0.450	0.884	0.115	14.9	1.07
53	No	60.1	8.93	0.106	15.8	3.96	0.472	0.834	0.113	14.9	1.14
55*	No	68.7	9.21	0.099	17.3	4.67	0.472	0.830	0.061	13.9	1.27
55 56*	Yes	72.3	9.86	0.107	17.3	4.07	0.427	0.937	0.001	15.6	0.96
57	XİNKO	64.4	9.01	0.101	16.2	4.17	0.446	0.882	0.115	15.4	1.07

S9   No   61.6   8.54   0.094   15.2   4.21   0.439   0.910   0.088   13.7   1.12     60   Yes   58.9   7.74   0.081   15.6   4.17   0.418   0.395   0.061   13.8   1.17     62   No   55.0   7.89   0.090   17.6   3.88   0.458   0.856   0.141   1.22     64*   No   67.8   9.42   0.105   14.1   4.71   0.444   0.898   0.101   14.5   1.16     65   Yes   64.1   9.02   0.101   16.5   4.33   0.452   0.880   0.110   14.5   1.102     67   Yes   63.8   9.04   0.102   16.9   4.38   0.451   0.870   0.114   1.3   1.02     67   Yes   64.9   9.46   0.110   16.9   4.38   0.451   0.870   0.114   1.3.4   1.02     72   No   64.0	58*	Yes	68.8	9.20	0.098	15.6	4.63	0.425	0.930	0.068	14.2	1.11
60   Yes   58.9   7.74   0.081   15.6   4.17   0.418   0.935   0.061   13.8   1.17     62   No   55.0   7.89   0.090   17.6   3.88   0.458   0.356   0.143   12.3   1.26     64*   No   67.8   9.42   0.105   14.1   4.71   0.444   0.898   0.100   16.3   0.88     65   Yes   64.1   9.02   0.101   16.5   4.38   0.452   0.870   0.127   15.1   1.02     66   No   66.3   9.37   0.106   15.2   4.38   0.451   0.870   0.125   13.3   1.32     67   Yes   64.0   9.07   0.103   14.5   4.42   0.449   0.870   0.125   14.3   1.14   1.09     72   No   64.5   9.09   0.102   15.8   4.044   0.449   0.427   0.171   13.8   1.34   1.14												
62   No   55.0   7.89   0.090   17.6   3.88   0.458   0.856   0.13   12.3   1.26     63   Yes   61.3   8.53   0.095   16.8   4.00   0.443   0.898   0.100   14.1   1.22     64*   No   67.8   9.42   0.105   14.1   4.71   0.444   0.890   0.109   16.3   0.88     655   Yes   64.1   9.02   0.101   16.5   4.38   0.452   0.870   0.127   15.1   1.02     67   Yes   63.8   9.04   0.102   16.9   4.38   0.451   0.873   0.125   13.3   1.32     68   Yes   64.0   9.07   0.103   14.5   4.42   0.449   0.873   0.114   13.4   1.09     72   No   64.0   9.62   0.103   15.7   4.17   0.462   0.838   0.151   1.10     74   No   65.1   <												
63   Yes   61.3   8.53   0.095   16.8   4.00   0.443   0.898   0.100   14.1   1.22     64*   No   67.8   9.42   0.105   14.1   4.71   0.444   0.890   0.109   16.3   0.88     65   Yes   64.1   9.02   0.101   15.5   4.38   0.452   0.888   0.101   14.5   1.16     66   No   66.3   9.37   0.106   15.2   4.33   0.452   0.888   0.109   14.1   1.09     67   Yes   64.0   9.07   0.103   14.5   4.42   0.449   0.873   0.125   13.3   132     68   Yes   64.0   9.07   0.103   14.5   4.42   0.449   0.873   0.12   15.3   1.32     73   No   64.9   9.62   0.103   15.7   4.17   0.462   0.838   0.151   1.1   1.07     74   No												
64*No67.89.420.10514.14.710.4440.8900.10916.30.8865Yes64.19.020.10116.54.380.4520.3700.12715.11.0266No66.39.370.10615.24.330.4520.3700.12715.11.0267Yes62.98.600.09415.04.380.43510.8790.1141.41.0968Yes63.89.040.10216.94.380.4510.8790.1141.3.41.0972No64.99.460.11016.94.580.4640.8270.12514.31.1674No64.59.090.10215.84.040.4490.8740.12515.11.0775No65.19.440.10915.74.170.4620.3880.15914.31.1476*No69.29.430.10315.24.520.4350.9090.891.661.1677Yes58.27.960.08715.74.000.4400.8650.13012.11.231.5278Yes67.17.680.08218.14.000.4280.9150.18112.31.5279Yes64.28.770.10317.44.290.4440.9000.09714.71.3181Yes67.6 </td <td></td>												
65Yes64.19.020.10116.54.380.4500.8880.11014.51.1666No66.39.370.10615.24.330.4520.3700.12715.11.0267Yes62.98.600.09415.04.380.4350.4880.012715.11.0268Yes63.89.040.10216.94.380.4510.8730.12513.31.3269Yes64.09.070.10314.54.420.4490.8790.11413.41.0972No68.09.620.10816.44.630.4500.8720.12514.31.1674No68.09.620.10215.84.040.4490.8740.12315.11.0775No65.19.440.10315.24.520.4350.9090.08913.61.1677Yes58.27.960.08715.74.000.4020.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.79.170.10115.04.380.4400.9120.8414.91.0383Yes61.78.71 <td></td>												
66   No   66.3   9.37   0.106   15.2   4.33   0.452   0.870   0.127   15.1   1.02     67   Yes   63.8   9.04   0.102   16.9   4.38   0.4351   0.873   0.125   13.3   13.2     69   Yes   64.0   9.07   0.103   14.5   4.42   0.449   0.879   0.114   13.4   1.09     72   No   64.9   9.46   0.110   16.9   4.58   0.464   0.827   0.127   15.1   1.07     73*   No   64.5   9.09   0.102   15.8   4.04   0.449   0.874   0.123   15.1   1.07     75   No   65.1   9.43   0.103   15.2   4.52   0.432   0.910   0.89   13.6   1.14     76*   No   69.2   9.43   0.087   15.7   4.00   0.440   0.80   1.03   12.1   1.23     78   Yes <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>												
67Yes62.98.600.09415.04.380.4350.8880.10914.11.0968Yes63.89.040.10216.94.380.4350.8730.12513.313.269Yes64.09.070.10314.54.420.4490.8730.11413.41.0972No64.99.460.11016.94.580.4640.8270.17113.81.3273*No65.19.440.10915.74.170.4620.8380.15914.31.1476*No65.19.440.10915.74.170.4620.8380.15913.61.1677Yes52.79.630.08218.14.000.4480.9150.08112.31.5278Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.79.290.10317.44.290.4440.9000.9714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes61.48.74 </td <td></td>												
68Yes63.89.040.10216.94.380.4510.8730.12513.31.3269Yes64.09.070.10314.54.420.4490.8790.1141.0972No64.99.460.11016.94.580.4640.8770.12514.31.1674No64.59.090.10215.84.040.4490.8740.12315.11.0775No65.19.440.10315.24.520.4350.9090.08913.61.1676*No65.29.430.10315.24.520.4350.9090.08913.61.1677Yes58.27.960.08715.74.000.4400.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9030.871.421.3183Yes61.78.740.09716.54.170.4330.9100.08714.21.3184Yes61.78.740.097 <td></td>												
69Yes64.09.070.10314.54.420.4490.8790.11413.41.0972No64.99.460.11016.94.580.4640.8270.17113.81.3273*No68.09.620.10816.44.630.4500.8720.12514.31.1674No64.59.090.10215.84.040.4490.8740.12315.11.0775No65.19.440.10915.74.170.4620.8380.15914.31.1476*No69.29.430.10315.24.520.9090.08913.61.1677Yes58.27.960.08715.74.000.4400.8650.30112.11.2378Yes64.28.870.09817.24.330.4390.9100.08112.31.5279Yes64.28.870.09117.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes65.68.930.09716.54.170.4330.9100.08714.21.3186*Yes61.48.740.100<												
72 No 64.9 9.46 0.110 16.9 4.58 0.464 0.827 0.171 13.8 1.32   73* No 68.0 9.62 0.108 16.4 4.63 0.450 0.872 0.125 14.3 1.16   74 No 64.5 9.09 0.102 15.8 4.04 0.449 0.874 0.123 15.1 1.07   75 No 65.1 9.44 0.109 15.7 4.17 0.462 0.838 0.159 14.3 1.14   76* No 69.2 9.43 0.103 15.2 4.52 0.435 0.909 0.89 13.6 1.16   77 Yes 58.2 7.96 0.087 15.7 4.00 0.442 0.915 0.081 12.3 1.52   78 Yes 64.2 8.87 0.098 17.2 4.33 0.447 0.866 0.112 1.41   81 Yes 65.6 8.93 0.097 16.4 4.08 0.442 0.818 1.12 1.31												
73* No 68.0 9.62 0.108 16.4 4.63 0.450 0.872 0.125 14.3 1.16   74 No 64.5 9.09 0.102 15.8 4.04 0.449 0.874 0.123 15.1 1.07   75 No 65.1 9.44 0.109 15.7 4.17 0.462 0.838 0.159 14.3 1.14   76* No 69.2 9.43 0.103 15.2 4.52 0.435 0.909 0.089 13.6 1.16   77 Yes 58.2 7.96 0.087 15.7 4.00 0.440 0.865 0.130 12.1 1.23   78 Yes 57.1 7.68 0.082 18.1 4.00 0.428 0.915 0.081 1.20   80 No 66.7 9.17 0.101 15.0 4.38 0.440 0.912 0.084 14.9 1.03   83 Yes 65.6 8.93 0.097 16.5 4.17 0.433 0.910 0.087 14.2 <												
74No64.59.090.10215.84.040.4490.8740.12315.11.0775No65.19.440.10915.74.170.4620.8380.15914.31.1476*No69.29.430.10315.24.520.4350.9090.08913.61.1677Yes58.27.960.08715.74.000.4400.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No62.29.170.10017.04.290.4530.8710.12113.71.3789No62.69.03<												
75No65.19.440.10915.74.170.4620.8380.15914.31.1476*No69.29.430.10315.24.520.4350.0990.08913.61.1677Yes58.27.960.08715.74.000.4400.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes61.38.540.09716.44.080.4520.8180.17813.81.2586*Yes61.78.710.09816.14.020.4510.8790.11113.71.2187No62.29.170.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1893aNo62.69.03 </td <td></td>												
76*No69.29.430.10315.24.520.4350.9090.08913.61.1677Yes58.27.960.08715.74.000.4400.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.8414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.9315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.75 <td></td>												
77Yes58.27.960.08715.74.000.4400.8650.13012.11.2378Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.9315.41.1488Yes61.48.740.10017.84.670.4570.8500.14614.21.1892bNo62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.20<												
78Yes57.17.680.08218.14.000.4280.9150.08112.31.5279Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08114.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.84.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes65.59												
79Yes64.28.870.09817.24.330.4390.9100.08915.01.2080No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4270.9110.08614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.8												
80No66.99.290.10317.44.290.4440.9000.09714.71.3181Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes65.59.110.10115.94.330.4410.8920.1001.201.1794aYes59.88												
81Yes57.68.100.09117.83.810.4470.8860.11212.41.4182No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.5 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.097</td><td></td><td></td></td<>										0.097		
82No66.79.170.10115.04.380.4400.9120.08414.91.0383Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4270.9110.08614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes59.88.400.09416.04.380.4470.8440.03514.91.0094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.5 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0.112</td><td></td><td></td></t<>										0.112		
83Yes60.38.540.09716.44.080.4520.8180.17813.81.2586*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.1001.291.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes55.59.110.10115.94.330.4440.8530.1441.441.05116aNo64.39.280.10715.04.290.4610.8530.1441.441.05116aNo64.3 <td></td> <td>No</td> <td></td> <td></td> <td>0.101</td> <td></td> <td></td> <td>0.440</td> <td>0.912</td> <td>0.084</td> <td>14.9</td> <td></td>		No			0.101			0.440	0.912	0.084	14.9	
86*Yes65.68.930.09716.54.170.4330.9100.08714.21.3186aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.3 <td>83</td> <td>Yes</td> <td>60.3</td> <td>8.54</td> <td>0.097</td> <td>16.4</td> <td>4.08</td> <td>0.452</td> <td>0.818</td> <td>0.178</td> <td>13.8</td> <td>1.25</td>	83	Yes	60.3	8.54	0.097	16.4	4.08	0.452	0.818	0.178	13.8	1.25
86aYes61.78.710.09816.14.020.4510.8790.11113.71.2187No67.29.170.10016.74.460.4360.9040.09315.41.1488Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.8 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>0.087</td> <td></td> <td></td>										0.087		
88Yes61.48.740.10017.04.290.4530.8710.12113.71.3789No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.7	86a	Yes	61.7	8.71	0.098		4.02	0.451	0.879	0.111	13.7	1.21
89No62.69.030.10416.44.670.4570.8500.14614.21.1892bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58	87	No	67.2	9.17	0.100	16.7	4.46	0.436	0.904	0.093	15.4	1.14
92bNo64.98.750.09516.94.670.4270.9110.08614.21.2493aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	88	Yes	61.4	8.74	0.100	17.0	4.29	0.453	0.871	0.121	13.7	1.37
93aNo64.49.200.10517.84.460.4540.8970.12513.61.3593b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	89	No	62.6	9.03	0.104	16.4	4.67	0.457	0.850	0.146	14.2	1.18
93b*Yes66.29.120.10015.94.330.4410.8920.10012.91.1793c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4450.8730.12413.81.29	92b	No	64.9	8.75	0.095	16.9	4.67	0.427	0.911	0.086	14.2	1.24
93c*Yes55.17.180.07519.63.850.4170.9420.05310.71.9494aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4450.8730.12413.81.29	93a	No	64.4	9.20	0.105	17.8	4.46	0.454	0.897	0.125	13.6	1.35
94aYes59.88.400.09416.04.380.4470.8940.10314.01.2094bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	93b*	Yes			0.100			0.441	0.892	0.100	12.9	1.17
94bNo64.58.840.09716.44.040.4380.9010.09514.71.1697bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	93c*	Yes	55.1	7.18	0.075	19.6	3.85	0.417	0.942	0.053	10.7	1.94
97bYes65.59.110.10115.94.330.4440.9050.09314.91.10116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	94a	Yes			0.094		4.38	0.447		0.103	14.0	1.20
116aNo64.39.280.10715.04.290.4610.8530.14414.41.05116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	94b	No	64.5	8.84	0.097	16.4	4.04	0.438	0.901	0.095	14.7	1.16
116cYes60.88.300.09017.14.290.4350.9090.08814.11.24119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	97b	Yes			0.101	15.9	4.33	0.444	0.905	0.093	14.9	1.10
119aYes64.28.670.09415.24.420.4320.9070.09114.61.08119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29												
119bNo66.79.060.09814.04.500.4340.9190.08013.51.08119cYes58.58.200.09217.13.960.4450.8730.12413.81.29	116c	Yes						0.435		0.088	14.1	
119c   Yes   58.5   8.20   0.092   17.1   3.96   0.445   0.873   0.124   13.8   1.29												
S.E.D. (616 d.f.) 3.64 0.506 0.006 1.19 0.206 0.009 0.02 0.019 1.00 0.177	119c	Yes	58.5	8.20	0.092	17.1	3.96	0.445	0.873	0.124	13.8	1.29
	S.E.D. (	(616 d.f.)	3.64	0.506	0.006	1.19	0.206	0.009	0.02	0.019	1.00	0.177

\* are selected lines used in the rhizotrons experiment



Supplementary Figure A1. 2013/14 season A) Average greenness of crop derived from far red: red ratio over thermal time from sowing. B) Proportion of PAR intercepted, averaged over field trial over thermal time from sowing. C) Above canopy PAR provided by solar radiation over whole season. D: Average PAR intercepted by crop as calculated by the logistic model. First dashed line represents anthesis; second dashed line is the onset of senescence.



Supplementary Figure A2. 2014/15 season A) Average greenness of crop derived from far red: red ratio over thermal time from sowing. B) Proportion of PAR intercepted, averaged over field trial over thermal time from sowing. C) Above canopy PAR provided by solar radiation over whole season. D: Average PAR intercepted by crop as calculated by the logistic model. First dashed line represents anthesis; second dashed line is the onset of senescence.