# BARLEY ROOT TRAITS FOR IMPROVED SUBSOIL EXPLORATION AND RESOURCE CAPTURE

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**CONTENTS**

# Abstract

Subsoil physical characteristics are often limiting to root growth, one of the major reasons being high density soil. However, deeper and more efficient root systems could help to explore a larger soil volume and reduce the input of nitrogen fertilisers if roots make more use of the nitrogen at depth.

The first target was to develop a screening method which allowed barley root extension rates to be quantified after four days of growth in loose and compacted soils. Firstly, seed quality (loss of germination ability caused by poor conditions in storage and long storage time) was identified as a potential source of variation for root extension rate in seedlings. The screening showed that roots growing in compacted soil had a slower extension rate than roots growing in loose soil. In addition, there was an interaction between soil conditions and cultivars meaning that not all of them showed the same ability to overcome high soil density.

Root architecture was characterized at days eight and 12 after planting for four selected divergent cultivars. Measurements were made using X-ray micro-computed tomography (μCT)-scanning. The differences between the four genotypes in root architecture (number of primary

roots, root extension rate, root length, root area, root volume, convex hull, centre of mass, lateral density, lateral length) were significant at eight days after planting but disappeared at 12 days after planting for most of the traits measured (i.e. growth rate of primary roots). Soil density influenced the root system architecture at both two-time points, roots elongated less and explored less soil in the high compaction treatment.

A third experiment was conducted to test the hypothesis that the differences in root architecture observed between the genotypes in response to the soil bulk density in the μCT-scanning would lead to different patterns of nutrient uptake from topsoil and subsoil. Layered soil columns of topsoil and subsoil were constructed with different subsoil physical parameters (loose, compacted and compacted with macropores) and a nitrogen tracer to measure nitrogen capture from the subsoil. Root length density and other traits determining root architecture differed between two barley cultivars and oat, but increased root length density in the subsoil did not improve nitrogen uptake from the subsoil. Hence showing that nitrogen uptake from the subsoil was not directly related with a greater presence of roots in the experiment.

# Abbreviations

| | |
|---|---|
| Air-filled porosity | AFP |
| Ammonium | $NH_4^+$ |
| Ammonium nitrate | $NH_4NO_3$ |
| Analysis of variance | ANOVA |
| Bulk density | BD |
| Carbon | C |
| Cell wall extensibility | φ |
| Compaction | C |
| Cultivar | cv. |
| Degrees of freedom | df |
| Dry weight | DW |
| Initial seed quality | Ki |
| Least significant differences | LSD |
| Loose | L |
| Macropores | M |
| Manganese | Mn |
| Mean germination time | MGT |
| Mega Pascal | MPa |
| micro-Computed Tomography | μCT |
| Moisture content | MC |
| Nitrate | $N0_3^-$ |
| Nitrogen | N |

| | |
|---|---|
| Phosphorus | P |
| Photosynthetically active radiation | PAR |
| Potassium | K |
| Rate of elongation | r |
| Resistance exerted by the soil against the cell wall | Ys |
| Root length Density | RLD |
| Simple Interactive Object Extraction | SIOX |
| Standard error of the mean | SEM |
| Sulphur | S |
| Tension required for the load bearing bones | Yr |
| Yield threshold | Y |

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# Chapter 1: Literature review

## 1.1 Introduction to barley

Barley (Hordeum vulgare L.) is a cereal that originated in the Middle East more than 10,000 years ago and was domesticated for human consumption. There are over 400,000 barley accessions in gene banks (Newton *et al.*, 2011). Presently it is cultivated worldwide in intensive agricultural systems as well as extensive because of its ability to adapt to biotic and abiotic stresses and because it produces more stable yields than wheat and other cereals (Newton *et al.*, 2011).

Barley is the 4$^{th}$ most widely cultivated cereal worldwide and is as high as 2$^{nd}$ in those places where the climatic conditions are severe. It can be grown under very different environmental conditions, from the high altitudes of the Himalayan Mountains to the arid conditions of the Middle East. Worldwide barley is grown over approximately 50 Mha with a yield of approx. 144.5 Mt annually (FAO, 2014). The highest levels of production are North America, Europe, Russia, China and Australia. The major producers in terms of tonnage of barley are Russia, Ukraine and Australia with low yields but harvested over large areas. In Northern Europe, it is grown in intensive agricultural systems; in the last 50 years

the total number of cultivated hectares has decreased but the total yields produced have not. The average yield in the UK is close to 6 tonnes per hectare. In the UK barley is the 2nd crop grown with 1.2Mha and an average yield of 5.8 t $ha^{-1}$ in 2013 (Department for Environment Food & Rural Affairs, 2013).

Barley's main uses are feed for livestock (more than 60% of its overall yield) and alcoholic beverages, with direct human consumption a relatively minor use of barley (Newman and Newman, 2006). It is the major food stuff in places where the environmental conditions are suitable mainly for the growth of barley such as the mountainous regions of Africa, Asia and South America (Newton *et al.,* 2011). However, due to the betaglucans present in barley (compounds linked to reduction of cholesterol levels (Yang *et al.*, 2003)) and the increased interest of society in healthy diets, it may also become more popular in other countries in the future.

Depending on its use, barley grains can have different properties which can be controlled with agronomic practices such as the input of fertiliser or the sowing date. For example, if the purpose of barley is animal consumption it requires a higher nitrogen (N) concentration than barley for malting purposes. This is because animals need the input of protein

in their diet whereas malting barley needs to have a low protein content and high malt extract in the seeds for the distilling process (Newton *et al.,* 2011).

Barley can be sown in autumn or spring. Winter barley is used mainly for feeding livestock. Environmental factors influence yields and limit the management strategies. The growth of crops and final yield will depend on the amount of captured light and on the nutrient and water uptake and the local availability of them in the soil. Barley is very useful in the rotation of the crops by helping in the control of pests and diseases (Newton *et al.,* 2011). It has also been suggested that the combination of wheat and barley in the same land decreases the risk of suffering diseases and lodging, while increasing the grain yield and quality (Woldeamlak *et al.*, 2008).

## 1.2 Food security

According to the FAO, 2009, the world population is expected to exceed 9.2 billion people by 2050. Agriculture must meet the requirements to produce enough food for direct human consumption, feed animals and provide fuel, chemicals and other materials for the population. In order for crops to reach their genetic yield potential, plants must be able to perform well under biotic and abiotic stresses (Slafer *et al.*, 2005).

It is generally accepted that an increase in yields should be achieved by intensive agriculture, if not, the land required to feed the population would be 225% of the present agricultural land (Dawson and Hilton, 2011). Agricultural soils usually need to be supplemented with plant-available nutrients which are applied to the soils as manure or fertilisers. The most limiting resources in soils are usually water and the mineral elements N, Phosphorus (P), Potassium (K) and Sulphur (S). N, P, K and S are macronutrients so plants need them in relatively high quantities and thus they are applied to the soils by regular input of fertilisers; some other macronutrients are usually in abundance in fertile soils as well as most of the micronutrients (Dawson and Hilton, 2011). N-fertilisers are expensive, the P is a shrinking resource and both fertilisers can pollute the environment where they are applied.

The new "green revolution" is looking for new models of intensification and to increase the efficiency of nutrient and water uptake, as well as alternative agricultural models which will improve the soil fertility and structure making better use of all the resources available (Branca *et al.*, 2011). Nutrient and water use efficiencies are defined in a simplistic way as the quantity of output provided for each unit of input. The uptake of water and nutrients depends on the root system, its growth and distribution and also on the soil conditions, such as the local availability

of nutrients and climate, physicochemical soil properties and the management techniques applied to the soil.

## 1.3 Structure of roots

Root systems have several functions for the plants: anchorage of the plant to the ground, acquisition and storage of water and nutrients necessary for plant growth and the synthesis of growth regulators (Gregory, 2008). The efficiency of the crops is determined to a large extent by the ability of roots to grow under different environments. The architecture of the root system is crucial for the efficient capture of water and nutrients and can be a key determinant of the survival of plants in difficult environmental conditions.

The root system is highly plastic and capable of adapting to and growing in a wide range of environments according to the signals and information captured by root tips (Malamy, 2005). At the same time, the developmental patterns followed by root systems are determined by their genetics and the environment. Some of the root characteristics influencing the plant's ability to penetrate soil are the growth angle, the presence of root hairs, and the diameter of the root (Jin *et al.*, 2013).

### 1.3.1 Anatomy and morphology of the root

The root system is initiated by the growth of the primary roots; it is a short stage before shoot elongation (Figure 1-1). The basal roots develop after the primary roots from the basal nodes of the shoot in barley (Zobel and Waisel, 2010).

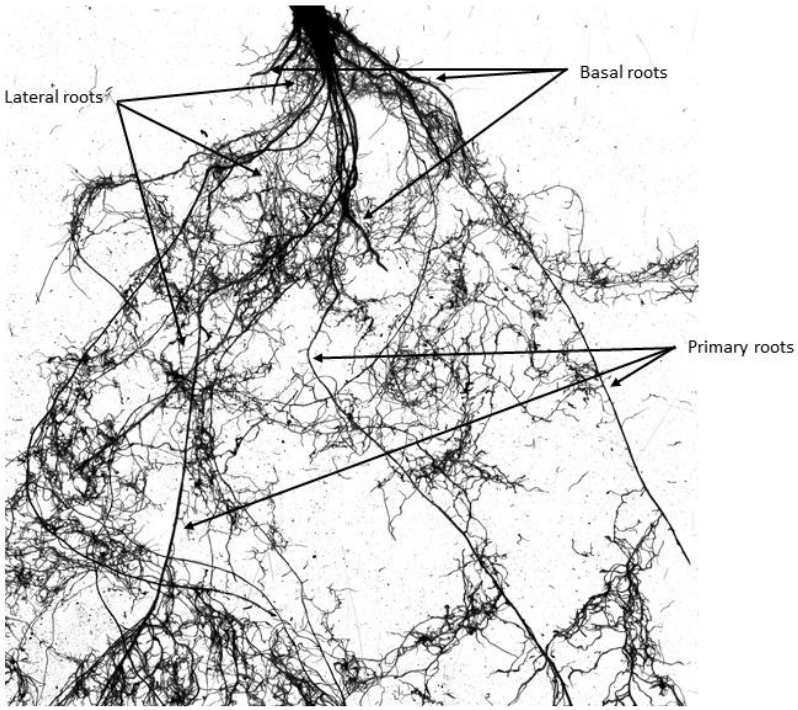


**Figure 1-1 Flat bed scanned 6-week old barley root. Arrows pointing to primary roots developed from the seed, basal roots developed from the stem and lateral roots of different orders.**

The outer cell layer of a root is the single cell layer of the epidermis, which is the layer in contact with the soil; on the inside of the epidermis is the cortex (Figure 1-2). Between the cortex and the stele there is the single cell layer of the endodermis. The stele contains the vascular tissue. The endodermis possesses the Casparian Bands which are suberized

regions within endodermal walls. They are developed at a late stage of maturity of the cells. The casparian band stops the flow of water and other substances in the apoplastic pathway.

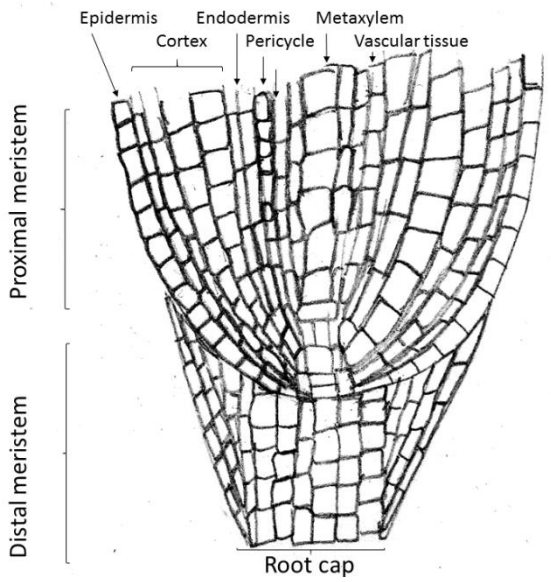


**Figure 1-2 Schematic representation of the longitudinal section of *H. vulgare* root meristem. Adapted from Braszewska-Zalewska *et al.*, 2013.**

## 1.3.2 Root microstructure

### 1.3.2.1 Regulation of root extension and lateral formation

There is an increase in the cell production rate at the root tip (Walch-Liu *et al.*, 2006) although long-range signalling (auxin and ABA) is also involved regulating the nutrient movement between the shoot and the root (Forde, 2002). ABA is linked to osmotic control within the plant and blocks lateral root development, while auxin is necessary for the initiation of the lateral roots (Babé *et al.*, 2012). A higher supply of sucrose

and glucose to the roots also causes an increase in lateral root initiation. It is the increase of sugars in the root system which causes the proliferation of roots when there are nutrient patches (Bingham *et al.*, 1998).

Lateral roots are formed by the anticlinal division of pericycle cells adjacent to protoxylem poles (Malamy, 2005; Yu et al., 2016), not from cells close to the root tip. Their growth depends on the internal nutritional status of the plants and external factors (Hawes *et al.*, 2002). The root cap determines the root distribution because it perceives the properties of the soil such as pressure, moisture or gravity (Gregory, 2008).

#### 1.3.2.2 Internal structure of the cap, meristem, older roots.

The root cap protects the apical meristem by the production of mucilage. There is evidence that mucilage and border cells may be involved in the elongation of root cells because the removal/addition of them alters the rate of mitosis and elongation (Hawes et al., 2002). Mucilage is delivered into the soil as the root cap grows. Cells in the cap periphery separate as a result of enzymatic activity from the root cap and from the other cells once they have differentiated. That process is genetically controlled by

programmed cell death and varies the length of time the cells survive once they are detached from the cap (Hawes et al., 2002).

The root tip is formed by a quiescent centre which has a very slow rate of cell duplication, a meristem regulating the growth and replacement of the root tissues (Figure 1-2). The cell duplication process allows the root to grow in the soil. In barley, the transverse divisions predominate (Luxova, 1975). If the meristem is removed, the roots stop elongating (Hawes et al., 2002).

Root hairs are formed in the zone of maturation of the roots, about 4 mm from the root tip (Pritchard, 1994). They are specialized projections from modified epidermal cells in the elongation zone (Gregory, 2006; Watt *et al.*, 2006). The functions of root hairs are nutrient and water uptake and anchorage of the root axis by increasing the contact between the roots and the soil (Gilroy and Jones, 2000). Their growth is determined by genetic and environmental constraints.

### 1.3.3 Impact of various parts on the growth of the root and uptake

The root system architecture depends on genomic attributes determined by the variety and also by external causes such as the physical properties of the soil, the access to nutrients and the water supply. For example,

variation in the concentration of phosphate, nitrate or ammonium in the soil results in changes in the dry matter distribution and in the lateral root architecture of barley (Drew, 1975). Barley main root axes are usually <1 mm diameter and are fasciculate, developed from the base of the stem (McKenzie *et al.*, 2009). There is evidence showing that the lateral roots develop according to the nutrient distribution in the soil profile (Hodge, 2004), having a higher density wherever physical and chemical soil conditions are good (Gregory, 2006) and the density of nutrients is higher too. For K acquisition, however, there is no higher lateral root extension because K it is a very mobile nutrient and not metabolized by the plant (Robinson, 1994). The way the roots behave under different N supply has been well researched. Roots proliferate in N- and P-rich patches because they are sensitive to the external nutrient environment (Drew, 1975; Hodge, 2004).

The root tip experiences the maximum physical stress encountered by the roots (Tracy *et al.*, 2011). The narrowly pointed root tip influences the root penetration into the soil positively as it enables the creation of cylindrical cavities (Bengough *et al.*, 2011). In addition, the secretion of mucilage or rhizodeposits facilitate root tip penetration into the soil by decreasing the friction at the soil-root interface and it may change the properties of the surrounding environment of the soil by influencing the

microbial environment and nutrient dynamics (McCully, 1999; Carminati and Vetterlein, 2013). The rhizodeposits also improve water uptake from the soils (Ahmed *et al.*, 2014). Mucilage is a water-soluble substance formed by the synthesis of starch and polysaccharides produced in the cap (Hawes et al., 2002).

## 1.4 Mechanisms of root growth

### 1.4.1 Cell division

The root growth process is divided into phases which are physically differentiated, although the limits are not completely marked. The first phase is the division of the cells present in the proximal meristem. The initiation of the cell division involves auxin and cytokinin. These cells elongate until they can divide again. This process is repeated several times before the cells start the expansion process. The cells in the meristem typically have a high content of cytoplasm and no clearly defined vacuole (Pritchard, 1994). The cell division phase occurs up to 4 mm from the root tip apex.

### 1.4.2 Biophysical processes involved in expansion

Cell expansion is a process where biochemical changes in the cell wall cause breakage of wall bearing bonds. The cell wall relaxes because the

microfibrils and hemicelluloses unions are broken by enzymatic activity and they become aligned. This lowers the turgor pressure, creates differences in the osmotic potential inside and immediately outside the cell, and creates a water potential between the inside and outside of the cell. Water thus moves into the cell increasing turgor pressure. If the pressure exceeds a yield threshold, the wall extends plastically and the cell grows. The axial pressure is directly related to the cell enlargement and the radial pressure is related to the thickening of the roots, increasing the strength over the axial section (Barley *et al.*, 1965) and the anchorage to the soil (Stolzy and Barley, 1968).

Continued growth requires a sustained flux of water and solutes into the cell. The maximum requirement of solutes would be at the maximum growing point. An influx of solutes is required to maintain the osmotic potential and thus turgor pressure as the cell volume increases to compensate their dilution because of the water influx. The flux of water and solutes into the root cells is generally considered not to be limiting. For the growth of the cells two transport pathways for nutrients are used, via the apoplastic (extracellular) and symplastic (intracellular) paths. The phloem has a higher osmotic pressure than the cells and infiltrates solutes into the cells by water flow (Pritchard, 1994). The water potential is always lower in the outer cortex than in the external medium (Tomos

and Pritchard, 1994). The growth is maintained even though the water potential is low because of the plastic and elastic properties of the cell walls (Tomos and Pritchard, 1994). Most of the radial movements of water in the roots follow the apoplastic path which shows low resistance (Pritchard *et al.,* 1988).

## 1.5 Solute and water fluxes to support cell expansion

For roots growing in unrestricted conditions such as hydroponics or moist vermiculate, the rate of elongation ($r$) is a function of the cell wall extensibility($\varphi$) and the maximum turgor pressure ($P$) and the turgor pressure required for growth (yield threshold, $Y$) (Pritchard *et al.,* 1988; Pritchard *et al*., 1989).

$$r = \varphi \times (P - Y)$$

$P$ is the difference in pressure created between the pressure of the soil and the pressure of the roots and the cell wall. For the growth to start $P$ has to overcome $Y$. Firstly, $Y$ decreases and the growth rate is increased until cells have reached their final length (Pritchard *et al.,* 1990). As the cell increases distance from the root tip the elongation rates decreases (Pritchard, 1994) by a decreased $\varphi$ or an increase in $Y$. As turgor pressure is considered to be constant along the growing zone, growth should

occur due to changes in the cell wall rheological properties. Changes in $\varphi$ are thought to occur because of enzymatic activity breaking load bearing bonds in the cell walls or cell wall components that control the microfibrils orientation (Bengough *et al.*, 2006) and $Y$ is the threshold tension required for the load bearing bonds to be suitable for starting the enzymatic activity.

Cell wall expansion is not constant; it is divided into two phases before the cells are mature and can differentiate. Those phases are the acceleration phase and the deceleration phase. During the acceleration phase the cell walls are loosened and grow because of water stress and osmotic changes until the saturating length. After that, the cell walls get tighter again because of an increase in the wall cross-linkages and because the microtubules and microfibrils become unaligned again (Bengough et al., 2006). Roots also face mechanical impedance during growth in physical media such as soil thus cell turgor pressure has also to overcome the resistance exerted by the soil ($Y_s$):

$$r = \varphi \times (P - Y_r - Y_s)$$

After elongation cells reach the maturation zone where cells differentiate and join one of the tissues of the roots.

## 1.6 Influence of soil on root growth

### 1.6.1 Soil characteristics

Soil is the medium where roots grow and develop; therefore, all factors related to the soil affect the growth of the root systems and, by extension, the whole plant. The most important factors for root growth are the soil moisture, mechanical resistance structure, aeration, soil temperature, nutrients, $CO_2$ and $O_2$. The structure of the soil and its hydraulic properties are dynamic and change over time because of environmental conditions or agricultural practices.

Soil has a characteristic pore distribution dependant on the soil particle size, the texture of the soil, density of the soil and the amount of shear deformation and other biological factors such as composition of faunal community. The inherent pore sizes in soil are often smaller than the root diameter but macropores are independent pores created by different factors such as the shrinkage of the soil, agricultural machinery, the growth of roots through the soil profile or earthworm burrows (Jin *et al.*, 2013). The last two macropores types are usually vertical and very stable while the ones created by agricultural machinery are not as stable against water and soil compaction (McKenzie and Dexter, 1993).

The pores existing in soil are very important for the growth of roots, especially in those soils where the mechanical strength is high (Valentine *et al.*, 2012). To penetrate into strong soil roots need enough pressure to create a cavity for growth plus the pressure to overcome the frictional resistance between the soil and the root. This resistance may be up to 80% of total penetration resistance and under drier conditions it can be even greater (Bengough *et al.*, 2011). When a root enters a pore it will follow the pore path until the angle changes and the surrounding soil is suitable for root penetration. However, if the macropores are very large, they can affect the roots negatively as there is not enough root-soil contact and the roots may desiccate (Passioura, 2012).

Tillage systems play a fundamental role in the structure of soil and its capacity to increase the available water and decrease in the porosity and pore size (Mangalassery *et al.*, 2014). Non-tilled systems tend to conserve the natural soil properties and biopores and increase water infiltration. The influence of the tillage system on soil structure and in the development of the root systems has been documented (McKenzie *et al.*, 2009). Initially, non-tilled soils show an increase in the lateral development of the roots and higher compaction in the topsoil compared to tilled soils (Lampurlanes *et al.*, 2001), but in the long term soils of non-tilled systems are thought to become stable and with improved structure

created by increased biological activity. It has been shown that the physico-chemical changes occurring in non-tilled soils after 5-10 years lead to a higher soil organic matter and microbial N in the surface layer of the soil and down to 20 cm depth (Mangalassery *et al.*, 2014)

## 1.7 Topsoil and subsoil

Topsoil, defined as the A horizon in the soil profile, covers the normal annual cultivation depth, usually reaches up to 20-30 cm. Subsoil is the subsurface material which lies below the topsoil and is defined as horizon B (Jones *et al*., 2003). The topsoil and subsoil differ in their physical, chemical and biological characteristics. Most agricultural practices are applied to the topsoil, for example tillage disturbs the topsoil from its original structure.

Topsoil is characterized by an accumulation of soil organic matter mixed with a mineral fraction, most of the mineral fraction emanating from the original underlying rock (FAO, 2006). Also, most of the microbial activity is found in the upper layers of the soil. The subsoil is also formed by the mineral fraction emanating from the original underlying rock but it has a smaller concentration of organic matter (FAO, 2006). The subsoil and topsoil differ in bulk density, the subsoil usually has a higher bulk density and smaller pore volume, which results in a lower air-filled

capacity and saturated water holding capacity but a higher cohesion between subsoil particles.

According to Salome *et al.*, (2010) the N content is about 1/3 of that in the topsoil and the carbon (C) content about 1/4. As subsoil is not disturbed as extensively as topsoil by agricultural practices its structure, including macropores, is more stable. Macropores are a hot spot for soil organic matter and C if they have been created by earthworms or previous roots influencing the microbial activity or the growth of future roots. They also facilitate water and gas fluxes (Kautz *et al.*, 2013). The accumulation of available water in the subsoil depends on the soil structure and the climatic conditions. The water at depth is particularly important for crops growing in dry periods (White and Kirkegaard, 2010).

## 1.8 The subsoil is an underutilized supply or resources

It has been suggested that the subsoil could contribute to more than 60% of plant nutrients required for plant growth if the topsoil lacks nutrients or the water resources are scarce (Kautz *et al.*, 2013). If plant roots reach the subsoil the nutrients from the subsoil will be allocated temporarily to the upper parts and eventually, after harvest some of the nutrients will be returned to the soil as unharvested organic matter; and due to environmental processes, such as infiltration or leaching, some of these

nutrients will be returned to the subsoil. Root elongation into the subsoil will also change hydraulic conditions in the subsoil due to the biopores creation and, therefore, change the soil physical properties (e.g. creating more aggregates, accumulation of organic compounds from the roots).

## 1.9 Root growth in the subsoil

In well-structured soils the maximum root depth is between 1.4-2 m for the winter cereals although when the root length density is less than 1-2 cm $cm^{-3}$, the water from the subsoil is not completely extracted (King *et al.*, 2003; Bingham, 2005). For effective N uptake the required root length density is considered to be the same as for the water uptake. This is based on the assumption that most of the mineral N present is in the form of nitrate. Deeper rooting with the correct root length density will enable plants to extract nutrients from the subsoil more efficiently and avoid problems related to drought or lack of nutrients in the top layers as, for example, due to poor infiltration by fertilisers, although the less mobile nutrients should still be obtained from the topsoil (King *et al.,* 2003).

The growth of the roots will be heavily modified by the resources available at depth. The greatest exploration of the subsoil is usually found in places where the crops suffer from drought conditions (McKenzie *et al.,* 2009). There is contradictory evidence whether deeper

rooting may be beneficial for plants or not. It has been shown that depending on the soil properties, the plants may or may not be capable of taking the nutrients available in the subsoil because while the bulk density increases the pore space diminishes creating anoxic conditions or even making the roots grow in clusters (Kirkegaard and Lilley, 2007; White and Kirkegaard, 2010). On the other hand, it has also been shown that the concentration of macronutrients such N in the subsoil profile is enough for the growth of the plants and that root growth in the subsoil may improve the soil conditions at depth by rhizosphere activity, increasing the microbial fauna and the C storage created by the death roots (Kautz *et al.,* 2013). McKenzie *et al.* (2009) investigated the increases in the leaf area and height of barley in relation to depth of the roots in the soil profile and found that when roots reach the subsoil, the leaf area and height of the stem are larger. This deeper rooting may also be beneficial to the soil microbial community and further development of the subsoil. It was first thought that only a small number of roots would grow through the biopores to reach the subsoil. Subsequent studies have showed that the main path that roots found to reach deeper into the soil profile was via the biopores and that almost 80% of the roots found in the subsoil were found clustered in soil biopores (Kirkegaard *et al.*, 2007; Lilley and Kirkegaard, 2011).

## 1.10 Rhizosphere

The rhizosphere is a dynamic system comprising the apoplastic parts of the roots, the epidermal tissue and the mucilage plus the soil in contact with them (York *et al.*, 2016). The rhizosphere is chemically, physically and biologically distinct from the bulk soil, because of the interaction between the roots, soil and the organisms present in soil (Paterson *et al.*, 2006). Plant roots and soil microbes alter the chemical composition of the rhizosphere. Roots exude C to the rhizosphere, which in conjunction with N, is used by microbes present in the soil. C is usually present in the soil in sufficient quantity but N can be a limiting resource in the rhizosphere.

The rhizodeposits can modify the surrounding soil up to 1 cm distance from the root surface (Kautz *et al.,* 2013). The rhizodeposits are potentially more important for subsoil because there are typically fewer microbes and less exchange of nutrients. Where the rhizodeposits are found, there would presumably be a larger community of microbes and greater microbial activity partly because of the nutrients supplied by the roots and the associated soil chemical and physical changes occurring (Kautz *et al.,* 2013). The identity of the microbial fauna present is determined by the crop species and the nature of exudates they produce

since the composition and biological activity varies among species. Depending on the type of bacteria around the growing zone, there can be beneficial effects for the roots or deleterious effects if the microbes compete with the roots for the nutrients (Watt *et al.*, 2006). The rhizodeposits can speed the decomposition of soil organic matter already present in the soil (Gregory, 2006). The amount of rhizodeposits released by spring barley is estimated at *c.* 33% of the C assimilated during photosynthesis, and between 65-99 g $cm^{-2}$ which is twice the amount of root biomass remaining at crop maturity (Gregory, 2006). The amount of C-exudates by the roots is also dependent on the nutrient environment; barley roots growing in N-patches exude a greater amount of C from the root tips (Paterson *et al.*, 2006).

The residues produced from the degradation of the roots are added to the soil increasing its heterogeneity. Aggregates can be formed in the rhizosphere by wetting and drying, the accumulation of cementing chemicals, exudation of organic compounds or senescent roots (Hinsinger *et al.*, 2009) or because roots crack parts of the soil creating smaller aggregates. If roots deform the soil by their growth, the bulk density in their surrounding soil is increased by a loss of the space used by the growing root (Gregory, 2006). The creation of new pores or cracks improves the aeration of the soil facilitating the future growth of roots

through them or through the compacted soil. The water transport is also influenced by the roots in the rhizosphere (Carminati *et al.*, 2010). The rhizosphere of barley is drier than the bulk soil, possibly due to the uptake of water by the roots (Whalley *et al.*, 2007; Hinsinger *et al.*, 2009) which could deplete the water from the biopore wall faster than the bulk soil or by the drying cycle. There is also an increase in soil compaction at the pore wall and a decrease of the hydraulic properties of the biopores creating a stressful environment for root growth.

## 1.11 Soil compaction

The function of anchorage of the plants by the roots is also related to the soil properties. Soils need to have enough strength to support the whole plant, although a high strength can create stress to the plant, not only because the crops find it too hard to penetrate the soil but also because the high strength is associated with other influential characteristics of the soil; water retention and porosity are two examples. The strength of the soil increases as the soil gets drier or if the aggregation of the soils increases at the same time, water availability may decrease as well (Horn *et al.*, 1994).

Soil compaction is one of the most important abiotic stresses suffered by crops in intensive agricultural systems. The Soil Society of America, 1996,

defines it as *"the process, by which the soil grains are rearranged to decrease void space and bring them into closer contact with one another, thereby increasing bulk density"*. In Europe alone, the degradation of about 33 Mha is thought to have been caused by soil compaction (Jones *et al.,* 2003). Heavy machinery used in agriculture causes soil compaction (Bengough and Mullins, 1990). The heavy load of those vehicles, the number of passes, the nature and depth of ripping or tillage practices can increase the compaction of the soil at lower depths. Subsoil compaction is often difficult to detect and hard to rectify. The susceptibility of a soil to compaction depends on its texture, clay mineralogy and organic matter content (Alakukku and Elonen, 1995; Alakukku *et al.*, 2003). In addition, wet soils support a lower machinery axle load and are easily compacted.

Several studies have shown that as the strength of the soil increases, crop root length decreases (Croser *et al.,* 1999; Bengough, 2003). When the root length is shorter the availability of nutrients decreases too, as roots are confined to a smaller soil volume. Soil strength and water status ideal for root growth differs between crops, but as the soil profile is typically very heterogeneous almost all roots suffer from mechanical impedance at some point (Bengough *et al.,* 2006). Very loose soils are not good for root growth as they do not provide enough soil-root contact and the nutrient

and water uptake will be reduced too (Bengough and Mullins, 1990; Stirzaker *et al.,* 1996).

## 1.12 Macropores and roots

Soil compaction affects pore size and continuity by decreasing the pore space; it also affects the water availability by changing the matric potential properties of the soil and, consequently, plants can suffer from anoxic or drought conditions in compacted soil depending on the soil water content (Bengough *et al.*, 2011). All these factors will influence the pressures exerted over the root cap, axially and radially. It is estimated that a soil pressure exceeding 2 MPa limits root elongation rate dramatically (Bengough *et al.,* 2011). Bengough (2012) showed how barley roots grew in undisturbed soil in macropores with diameters of 60-300 μm, suggesting that macropores are the main way for root tips to elongate in strong soils. Macropores offer roots a discontinuity to the soil strength and if the bulk density is high roots will grow through them (Lipiec and Hatano, 2003).

However, roots growing in soils with high strength usually show a greater extension of the lateral roots or a proliferation of the roots if they find a volume of soil where the strength is reduced such as a wetted zone because of rainfall events. In an experiment carried out with barley plants

grown in soils with artificial biopores and different bulk densities it was found that the biggest biopores (3.2 mm) reduced the growth of the leaves by about 30% (Passioura and Fry, 1992). The pores were too large for the size of the roots so less contact occurred between the roots and the soil, limiting the water continuity between the soils and the root thus impeding nutrient and water acquisition. Barley roots are usually found clustered in biopores, making it more difficult for the lateral roots to penetrate the pore wall, which has a higher strength than the bulk soil (Stirzaker *et al.*, 1996).

White and Kirkegaard (2010) found that in the topsoil, 30-40% of wheat roots grew in established pores while for the subsoil the percentage increased to 85-100%. Although the water resources were sufficient in the subsoil and the root density too, the result was a water-stressed crop. This may have been due to high physical and osmotic resistances which delay soil-root water flux in roots growing in clusters (Tardieu, 1994). Jin *et al.*, (2013) concluded that wheat did not exploit the pores at depth because the amount of roots found in pores at 1.5 m in depth was decreased to <5% from about 20% of the available pores at each depth. Increasing the amount of macropores explored by the roots is thought to be beneficial for root growth due to development of the macropore sheath. Pierret *et al.*, (1999), described as the local environment created

around the macropores by the growth of the roots differing from the bulk soil. It is thought that this macropore sheath could have effects on the growth of the roots because there is a population of bacteria and fungi growing in that special environment caused by the release of C into the soil by the roots (Stewart *et al.,* 1999).

## 1.13 Morphology of roots growing in compacted soils

Soil compaction creates a force on the axial direction of the root which creates pressure over the cell walls and prevents root elongation. The high impedance leads to a decrease in the length of the roots and a slower production of cells. Usually, under high mechanical impedance, the roots grow thicker and, in certain soils they develop more lateral roots. As soils are very heterogeneous, compensatory adjustments may be found in the roots as it has been shown with split root treatments and soil compaction (Bingham and Bengough, 2003; Tracy *et al.*, 2012). Main root axes are more affected by compaction than the lateral roots (Bingham and Bengough, 2003). However, there is evidence that roots penetrate more successfully into compacted soil when the strength is increased gradually instead of a sudden increase in strength and that the gradient of stress is more important than the magnitude of the stress (Clark *et al.*, 2008).

Mature roots and the root tip are differentially affected by compaction; a reduction in root growth rate will be greater if the pressure is over the root tip. Soil compaction affects more to the cells of the roots if they are in the fast growth rate stage and it takes longer to reach the steady growth rate stage of the cells (Croser *et al.,* 1999). If root cells are suffering from mechanical impedance at the fast cell growing period it takes also longer to recover if the impedance is removed (Bengough, 2003).

### 1.13.1 Anatomical and physiological changes of the roots growing in compacted soils

Root morphology is affected by high soil strength. In barley, the roots may change their cylindrical shape and become flattened in their transverse section (Lipiec and Hatano, 2003), which deforms the cortical and vascular cylinder. This may be a way to adapt the shape of the roots to the pores.

Usually, impeded barley roots are shorter in length but are thicker (Masle, 1992b); there is a radial expansion in the root axes. The size of the roots affects their capacity to penetrate into high bulk density soils.

Thicker roots can exert higher growth pressure (Rosolem *et al.*, 2002), and also create intense stress in front of the axially expanding root increasing the soil cracking potential (Croser *et al.*, 2000) and relieving some of the strength near the root cap. They have more resistance to bending (Materechera *et al.*, 1992). Thicker roots penetrate hard soil layers more effectively, and are better at maintaining their penetration rate in very hard soils (Materechera *et al.*, 1991).

The thickening of roots in compacted soils has been recorded for several species such as barley, wheat, lupin and pea. The growth rate is slowed under impeded soils, along with the meristematic activity (Croser *et al.*, 1999; Croser *et al.*, 2000). For lupin, the diameter of roots is more than doubled near the basal region of the root apex (Hanbury and Atwell, 2005). Roots grown into compacted soil show an increased vascular cylinder area (Lipiec *et al.*, 2012). The cortical cells enlarge preferentially radially rather than axially (Clark *et al.*, 2003); in lupin roots the epidermal cells are about 50% shorter (Hanbury and Atwell, 2005). It has been shown that the thickening of the roots comes preferentially from the enlargement of the cells, rather than a increased production of cells (Pritchard, 1994; Croser *et al.*, 2000). After mechanical impedance is applied to barley there is an increase in root osmotic pressure which creates an increase in turgor pressure provoking the swelling of the cells

and thicker roots (Pritchard, 1994). However, changes in turgor pressure are not the important response of roots to impedance (Clark *et al.,* 2003). The cell wall extensibility ($\varphi$) determines the growth of the cells. It has been observed that changes in the cell wall microfibril orientation following mechanical impedance decrease the longitudinal wall extensibility (Pritchard, 1994). This is regulated by cell wall components (Bengough *et al.,* 2006) because the turgor pressure is constant in the root tip of roots growing in soil with high strength (Bengough and Young, 1993).

If soil impedance is relieved, the roots still suffer from the effects of compaction, depending on species. For pea, the effects of impedance on growth rate last for 72 hours after the stress is relieved (Croser *et al.,* 2000; Clark *et al.,* 2003), and it was regulated by the enzymatic activity controlling the cell wall extensibility during the cell extension phase (Bengough and Young, 1993).

### 1.13.2 Additional root branching

The additional development of lateral root branches as a result of mechanical impedance may happen when the growth of the main root tip is blocked. In barley, it is more likely to occur in varieties that are genetically hairless (White and Kirkegaard, 2010). However, there are

usually compensatory adjustments involving lateral root extension in the loose soil zone, when there is impedance in the subsoil (Atwell, 1993).

### 1.13.3 Root angles

Narrow angles of root growth in relation to the vertical axis are beneficial for the growth of roots to depth, especially under drought conditions. In a comparison between different genotypes of wheat, those with an angle of spread closer to the vertical had a better penetration rate under compacted soils as the root growth is invested at depth. This beneficial response is associated with the strength of the physiological response to gravity (Jin *et al.,* 2013).

### 1.13.4 Root hairs

Root hairs are thought to be essential for the growth of the roots in compacted soils. It has been shown that for barley a non-root hair genotype was not capable of penetrating through dense soil compared to a genotype with root hairs. Although the number of seminal roots for both genotypes was the same, the length of the roots was significantly higher for the genotype with root hairs (Haling *et al.*, 2013). Root hairs serve as anchorage to the roots behind the growth zone. In addition, root hairs may help roots survive in biopores by increasing root-to-soil

contact. If the roots grow through large pores, they develop more hairs than roots in smaller ones (White and Kirkegaard, 2010).

## 1.14 Influence of water and nutrient acquisition on root growth

In soils with high impedance the amount of photosynthate required for the growth of roots is the same than in non-compacted soils because the root systems produce the same amount of root biomass. However, the translocation of carbohydrates to the roots is reduced due to a reduction in aboveground organs (Rosolem *et al.,* 2002).

The water available in the soil is largely related to the soil physical characteristics. In wet soils, the mechanical impedance decreases but plants may suffer from anoxic conditions if the air-filled pore space is less than 10% (daSilva *et al.,* 1994). In drying soils the capillary forces cause an increase in soil strength so in drought environments the mechanical impedance of the soil limits the growth of the crops more than the water stress (Cairns *et al.*, 2004; Bengough *et al.*, 2011). This depends on the type of soil.

Soil-water dynamics influence the root growth pattern through changes in root density and root length in drying soils (Kell, 2011). Wheat roots

grew in the subsoil through macropores rather than through the bulk soil because the strength in the macropores was lower. These roots also had a greater density of root hairs, possibly to improve the root-soil contact and a greater water uptake (White and Kirkegaard, 2010).

Water availability influences the growth of barley roots more negatively than the changes in soil nutrient availability. There is an increase in the root surface area when water is a limiting resource (Vègh, 1991), which icreases root: shoot ratio (Chapman *et al.*, 2012).

Crop growth and nutrient use efficiency is significantly affected by soil compaction as can be seen in the reduction of total biomass, productive tillers, grain and straw yields in barley, wheat, cotton and maize (Arvidsson, 1999; Ishaq *et al.*, 2001; Gregorich *et al.*, 2011) and also a reduction in nutrient uptake in wheat and oats (Lipiec and Stepniewski, 1995). In the study performed by Ishaq et al., 2001, compaction induced a reduction in above ground biomass associated with a reduction in the root elongation rate between 19-36% for wheat. Also a 16% reduction in nutrient use efficiency was observed due to a decreased uptake of N and P but not K, which could be derived from a reduction in soil exploration.

## 1.15 Root-shoot influenced by the subsoil

Leaf expansion is particularly sensitive to mechanical impedance of root growth. Bingham *et al.,* (2010), showed the root length and the leaf area of barley were reduced by 23% and 21% in a compacted soil, but the total biomass was not affected. In a different experiment using glass ballotini (Young *et al.*, 1997), the leaf elongation rate was reduced by 22.6% after the impedance was increased, despite a sufficient nutrient supply. In this respect both experiments showed a similar result.

Leaves are more sensitive to root mechanical impedance when they are in the early stages of their development (Masle, 1998), after that, the rate of leaf growth remains constant with smaller leaves and less development when roots suffer impedance compared with plants growing in loose soil (Passioura, 2002). The extent of the reduction depends on the proportion of the root systems that is impeded. In Monatgu's experiment (2001), using broccoli plants, the total leaf area was reduced by 54% but when compaction was localized, the reduction was only to 30%. The smaller leaf area was attributed to a reduction in the production of leaves and their reduced expansion. Mature cell size was reduced in the leaves as well as the number of leaves (Monatgu *et al.,* 2001).

There is genotypic variation in the responses of barley and wheat to root impedance (Masle, 1992a). In barley, most of these differences are associated with root growth. In general, for barley at high soil resistance, the slower leaf development is linked with faster photosynthetic assimilation rate.

## 1.16 Chemical signalling and physiology aspects

Chemical signals (phytohormonal) are the initial precursors of the first response of shoot-to-soil compaction, although if soil strength persists nutritional factors have influence too. The chemical signals come from an increase of the ABA concentration in the roots which travel to the leaves by the xylem (Jin *et al.,* 2013). There is an exception for this increase in ABA concentration, if the topsoil is watered the mechanism does not occur. There is also an increase of ethylene by roots under soil compaction (Tardieu, 1994).

It has been investigated for barley the ABA concentration in xylem of roots growing in compacted soils and related also with the growth of the leaf area and shoot dry weight. Both leaf area and shoot dry weight are reduced at a bulk density of 1.7 g $cm^{-3}$ and the xylem sap ABA is increased, meaning a reduction in stomata conductance. There is also a decrease in stomatal conductance found in those plants whose roots were

growing in compacted soils compared to non-compacted soils (Hussain *et al.*, 1999). Mulholland *et al.* (1996) found an increase in ABA xylem concentration in barley grown in compacted soil not related with a decrease in the leaf or shoot growth but related to the stomata responses.

The decrease in leaf development could be explained also as a decrease on the transpiration rate by a reduction of stomata conductance which is proportional to the reduction in growth (Masle, 1992b). The levels of reduced nitrogen indicate that the photosynthetic activity is not affected as the stomatal behaviour.

ABA is related with the control of ethylene production which inhibits the growth of the shoots and roots in plants (Sharp, 2002). It has been found an increase in ABA concentration in plants suffering diverse stresses such as limited water resources. ABA increases in concentration are usually correlated with a decrease in growth rate. But for plants suffering soil compaction the increase in ABA was not enough to interfere with the ethylene production. Ethylene, according to Mulholland *et al.* (1996) is the major chemical compound acting in the decrease of the growth of tomato plants with their roots growing in split systems, one half of the root growing in compacted soil and the other half growing in non-compacted soil.

## 1.17 Root ideotypes, in search of the perfect root system

The importance of the root systems and their role in nutrient acquisition has already been discussed. Although root systems are very plastic and have the ability to adapt to different environments, some root ideotypes have been described with the aim of improving root foraging efficiency and at the same time reduce the C costs to the plant.

The main three targets for phenotyping roots are N, P and water acquisition. The two first are important because they are the most limiting nutrients in agriculture and the main problem for phenotyping is that they do not have the same level of mobility in the soil profile. P is a non-mobile nutrient and is generally found in the top layer of the soil whereas N can be found at depth more readily (Babé *et al.*, 2012). Regarding water acquisition, it can also be a limiting factor in certain environments where for example water may be restricted to depth later in the season (Lynch, 2013).

Several ideotypes have been proposed. P acquisition would need a shallow root system (Figure 1-3 a), with high density and long root hairs (Gahoonia and Nielsen, 2004; White *et al.*, 2013) to improve the root soil contact (Gregory *et al.*, 2009) which will also help in water acquisition and decrease the risk of lodging for wheat or barley with wide root

angles and stiff roots (Crook and Ennos, 1994; Berry *et al.*, 2006), while N and water at depth would be taken up more efficiently by a deep root system (Figure 1-3 b), with narrow vertical angles between main roots to increase the exploration of the soil profile at depth (Lynch, 2013). To match these two statements the root system would have to develop a main axis which will grow vertically with lateral roots developing in almost 90$^{o}$C angles to explore the profile horizontally. The proposed deep root system would also help to get water from depth and avoid terminal drought in hostile environments (Wasson *et al.*, 2012). The root system architecture would have a high density of laterals in the topsoil and fewer laterals in the subsoil. As the main axis of the root system would be growing in dense soils, to reach further in the profile, McKenzie *et al.*, (2009) suggests that roots would need increased ability to find biopores.

The metabolic cost of the root system cannot be increased if the aim is to make better use of the resources, which is why the proposed ideotypes would have either thinner roots (Comas *et al.*, 2013) or more aerenchyma (Lynch, 2013, White *et al.*, 2013). Thinner roots can be obtained either by a decreased number of root cells or fewer cell files (Wasson *et al.*, 2012). On contrast, increasing the C allocated to the root system would be beneficial for C sequestration and $CO_2$ reduction from the atmosphere

(Kell, 2012), hence reducing the impact of the greenhouse effect caused by the accumulation of $CO_2$ on the atmosphere. According to the carbon sequestration calculator developed by the University of Manchester, one extra meter of root length in crops and grassland can reduce global atmospheric $CO_2$ about 30% from current values over a two years period (http://dbkgroup.org/carbonsequestration/rootsystem.html) Allocating more C into the root system can help to prevent lodging

However, these three phenotypes change the root hydraulic properties. A decreased number of root cells or more aerenchyma reduces water conductivity while fewer cell files will increase water conductivity due to the shorter path that water has to travel to reach the stele (Comas *et al.,* 2013). To overcome problems with water transport, Comas *et al.,* (2013) suggests a higher density of cell membrane aquaporins (proteins that transport water) and narrower xylem vessels to avoid caviation in drought environments. All these ideotypes have a focus on improving root growth by means of enhanced nutrient acquisition.

a) b)

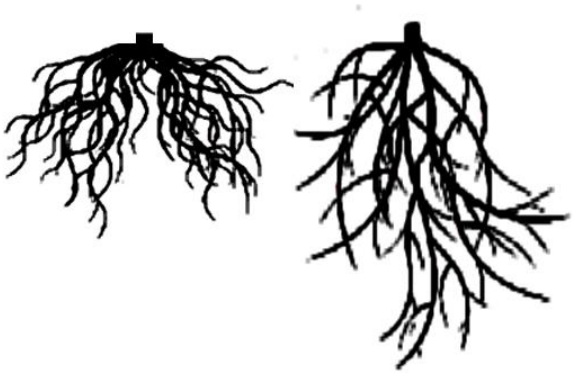

**Figure 1-3 Root phenotypes for foraging a) topsoil foraging for less mobile nutrients such as P; b) subsoil foraging for water and mobile nutrients such as N. Adapted from White *et al.,* (2013).**

Unfortunately soils present other constraints to root growth because of their physical, chemical and biological characteristics. One of the problems presented here was soils with high density which have been proven to result in roots growing thicker, with increased root hair density close to the root tip to provide anchorage for the growth of the root system and allow further growth and mucilage and border cells to be released to facilitate the growth through the soil (Bengough *et al.*, 2011; McKenzie *et al.*, 2013). More investigations are needed to further understand how these ideotypes would meet and succeed in soil and which traits are more desirable in a constraining soil physical environment.

## 1.18 Genotypic variation

Cereal species have been investigated for genotypic variation in their root systems between species and within species. They differ in their root system architectural traits. Phenotyping of the traits of interest is needed to fully understand the genetic control and environmental interactions and the mechanism by which they affect plant performance (Furbank and Tester, 2011; Fiorani and Schurr, 2013; Ghanem *et al.,* 2015). The aim of phenotyping is, ultimately, to improve breeding programs (Richards, 2006). The selected traits are matched with the identification of the genes or the regions of the genome (quantitative trait loci, QTL) with marker assisted technologies and the understanding of the interactions between the genes and the environment (Richards *et al.*, 2010). To improve crops, the selection of traits needs to be done both below ground and above ground and from cell to whole organ. Future crops will have to meet the requirements in terms of market quality, yield quantity and their survival under abiotic and biotic constraints (Sinclair *et al.,* 2004; Sinclair, 2011; Ghanem *et al.,* 2015). Phenotyping can be challenging and expensive, and it has been classified as "the bottleneck for genomic improvement" (Furbank and Tester, 2011; Fiorani and Schurr, 2013). Nevertheless, most experiments are performed in the glasshouses with artificial environments, such as soil columns, or in agar plates or

hydroponics (Gregory *et al.*, 2009; Hargreaves *et al.,* 2009) and they need to be related back to field conditions afterwards. An alternative to such methods is to select the genotypes based on aboveground traits and performance and then studying the underlying traits of the root systems (Wasson *et al.*, 2014). Nevertheless, the techniques are currently being improved and developed to produce high-throughput phenotyping methods which will potentially help in the identification of desirable traits in a fast and accurate way and faithful to the behaviour of the crops under field conditions.

Some varieties and cultivars of cereal species such as wheat, maize, sorghum or rice have had their root systems investigated under abiotic constraining environments such as dry soil conditions or nutrient scarcity (Steudle, 2000; Clark *et al.*, 2008; Manschadi *et al.*, 2008; Lipiec *et al.*, 2012; Christopher *et al.*, 2013; White *et al.,* 2015; Zurek *et al.,* 2015; Colombi and Walter, 2016). It has become apparent that some traits such as high density of root hairs are desired or roots with narrow angles and early and vigorous seedling growth was crucial in dry environments (Richard, 2015) and their QTLs are being studied. Price *et al.*, (2002) identified QTLs for root: shoot ratio, root thickness, root weight and maximum depth in rice by examining root phenotypes and growth in drought conditions. However, they also detected that the same traits

were linked to different QTLs in different parts of the genome depending on the environmental conditions suffered by the crop. This indicates that testing the new cultivars under a high range of environments is a necessity when breeding for improved crops and for a range of factors for the new cultivars to be successful.

## 1.19 Conclusions

The compaction of soils is a problem affecting soils all around the world, and most crops are susceptible to soil compaction at certain stages of their growth. The structure of the subsoil may limit the growth of the plants for different reasons such as high strength or low air permeability if it is wet. The exploration of the subsoil by the roots will certainly be of interest for breeders as it will be related to a decrease in the input of fertiliser, as well as a better use of the water available at depth.

The problem arises when limitation of roots to grow through compacted layers of the soil increases when soil gets drier. Soil compaction is the reason why roots are typically observed growing inside the macropores of the soil. Physiological changes occur in the roots in order to penetrate the subsoil and sometimes the efficiency of capture of water and nutrients is also limited, although there may be sufficient resources for them to grow. It is not only the roots of crops that are influenced by soil

compaction; shoot growth is also limited as a consequence of signalling between the roots and the shoot.

Further investigation is needed to assess the genetic variation in the ability of barley roots to explore the deep layers of the soil, and to establish the relationship between root trait expression and the soil structure and properties. In addition, the influence of improved rooting systems on the yield must be explored.

# Chapter 2: Research aims and objectives

## 2.1 Aims

To investigate genotypic variation in the ability of spring barley roots to penetrate dense soil and to determine the potential for improving root exploration and resource capture from the subsoil.

The underlying hypothesis was that root traits that improve the exploration of the soil at seedling stage are maintained for mature plants and they improve the nutrient acquisition from the deeper layer of the soil, making better use of the resources available.

## 2.2 Objectives

1. Establish a suitable and reliable screening method for barley seedlings to investigate root growth in soils presenting physical constraints.

2. Explore genetic differences in the ability of barley roots to penetrate dense soil.

3. Describe and characterise the differences between genotypes in the size and architecture of root systems measured during early plant

growth in loose and compacted soil which are associated with differences in their ability to grow and explore the subsoil.

4. Investigate and quantify the benefit of deeper rooting during plant maturity in the subsoil and the potential to improve crop growth.

## 2.3 Thesis structure

Chapter 3 aimed to develop the screening method. It investigated the optimum soil conditions to use in the subsequent screening and also the influence of seed quality on the growth response of barley roots to soil physical conditions. The hypothesis tested was that the growth rate of barley roots early after germination is reduced by seed ageing and that the effects differ with the scale of the limitations imposed by soil physical conditions. The focus of the chapter was on the first objective.

Chapter 4 is where objectives 2 and 3 are addressed. The first hypothesis was that barley genotypes will have different abilities to explore soil, with low and high density soil creating differences in root growth extension rate. The second hypothesis was that the differences in root growth and interactions with the soil treatments will be maintained at later stages of growth and key root architectural traits associated with this can be identified.

Chapter 5 targeted objective 4. The aim of the chapter was to quantify the importance of deeper rooting for nitrogen capture under realistic subsoil conditions. The first hypothesis tested if soil structure influences root extension in the subsoil and consequently the ability of the root system to take up nitrogen from depth. The second hypothesis tested if cultivars with a greater rate of root extension and deeper root system will explore the soil matrix to a greater extent and uptake more N from the subsoil than the cultivars with a slower growing and shallower root system.

# Chapter 3: Development of a high throughput screening technique for seedling root growth in soil: the importance of seed quality

## 3.1 Introduction

It has been estimated that the yields of cereals will need to increase by 70-100% (Godfray *et al.*, 2010) from current values by 2050 in order to meet the rising demand from an expanding global population. This will require major improvements in the resource use efficiency of agricultural systems, because the yield increases must be achieved without correspondingly large increases in water use and applications of fertiliser. High inputs of nitrogenous fertiliser can cause major problems to the environment such as greenhouse gas emissions and contamination of water courses (Tilman *et al.*, 2011). Water is an increasingly scarce resource and agriculture must compete with other users for access to limited supplies (Vörösmarty *et al.*, 2010). Promoting the growth of deeper and more efficient root systems could help towards the goal of greater resource capture as roots would explore a larger soil volume thereby increasing the crop's access to subsoil water and nutrient supplies (Passioura, 1992; Kirkegaard and Hunt, 2010; Lynch, 2015). The

potential benefits of increased root growth in the subsoil have been quantified in a number of modelling studies (King *et al.*, 2003; Manschadi *et al.*, 2006; Jin *et al.*, 2015). There is also experimental evidence that resource capture might be improved if root growth were increased in deep soil layers (Wiesler and Horst, 1994; Price *et al.*, 2002; Palta *et al.*, 2011; Lynch and Wojciechowski, 2015).

Although soil management techniques, including cultivations and soil amendments, can be used to manipulate root growth in the topsoil, there would appear to be relatively little scope for directly modifying conditions in the subsoil (Berisso *et al.*, 2012). Thus, breeding for specific root characteristics offers the best opportunity for modifying root architecture and promoting root growth in deeper soil layers (White *et al.,* 2013). Several root architecture ideotypes have been proposed to improve resource capture from soil, but these can differ depending on the resource(s) of primary interest leading to potential trade-offs in root system design. Thus, for water and relatively mobile nutrients such as nitrate in leaching environments, deep root systems with main axes growing at small angles from the vertical and laterals growing at 90$^{o}$ from the main axes are considered desirable to improve soil exploration (Lynch, 2013; White *et al.,* 2013). By contrast for less mobile nutrients such as phosphate and manganese, root traits associated with topsoil foraging

appear more appropriate, such as main axes with large growth angles from the vertical and extensive branching (Haling *et al.,* 2013; White *et al.,* 2013; Lynch, 2015).

Within a range of crop species variation exists in a number of root morphological traits that could be exploited in breeding (Richards *et al.,* 2010). The major obstacle to progress is the lack of suitable techniques for rapidly screening large numbers of genotypes. Seedling screens are attractive because they facilitate high throughput measurements on roots at relatively low cost (Price *et al.,* 2002; Watt *et al.,* 2013). However, many of the screening methods reported to date have used growth media (e.g. hydroponics, paper towels, agar plates) that fail to replicate the physical conditions a root encounters in soil (Bengough *et al.*, 2004; Wasson *et al.*, 2012). This may explain why attempts to correlate variation in root growth in seedling screens with crop yield or root growth in field plots have had mixed success (Price *et al.,* 2002; Watt *et al.,* 2013). It is essential, therefore, to measure root growth under realistic soil conditions and to understand what the main constraints are in the target environments for the improved varieties (Cairns *et al.*, 2004). For many soils the physical conditions are the greatest constraint to growth, particularly at depth. Many European soils have compacted subsoils (Jones *et al.,* 2003). If dense soils are dry, root growth could be limited by the lack of water or

by high soil strength; in drying soils, soil strength can increase more rapidly than the soil moisture deficit (Cairns *et al.*, 2004). On the other hand, if dense soils are wet, roots could suffer from hypoxic conditions (Bengough *et al.*, 2006).

This work aims to develop a high throughput screening method for identifying variation in the ability of barley roots to penetrate dense soil under different physical stress conditions. Not only must the screening method try mimic realistic root environments, it must also be capable of identifying heritable differences in desirable root traits and not be confounded by other environmental factors. Typically, phenotypic screens involve testing a large number of diverse genotypes so it is common to source seed from different places, and hence seed lots, that may differ in their production environment and storage history. It is well established that seed quality declines during storage and that this leads to a reduction in vigour prior to a loss of viability (Matthews, 1985; Bernal-Lugo and Leopold, 1998). Ageing delays the onset and rate of germination following imbibition (Powell and Matthews, 2005), but there is some evidence that the subsequent rate of root growth may also be reduced. Thus in maize, the rate of primary root extension was lower following germination of artificially aged compared to unaged seed; an effect that was associated with a shortening of the root growth zone and

reductions in both the rate of cell production and expansion (Bingham and Merritt, 1999). If this is also the case for barley and other species, it will have important consequences for the selection of seed for use in experiments designed to quantify genetic variation in seedling root traits.

The objective of the experiment was to determine the influence of seed quality on the growth response of barley roots to soil physical conditions. The hypothesis tested was that the growth rate of barley roots early after germination is reduced by seed ageing and that the effects differ with the scale of the limitations imposed by soil physical conditions.

## 3.2 Materials and methods

### 3.2.1 Seed morphological characteristics and germination

Experiments were conducted in 2014 on two cultivars of two- row spring barley (*Hordeum vulgare* L. cvs. Vada and Kenia). Vada is a cultivar bred by the Institute of Agricultural Plant Breeding in Wageningen (The Netherlands) and registered in 1956. It is a cross between Hordeum laevigatum x Goudgerst. It has a short and stiff straw and some resistance to mildew. It has a slow development on spring and develops a high number of tillers improving the production (Dros, 1957). Kenia is a cultivar bred by Guiness and released to the market on 1931. Kenia

presents a short and fine straw with low weight grain and higher N content than other cultivars used for malting. At the time of release it was very favoured by growers because of high yields and it was also used for crossings with Scandinavian cultivars (Horne, 1952). The seeds were produced at the James Hutton Institute (Scotland) in field multiplication plots in 2011 and 2013. After harvest, they were dried to approximately 13% moisture content and stored. In 2011 seeds were stored initially in cotton bags in an unheated grain store for four months over winter. A subsample of seeds was then sealed in polyethylene bags and subsequently stored at 4$^{o}$C until the beginning of the experiment. In 2013, seeds were stored in polythene bags at 4$^{o}$C immediately after drying. Thus, the seed lots produced in 2011 and 2013 differed in their maternal environment as well as in storage time. Seed morphology was characterized by weighing individual seeds from three replicates of 20 seeds selected at random from each lot and scanning them afterwards in an Epson Expression 836XL scanner (Regent Instruments Inc., Canada). Seeds were set over a transparent PVC tray and covered with a blue plastic sheet. Three images were obtained, each of them containing a replicate of the 20 weighed seeds of the two barley cultivars and the two harvest years. Scans were made at a resolution of 300 dpi and stored in TIFF file format. Scans were later analysed using Fiji ImageJ software

(Schindelin *et al.*, 2012). A cut of the first image was used to create the Simple Interactive Object Extraction (SIOX) (Friedland, 2006) marking the seeds as the regions of interest or foreground and the blue colour as background. SIOX created a binary image in which the seeds were differentiated from the background. Then the SIOX was applied to the three images and they were segmented. The segmented images were used for the analysis of projected area, perimeter, length and width.

Germination characteristics of the seed lots were assessed by making modifications to germination temperature and number of seeds per replicate from the standard International Seed Testing Association procedures (Hampton and Tekrony, 1995). Three replicates of 50 seeds for each cultivar and each harvest year were placed between two layers of moistened tissue paper (Tork, Basic paper 1 Ply, dimensions 35 x 19.4 cm, SCA Hygiene Products, Dunstable, Bedfordshire) and then rolled into a cylinder. The outer surface of the rolled paper was covered with aluminium foil to exclude light from the seed and developing root and to help to maintain the roll in an upright position. The cylinder roll was placed on one end in an upright position inside a 250 ml beaker with its lower-most end immersed in a 25 ml reservoir of water. The embryo of the seed was facing downwards to allow the root to grow in a vertical direction. The cylinder rolls were placed in a controlled environment

cabinet at 15°C with 16:8 h light:dark photoperiod; irradiance at the height of columns was 121 μmol $m^{-2}$ $sec^{-1}$ PAR (photosynthetically active radiation) supplied by 15 fluorescent lamps (Sylvania GRO-LUX®, OSRAM Sylvania, Westfield, USA). Seeds were examined every 24 hours over the course of a week and counted as germinated when the radicle had reached a length of at least 2 mm; abnormal seedlings were not included in the count. Mean germination time was calculated as in Ellis and Roberts, 1980 following the formula:

$$\boldsymbol{MGT = \sum n * H / \sum n} \quad \textbf{(Equation 3-1)}$$

with H being the number of hours from the beginning of the experiment, n the number of newly germinated seeds and $\sum n$ final germination.

### 3.2.2 Artificial ageing

Seeds of spring barley cvs. Kenia and Vada harvested in 2013 were subjected to controlled deterioration following the methods described by Powell and Matthews, (1981). Samples of seed were weighed to 0.1 mg, oven dried at 105°C for 48 h and reweighed to determine their initial moisture content. Further samples were taken from the same seed lots divided into batches of 200 seeds and placed on moistened tissue paper until they reached a moisture content of 20%. This was achieved by repeatedly weighing the seeds until the desired moisture content had

been reached. The seeds were then sealed in plastic bags and stored at 4°C overnight to allow the moisture to distribute through the tissues. The seeds, still sealed in their bags, were then submerged in a water bath at 45°C for defined periods of time which differed for the two cultivars as pilot experiments suggested that Kenia deteriorated more rapidly than Vada. The incubation times for Kenia were 0; 48; 60; 66 and 72 h and for Vada 0; 72; 96; 108 and 120 h. Finally the seeds were air dried at room temperature to their initial moisture content and stored in polythene bags at 4°C for up to three weeks.

### 3.2.3 Soil columns and physical characteristics

Soil was collected from Boghall farm, SRUC, Penicuik, Scotland (latitude 55.876, longitude -3.203). The soil used was a Cambisol (FAO classification), a typical sandy clay loam from the MacMerry series (Soil Information for Scottish Soils, http://sifss.hutton.ac.uk/SSKIB_Stats.php). The soil was taken from the topsoil (0-20 cm). The soil was air dried and sieved to 2 mm. The gravimetric moisture content (gMC) of the soil was measured (Rowell, 1994) and then raised up to 20% or 26% gMC in polythene bags with 3 kg of soil. The bags were stored overnight at 4°C to allow the moisture to distribute homogeneously through the soil. Then the soil was packed into opaque PVC columns of 5 cm (inner diameter)

and 10.5 cm length. The lower end of the columns was covered with a polyester fabric mesh of 0.25 mm to prevent the roots growing through but allowing aeration. The soil columns were packed at 5 soil dry bulk densities (1.1; 1.2; 1.3; 1.4; 1.5 g $cm^{-3}$) for each gMC to simulate a wide range of soil conditions. The columns were packed with a manual press in 2.5 cm layers. After packing each layer the surface was scarified (Lewis and Sjöstrom, 2010) to minimise discontinuities between layers. Water release curves of packed columns were determined using tension tables and pressure plates following the method stated by Ball and Hunter (1988) at matric potentials of 0, -1, -6, -10, -20, -100, -200 and -1500 kPa. Available water (difference between water volume and water volume at wilting point) and air-filled porosity (AFP) for each condition in the soil columns were calculated from the water release curves. Penetration resistance was determined using the penetrometer 5966 Dual Column for Mechanical Testing (Instron, Illinois Tool Works Inc. ITW, UK.) to define the mechanical impedance to root growth in the five different dry bulk densities (BD) stated above at both 20% gMC and 26% gMC. The diameter of the tip of the penetrometer was 0.95 mm, the length of the tip was 1.7 mm, the angle of the cone $32.45^0$ and the shaft diameter just behind the cone was 0.75 mm. The probe was inserted into the soil to a

maximum depth of 15 mm in a period of 4 minutes. Three replicate columns and two individual measures per replicate were determined.

### 3.2.4 Determination of root growth rate

Seeds were germinated on moist paper towels under controlled environment conditions as described above for the germination test. Naturally aged and artificially aged seeds were set to imbibe 8-10 h earlier than new seed so that old and new seeds germinated and reached the appropriate root length at approximately the same time (*c.* 48-60 h after set up for germination). Germinated seeds whose radicle (primary root) reached a length of 15 – 20 mm were selected for use. The lengths of the primary roots were measured to the nearest millimetre prior to sowing. Two holes per column were made in the soil by twisting the tip of a glass Pasteur pipette into the soil and removing a plug of soil. The holes were slightly deeper than the length of the primary roots and had a diameter between 1 and 2 mm. Two seedlings were transplanted per column with their roots in each of the holes. The upper few mm of each hole was conical in shape allowing the seed to sit in a recess below the soil surface. In the high bulk density treatments, the dense soil forming the wall of this recess prevented later emerging roots from growing laterally along the soil surface. The seeds were then covered with a

further 5 mm deep layer of soil at the same water content as that in the column. Finally, the columns were covered with a paraffin wax sealing film (Parafilm M®, Bemis, Llansamlet, UK) to minimise the loss of water but facilitate some air diffusion (www.parafilm.com) and they were punctured with the tip of a glass Pasteur pipette in the centre to allow the shoots to grow through. The columns were placed randomly in a controlled environment cabinet under the same conditions as for the germination of the seeds. After four days of growth, the columns were taken out of the cabinet and the soil washed from the roots in a tray of water. The length of the longest primary root was measured to the closest mm. The root growth rate was calculated as:

$$\frac{\boldsymbol{Final\ root\ length - initial\ root\ length)\ (mm)}}{\boldsymbol{hours\ in\ the\ column\ (96\ h)}.} \quad \textbf{(Equation 3-2)}$$

The experiment was conducted 6 times with a single replicate column of each combination of cultivar x seed age x soil BD x MC on each occasion.

### 3.2.5 Experimental design and statistical analysis

Two-way analysis of variance (ANOVA) was performed to compare the morphological differences in area and weight of the seed lots. The final seed germination proportion was transformed using an arcsine square root function prior to statistical analysis. Two-way ANOVA was used to test if there were differences in the germination percentage between

cultivars and seed lot and assess differences in the controlled deterioration treatments for each of the cultivars. In order to evaluate the initial seed quality of the cultivars harvested in 2013, Ki value was calculated (Ellis and Roberts, 1980). This involved performing probit analysis on the final germination percentage and fitting a linear regression to the subsequent values to obtain the Ki value for each cultivar, which corresponded to the intersection between the y-axis and the fitted line for each cultivar. Linear regression by groups was used to compare the intercepts of the two slopes.

For the root growth experiments, each run of the experiment was considered a block. On each occasion individual seed age, soil treatment and cultivar combinations were completely randomized in the growth cabinet (i.e. in each block). The experiment was, therefore, analysed as a randomised block design with 6 blocks. A four factor ANOVA was used to investigate the differences in root growth created by the treatments and interactions between the treatments. The factors considered were the BD of the soil, the soil gMC, the age of seeds (or seed lot) and the cultivar. All data were checked for a normal distribution of residuals and homogeneity of variance prior to analysis. The initial length of primary root of each seedling was used as a covariate because it was found to be significantly different for the cultivars and it was used to reduce seed

selection bias. All the statistical analyses were performed with Genstat 16 (VSN International Ltd, Hemel Hempstead, UK.).

## 3.3 Results

### 3.3.1 Seed -morphology and ability to germinate

Seed measurements differed between harvest years for all measures except seed length and width (Table 3-1). Averaged across varieties, seeds harvested in 2011 (old seed) were smaller (10% smaller in perimeter and 11% in projected area) and had 13% smaller average dry weight than seed harvested in 2013 (young seed). There were also significant differences in size and morphology of seeds between varieties (Table 3-1). Seeds of cv. Vada had a larger perimeter, length, width, length to width ratio, projected area and dry weight than those of cv. Kenia. With the exception of perimeter, the cultivar x harvest year interaction was weak (Table 3-1). Thus, the size of Kenia and Vada seed differed to a comparable extent between years.

Seed viability differed between harvest years (Figure 3-1); differences in the proportion of seeds that germinated were detected (Table 3-2). For both varieties, young seeds germinated faster than old seeds (smaller mean germination time, MGT) (Table 3-2), and had a final germination

above 95%, while old seeds were below 80% (Figure 3-1). The germination percentage and MGT (h) of the seeds for each cultivar harvested in 2013 were similar, but there were differences between varieties harvested in 2011. Old seeds of cv. Kenia showed a more pronounced loss of quality during storage, having a final germination percentage after seven days of 19% lower compared to Vada (Figure 3-1).

**Table 3-1 Mean values and Two-way ANOVA results (df=1, F-value, P-value and LSD 5%) of seed morphology variables.**

| | Cultivar | Harvest year | Mean | | Cultivar | Harvest year | Interaction |
|---|---|---|---|---|---|---|---|
| Projected area | Vada | 2011 | 0.236 | F-value | 62.51 | 9.05 | 3.56 |
| $cm^2$ | | 2013 | 0.242 | P-value | <0.001 | 0.004 | 0.063 |
| | Kenia | 2011 | 0.186 | LSD 5% | 0.0102 | 0.0102 | 0.0145 |
| | | 2013 | 0.211 | | | | |
| Perimeter | Vada | 2011 | 2.100 | F-value | 92.45 | 19.77 | 4.93 |
| cm | | 2013 | 2.162 | P-value | <0.001 | <0.001 | 0.029 |
| | Kenia | 2011 | 1.783 | LSD 5% | 0.0531 | 0.0531 | 0.0378 |
| | | 2013 | 1.964 | | | | |
| Length | Vada | 2011 | 0.833 | F-value | 104.48 | 25.56 | 3.3 |
| cm | | 2013 | 0.870 | P-value | <0.001 | <0.001 | 0.073 |
| | Kenia | 2011 | 0.697 | LSD 5% | 0.0233 | 0.0233 | 0.0159 |
| | | 2013 | 0.775 | | | | |
| Width | Vada | 2011 | 0.360 | F-value | 9.49 | 0.01 | 1.93 |
| cm | | 2013 | 0.354 | P-value | <0.001 | 0.0159 | 0.168 |
| | Kenia | 2011 | 0.339 | LSD 5% | 0.0159 | 0.936 | 0.0316 |
| | | 2013 | 0.346 | | | | |
| Length: Width | Vada | 2011 | 2.31 | F-value | 47.96 | 22.64 | 0.17 |
| $cm\ cm^{-1}$ | | 2013 | 2.46 | P-value | <0.001 | <0.001 | 0.68 |
| | Kenia | 2011 | 2.07 | LSD 5% | 0.047 | 0.047 | 0.093 |
| | | 2013 | 2.24 | | | | |
| Weight | Vada | 2011 | 49.8 | F-value | 28.42 | 5.57 | 3.11 |
| mg | | 2013 | 50.9 | P-value | <0.001 | 0.021 | 0.082 |
| | Kenia | 2011 | 38.3 | LSD 5% | 2.30 | 2.30 | 4.583 |
| | | 2013 | 45.0 | | | | |

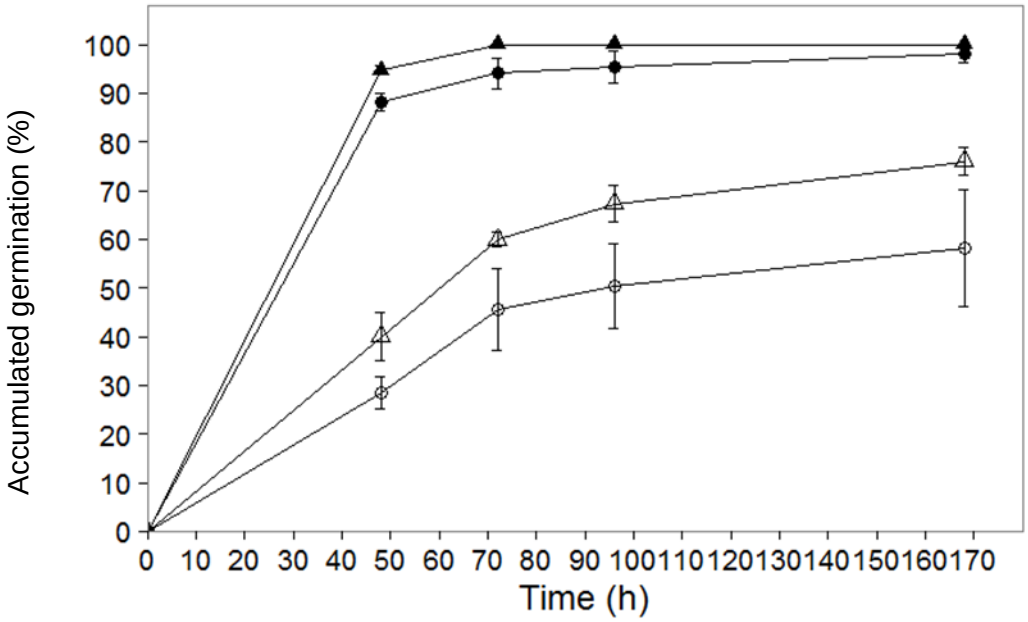


**Figure 3-1. Accumulated germination (%) over time (h) for different seed batches- Seeds harvested in 2011 (open symbols) and the seeds harvested in 2013 (closed symbols); cultivar Vada (triangles) and Kenia (circles). Values are means of accumulated germination, three replicates of 50 seeds ± SEM (shown when larger than symbols).**

**Table 3-2 Mean values and Two-way ANOVA results (df=1, F-value, P-value and LSD 5%) of mean germination time (MGT) for the cultivars at the two harvest years.**

| | Cultivar | Harvest year | MGT (h) |
|---|---|---|---|
| | Vada | 2013 | 30.2 |
| | | 2011 | 46.8 |
| | Kenia | 2013 | 29.4 |
| | | 2011 | 60.6 |
| | | | Interaction |
| F-value | 4 | 54.65 | 5.05 |
| P-value | 0.093 | <0.001 | 0.066 |
| LSD 5% | 7.92 | 7.92 | 11.23 |

### 3.3.2 Controlled deterioration

The seeds of both varieties harvested in 2013 and subjected to controlled deterioration suffered from a lower final germination percentage and an increase in MGT (Figure 3-2, Figure 3-3, Table 3-3). The extent of the changes depended on the severity of the ageing treatment imposed (period of incubation at 45°C). However, cv Kenia was more sensitive to controlled deterioration than Vada, Kenia suffered from a lower initial seed quality (Ki) compared to Vada (Table 3-3 and Figure 3-4). Kenia (Figure 3-2) showed a steady decrease in germination percentage with increasing incubation up to 72 h. The MGT showed similar changes, increasing 6.75 h between 0 h and 72 h of controlled deterioration. The same pattern was followed by Vada subjected to controlled deterioration. Vada for 120 h compared to the control (0 h), reduced the percentage germination a 5% and increased MGT 15.34 h (Figure 3-3).

**Table 3-3 Mean values two way ANOVA (F-value, P-value and LSD 5%, df $_{controlled\ deterioration}$=4, df $_{cultivar}$=1, df $_{interaction}$=4) results for mean germination time (MGT (h)). Ki values for each cultivar are also shown.**

| Cultivar | Controlled deterioration (h) | MGT (h) | Ki (probit transformation) |
|---|---|---|---|
| Vada | 0 | 31.02 | 7.01 |
| | 72 | 41.07 | |
| | 96 | 39.60 | |
| | 108 | 44.12 | |
| | 120 | 46.36 | |
| Kenia | 0 | 33.75 | 6.6 |
| | 48 | 37.94 | |
| | 60 | 39.17 | |
| | 66 | 40.44 | |
| | 72 | 40.49 | |
| | Vada | Kenia | Ki cultivar linear regression comparison |
| F- value | 10.64 | 116.63 | |
| P-value | 0.003 | <0.001 | 0.006 |
| LSD 5% | 5.884 | 5.233 | std error=0.134 |

Seeds of cv Vada deteriorated for 120 h were used for the root growth experiment because this treatment gave a MGT (46 h) comparable to that found with naturally aged seed (harvest year 2011) (Table 3-2 and Table 3-3), even though the final germination percentage was greater. For Kenia, seed deteriorated for 72 h was used. This gave a lower MGT of 8 h and greater final percentage germination than those observed with naturally aged seed, but was the longest period of deterioration available. A seed lot that had been aged for longer (96 h) showed an unexpectedly low viability and thus was not suitable for use. Subsequent tests indicated that the abnormally severe deterioration observed with

this treatment was likely to be the result of experimental error, possibly caused by seed moisture content above the set point (data not shown).

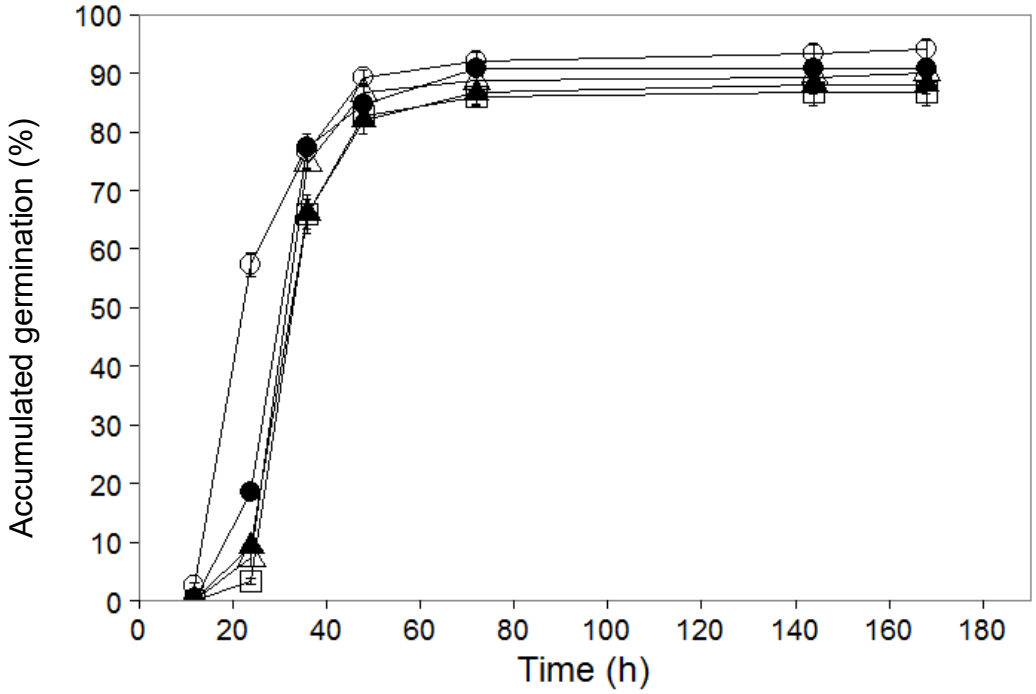


**Figure 3-2 Accumulated germination (%) over time (h) for cultivar Kenia subjected to controlled deterioration. Control (open circles), 48 h (closed circles), 60 h (open triangles), 66 h (closed triangles), 72 h (open squares). Values are percentage of accumulated germination for 3 replicates of 50 seeds ± SEM (when larger than symbol).**

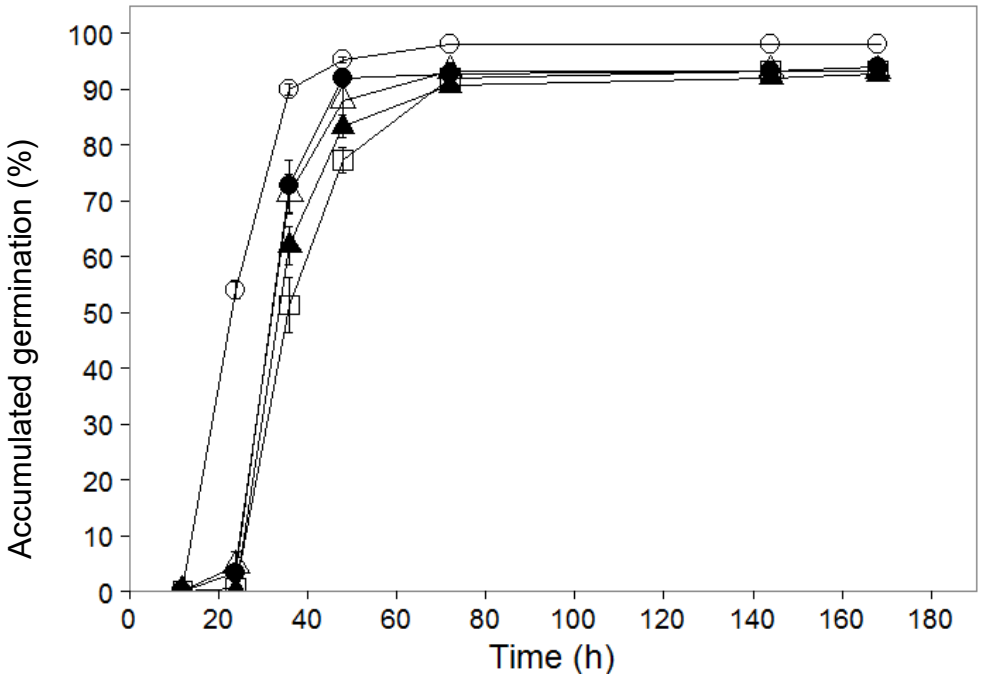


**Figure 3-3 Accumulated germination (%) over time (h) for cultivar Vada subjected to controlled deterioration. Control (open circles), 72 h (closed circles), 96 h (open triangles), 108 h (closed triangles) and 120 h (open squares). Values are percentage of accumulated germination for 3 replicates of 50 seeds ± SEM (when larger than symbol).**

### 3.3.3 Soil characteristics

The soil physical conditions (Table 3-4) varied with each soil bulk density and at each moisture content. According to the concept of the least limiting water range (daSilva et al., 1994), the best condition for root growth was expected to be the lowest BD (1.1 g $cm^{-3}$) and highest MC (26% w $w^{-1}$). For those soil conditions the matric potential was -20 kPa, soil strength was low (0.06 MPa), air-filled porosity was high (30% v $v^{-1}$) and the water availability was sufficient (14% v $v^{-1}$). The most limiting

soil conditions were associated with the highly-compacted soil at each moisture content. However, the restriction experienced by the roots in the compacted treatments is likely to have differed according to the moisture conditions. At high dry bulk density (1.5 g $cm^{-3}$) and low gravimetric moisture content (20%) the soil strength was 4.08 MPa and 13% air-filled porosity. However, at the higher moisture content (26%) soil strength was low (0.27 MPa), but air-filled porosity was also very low (4%). At each moisture content the matric potential increased with increasing bulk density, but the available water content changed relatively little. At high moisture content, the available water ranged from 13% to 16%. At low moisture content, it was between 7% and 8% over the bulk density range 1.1 - 1.4 cm $cm^{-3}$ and fell to 4% only in the most compacted soil.

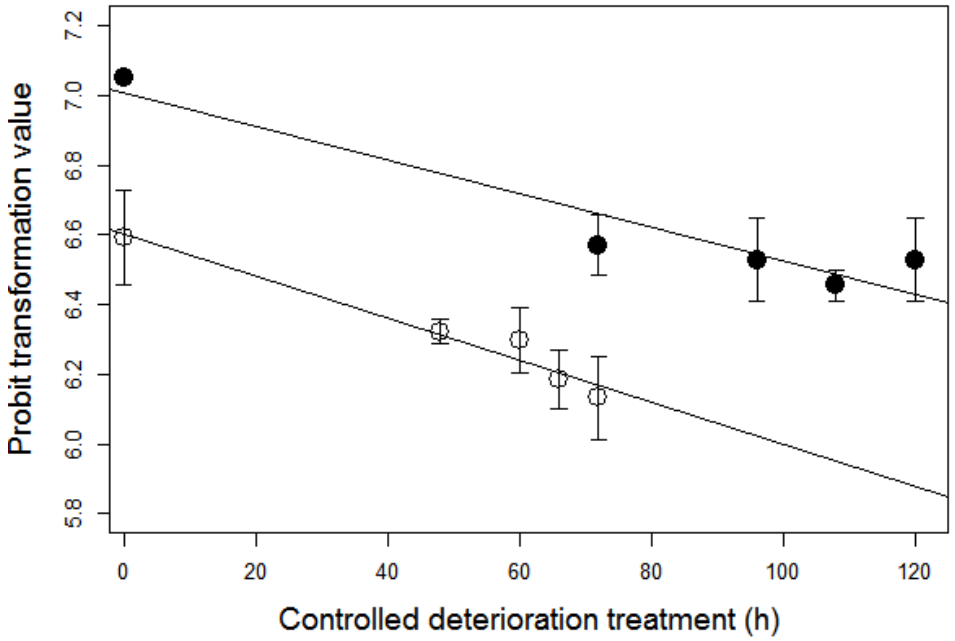


**Figure 3-4. Probit transformation of germination % after each controlled deterioration treatment (h) with fitted linear regression for cv. Kenia (open circles) and cv. Vada (closed circles). Values are percentage of accumulated germination for 3 replicates of 50 seeds ± SEM.**

**Table 3-4 Soil physical characteristics for each soil dry bulk density (BD) and each soil moisture content (gMC) treatments.**

| BD (g cm-$^{3}$) | gMC (%) | vMC (%) | Matric potential (kPa) | vMC at wilting point (-1.5 MPa) (%) | Pore volume (%) | AFP (%) | Available water (%) | Soil strength (MPa) |
|---|---|---|---|---|---|---|---|---|
| 1.1 | 20 | 22 | -120 | 15 | 58 | 36 | 7 | 0.21 |
| | 26 | 29 | -20 | | | 30 | 14 | 0.06 |
| 1.2 | 20 | 24 | -100 | 17 | 55 | 31 | 7 | 0.62 |
| | 26 | 31 | -15 | | | 24 | 14 | 0.07 |
| 1.3 | 20 | 26 | -40 | 18 | 51 | 25 | 8 | 1.11 |
| | 26 | 34 | -6 | | | 17 | 16 | 0.09 |
| 1.4 | 20 | 28 | -50 | 21 | 47 | 19 | 7 | 1.86 |
| | 26 | 36 | -4 | | | 11 | 15 | 0.18 |
| 1.5 | 20 | 30 | -20 | 26 | 43 | 13 | 4 | 4.08 |
| | 26 | 39 | -3 | | | 4 | 13 | 0.27 |

### 3.3.4 Root growth

There was a highly significant effect of dry BD, soil gMC and seed age on rates of root extension in the two experiments examining i) naturally aged seed (2013 and 2011 harvests) and ii) 2013 seed subject to controlled deterioration (Table 3-5). In addition, there were significant interactions in each experiment between dry BD and soil gMC plus cultivar and seed age. The three-way interaction between dry BD, soil gMC and seed age was only significant (Table 3-5) for artificially aged seed because the extension rate at 1.2 g $cm^{-3}$ and 20% gMC was reduced for the non-aged seeds compared to the aged seeds. For a small number of other treatments or treatment combinations significant effects were found in one experiment, but not the other. As the main interest was in the effects of seed ageing on the root growth response to soil physical conditions, and because cultivar showed no interaction with dry BD, soil gMC or their combination, the following description focusses on the effects of dry BD, soil gMC and seed age on rates of root growth averaged across cultivars.

Soil characteristics significantly influenced root extension rate. However, the main characteristic affecting root growth in both experiments was soil dry BD (Figure 3-5 a and; Figure 3-5 b). Root extension rate decreased

when dry BD increased for each gMC investigated. Increasing dry BD from 1.1 to 1.5 g $cm^{-3}$ reduced root extension rate by around 30 to 50%. At each moisture content and in each experiment, the decline tended to be greatest at bulk densities higher than 1.2 g $cm^{-3}$. Soil gMC also had a significant effect on the growth rate. At a given BD, the growth of roots was faster under the high gMC treatment. However, there was a significant interaction between dry BD and gMC for each type of seed ageing. Thus root growth rate was reduced more by the combination of high dry BD and low soil gMC than high dry BD and high soil gMC (Table 3-5).

Age of the seeds had a significant effect on root extension rate (Table 3-5). When averaged over variety, bulk density and moisture content treatments, natural ageing reduced the rate by 5% and controlled deterioration by 13% relative to their respective controls (data not shown). Cultivar had a significant overall effect on root growth rate only for seeds subjected to controlled deterioration. Vada generally had a slower growth rate than Kenia for both deteriorated and non-deteriorated seeds, the difference being particularly large for the deteriorated seeds (Figure 3-6) thus giving a significant cultivar x age interaction (Table 3-5). By contrast, for naturally aged seeds Vada had a faster root growth rate than Kenia when germinated from old seed, but

a slower rate when germinated from young seed. This gave rise to a significant cultivar by age interaction (Table 3-5), but no overall effect of cultivar in the experiment with naturally aged seed.

**Table 3-5 F-values, P-values and degrees of freedom (df) for analysis of variance for root extension rate in the different soil treatments (dry bulk density (BD) and soil moisture content (gMC)) for seedlings of the two cultivars (cv) with decreased quality (age) due to natural deteriotation because of storage.. Analysis included the seedlings of the two cultivars (cv) with decreased quality (age), using seed that had been natural aged or subject to controlled deterioration.**

| | df | F-naturally aged | P-Naturally aged |
|---|---|---|---|
| BD | 4 | 118.66 | <.001 |
| MC | 1 | 50.45 | <.001 |
| cv | 1 | 1.57 | 0.215 |
| age | 1 | 13.05 | <.001 |
| BD.MC | 4 | 3.09 | 0.016 |
| BD.cv | 4 | 0.39 | 0.819 |
| MC.cv | 1 | 1.19 | 0.277 |
| BD.age | 4 | 1.21 | 0.306 |
| MC.age | 1 | 1.43 | 0.233 |
| cv.age | 1 | 3.90 | 0.049 |
| BD.MC.cv | 4 | 0.29 | 0.888 |
| BD.MC.age | 4 | 2.16 | 0.073 |
| BD.cv.age | 4 | 0.56 | 0.689 |
| MC.cv.age | 1 | 4.40 | 0.037 |
| Covariate | 1 | 4.15 | 0.042 |

**Table 3-6 F-values, P-values and degrees of freedom (df) for analysis of variance for root extension rate in the different soil treatments (dry bulk density (BD) and soil moisture content (gMC)) for seedlings of the two cultivars (cv) with decreased quality (age) due to natural deteriotation because of storage.. Analysis included the seedlings of the two cultivars (cv) with decreased quality (age), using seed that had been subject edto controlled deterioration.**

| | df | F-controlled deterioration | P-Controlled deterioration |
|---|---|---|---|
| BD | 4 | 73.35 | <.001 |
| MC | 1 | 164.09 | <.001 |
| cv | 1 | 31.23 | <.001 |
| age | 1 | 41.37 | <.001 |
| BD.MC | 4 | 3.82 | 0.005 |
| BD.cv | 4 | 0.46 | 0.771 |
| MC.cv | 1 | 1.96 | 0.524 |
| BD.age | 4 | 4.95 | <.001 |
| MC.age | 1 | 2.66 | 0.104 |
| cv.age | 1 | 5.8 | 0.017 |
| BD.MC.cv | 4 | 1.73 | 0.143 |
| BD.MC.age | 4 | 5.6 | <.001 |
| BD.cv.age | 4 | 0.92 | 0.45 |
| MC.cv.age | 1 | 0 | 0.954 |
| Covariate | 1 | 9.76 | <.001 |

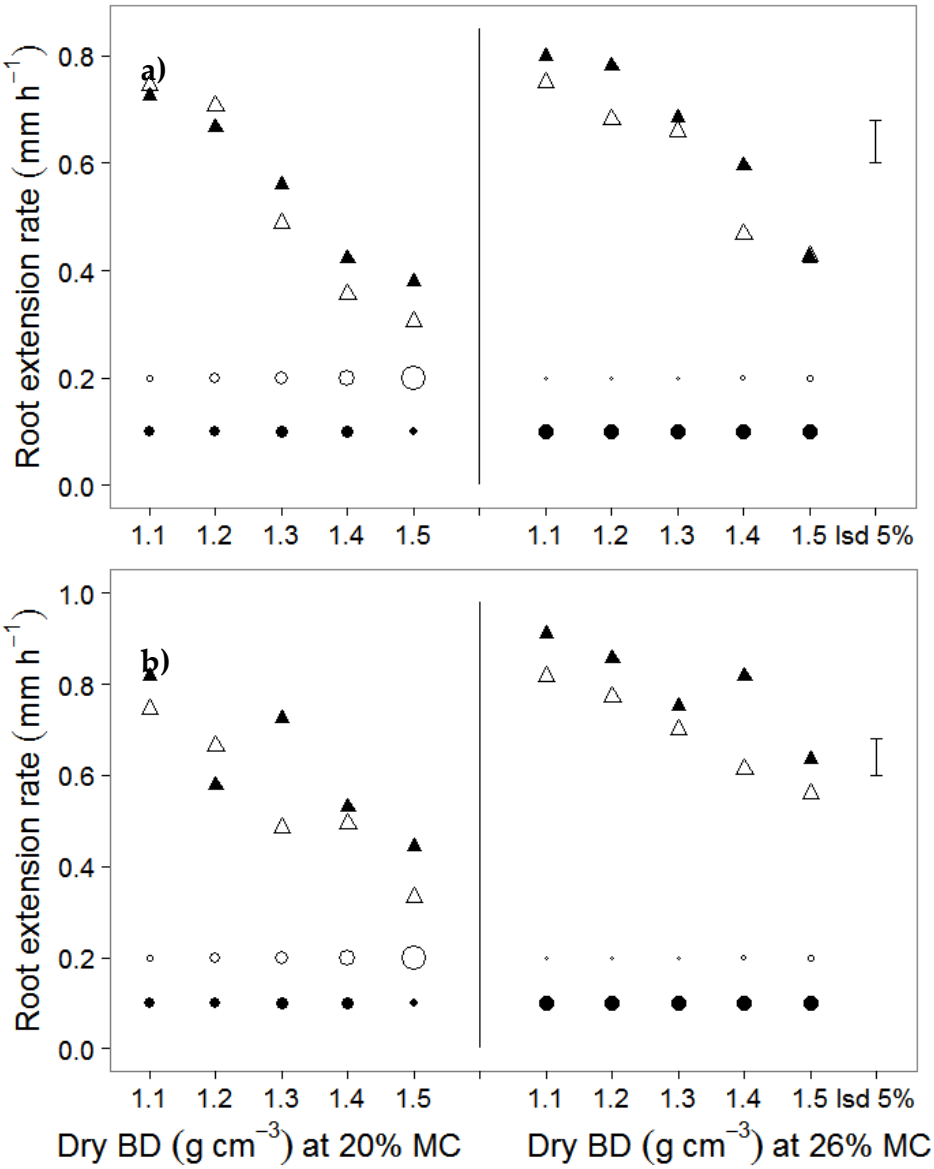


**Figure 3-5 Mean extension rate (mm h$^{-1}$) of primary roots a) from naturally aged seeds b) from seeds subjected to controlled deterioration –closed triangles are a) young (2013) and b) control and open triangles are a) old (2011 seeds) b) artificially aged- in the different soil treatments. Error bar shows LSD of soil treatment*seed quality (5%). Bubbles show soil physical limitations for root growth. Filled bubbles represent AFP and it decrease with soil BD for both gMC. Open bubbles represent soil strength and it increase with BD, having a large increase at 20% gMC. Size of the bubbles is scaled to the soil parameters. Values are means of n=12 samples.**

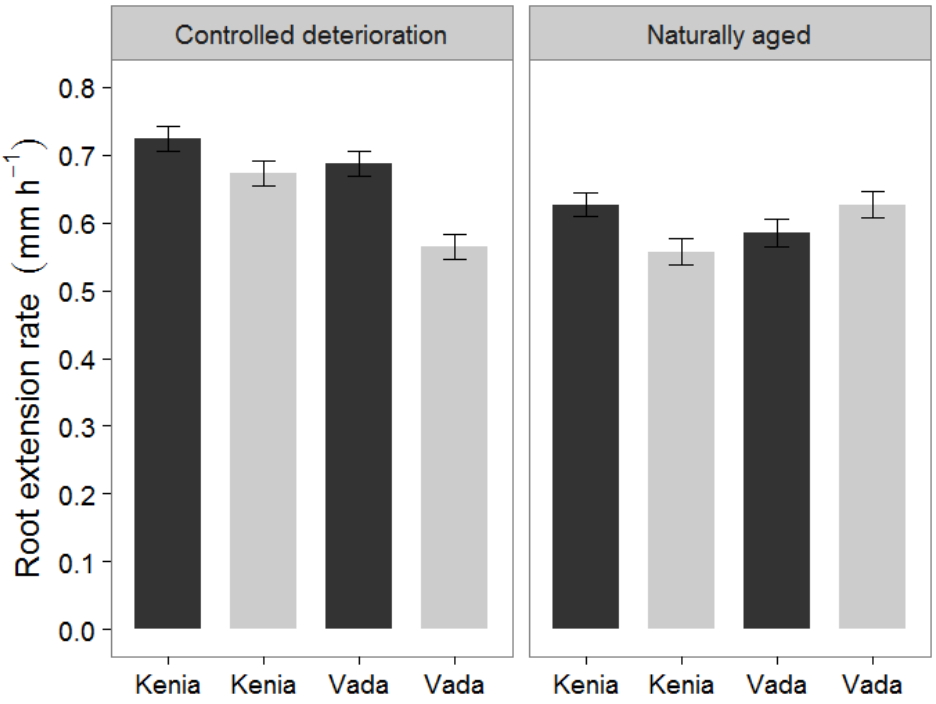


**Figure 3-6 Root growth rate (mm h$^{-1}$) for the two cultivars using seed aged by different methods in loose soil conditions (dry BD= 1.1 g cm$^{-3}$). Black bars are controls (non aged) and the shaded bars are the aged seeds. Values are means of n=6 samples. Error bars represent SEM.**

## 3.4 Discussion

### 3.4.1 Seed quality and implications for phenotypic screening

Seedling screens are attractive for high throughput phenotyping of root growth and architectural characteristics because they enable rapid measurements to be made on a large number of genotypes (Gregory *et al.*, 2009). Root extension rate and growth angle, both of which can be measured easily in seedlings, have been suggested to be important in

determining the depth of rooting (Bingham and Wu, 2011; Lynch, 2015). There is some evidence that variation in root systems of vegetative plants can be predicted from measurements made in seedling screens, although the relationships may not be as robust with mature plants (Wasson *et al.*, 2012). The results showed that seed quality can have a significant influence on the rate of root extension in seedling screens and if not controlled carefully could lead to the wrong conclusions being drawn regarding genetic variation in root growth rate. When averaged across soil BD and gMC treatments root extension rate was reduced in old seed compared to young.

Use of seed lots harvested in different years confounds the influence of differences in ageing during storage with that of the maternal environment during seed production, both of which have been reported to impact on seed germination and subsequent seedling establishment (White and Kirkegaard, 2010). In this experiment the average size of seeds of both cultivars differed between years with seed produced in 2013 being longer in length, area and weight than that in 2011. Cultivars also differed in seed size, but the interaction between cultivar and harvest year was weak suggesting that differences in maternal environment (encompassing soil, climatic and agronomic differences) affected grain development and filling of each cultivar similarly. In spite

of the contrasting seed morphology in seed lots from 2013 and 2011, the slower extension rate of roots from seed harvested in 2011 is mostly influenced by the effects of ageing during storage rather but there may still be some differences due to maternal environment. Seed taken from lots produced under the same conditions (2013) but subjected to artificial ageing using the controlled deterioration technique showed a reduction in rate of root extension relative to non-aged controls.

It is well established that seeds deteriorate during storage leading first to a reduction in vigour followed by a loss of viability (Powell *et al.,* 1984) and that cultivars with a lower initial Ki value will deteriorate sooner than those with higher initial quality (Alam *et al.,* 2006). Low vigour seed lots can have an acceptable final germination but a delayed onset and rate of germination compared to high vigour lots leading to delayed and often poor crop establishment in field sowings (Abdalla and Roberts, 1969). The results showed that root growth rate post-germination is also reduced by ageing in barley and that this occurs in soil and not just the simple growth media reported elsewhere with other species (Bingham *et al.,* 1994; Bingham and Merrit, 1994; Alam *et al.,* 2006). Moreover, since the effects of ageing persists beyond germination the impact of seed quality on root extension rate cannot be corrected for by pre-germinating

seeds and selecting seedlings of comparable size, or by measuring the initial root length and using it as a co-variate in the data analysis.

Figure 3-6 illustrates the importance of using seed of high initial quality when screening for varietal differences in root extension rate because even if they are stored under identical conditions the ranking of varieties can change with time in storage. Kenia ranked above Vada in growth rate when young seed were used, but lower than Vada when seedlings were grown from old seed. This is because Kenia had a lower initial quality and thus deteriorated more rapidly during storage compared to Vada. Thus, the effects of ageing on root extension eroded the initial advantage that Kenia showed with young seed.

### 3.4.2 Response to soil physical properties

Soils packed to high bulk density restricted root growth as has been shown for barley and other species elsewhere (Atwell, 1993; Bingham and Bengough, 2003; Tracy *et al.*, 2012; Andersen *et al.*, 2013; Chen *et al.*, 2014). Soil strength has been reported to be the most limiting constraint for root growth (Bengough and Mullins, 1990), as is confirmed by the findings here. Soils of higher strength at a given level of compaction (i.e. at 20% compared to 26% gMC) reduced the root growth rate to the greatest extent. Although the main restriction experienced by roots at

high BD and low gMC was soil strength and available water was within the limits of what is generally regarded as being necessary for root growth, matric potential below -10 kPa could have been an extra factor limiting root growth (Alaoui *et al.*, 2011).

Increasing bulk density caused a similar reduction in root extension rate at high soil gMC (26%) than at low soil gMC (20%). Here the increase in soil strength was negligible and available water was sufficient for root growth (Hakansson and Lipiec, 2000). Consequently, root extension is likely to have been restricted by poor soil aeration leading to hypoxic conditions at the root apex. The pattern of the reduction in root growth was similar to that with increasing soil strength. The roots responded to the soil constraint with a progressive decline in extension rate as the BD was increased in the experiment. However, high bulk densities were more restrictive to root extension at low gMC than high gMC giving rise to a significant BD x gMC interaction.

With naturally aged seed, interactions between seed age and soil BD, soil gMC and BD x gMC were weak, implying that whilst seed quality influenced root growth rate it did not modify the response of roots to soil strength and aeration to any great extent. With controlled deterioration on the other hand, significant interactions were found between ageing

and soil physical properties. However, these were associated with unusually high values for growth rate at a BD of 1.3 g $cm^{-3}$ and 20% gMC. When these values were excluded from the analysis the interaction was lost. At present, there is no biologically plausible explanation for these results.

## 3.5 Conclusions

Seed quality is an important factor to take into account for high throughput phenotyping of root traits, firstly because seed ageing influences the rate of seedling root extension and, secondly, because the deterioration of seeds during storage is not the same for all the cultivars (Alam *et al.*, 2006). Thus, even if seeds were collected and stored under the same conditions they may still show different germination and growth abilities. Moreover, if the aim is to compare genetic differences between cultivars, varieties or plant species, it is fundamental to be sure that any differences found are due to genetic differences and not environmental factors including seed quality. Evaluating factors such as germination percentages, MGT or Ki could be helpful ways to interpret results of phenotypic screens in a comprehensive way.

High seed quality can be achieved by using seed as soon as possible after harvest and by storing in conditions that will slow ageing (e.g. dry and

at low temperature). When collecting germplasm from different sources, seed should be multiplied up in the same field environment to ensure the best starting quality is achieved. When these conditions are met, the seedling screen developed here can be used to test for differences amongst genotypes in the ability of their roots to grow under realistic, but restrictive soil physical conditions at early seedling stage.

# Chapter 4: Genotypic variation of barley root systems at seedling stage.

## 4.1 Introduction

Roots, have been referred to as the hidden half by Waisel *et al.*, (2002) and are increasingly seen as a potential target for crop improvement with the aim of increasing yield and nutrient efficiency. Better understanding of root traits and root-soil interactions may help to these aims (Chapman *et al.*, 2012; Lynch, 2015b). Soils are typically heterogeneous and soil properties can change between geographical zones but also within a few centimetres or less (Rajaniemi, 2007).

Roots are therefore exposed to a wide range of stresses from abiotic factors (such as soil compaction, drought and heavy metals) and to biotic factors (pathogens) (Baligar and Fageria, 2015). Nutrient distribution in the soil can also be patchy (Jobbagy, 2001; Hodge, 2004). Deep rooting has been identified as one potential means of improved soil exploration and nutrient capture because it would allow access to water and nutrients stored in the deeper soil layers, thus potentially increasing resource acquisition (Passioura, 1992; King *et al.*, 2003; Foulkes *et al.*, 2009;

Kirkegaard and Hunt, 2010; Kautz *et al.,* 2013; Lynch, 2015). However, one of the most limiting factors for root growth at depth and soil exploration is soil densification and soil compaction (Jones *at al.,* 2003; Bengough *et al.,* 2006; Batey, 2009; Gao *et al.*, 2016). Soil compaction is a major problem for agriculture. It can be caused by agricultural machinery and by natural means such as freezing and thawing events (Jones *et al.,* 2013). Dense soils can exhibit high strength and in addition they may create two different abiotic restrictions for plant growth. If the soil is wet plants could suffer from lack of air-filled pore space and hypoxic conditions, whereas if the soil is dry there could be limited water availability.

This chapter will focus on soil physical characteristics as the main limitation for root growth. The focus is on soils with high soil strength because it was found in the previous chapter (3.3.3 Soil characteristics) that this factor most limited primary root extension rate of seedlings at very early stages of growth. It is known that soil strength affects root growth and very small increases in soil strength (from 0.85 MPa) can alter root growth and development (McMichael and Quisenberry, 1993; daSilva *et al.*, 1994; Lipiec and Hatano, 2003; Bengough *et al.*, 2006). Moreover, it has also been shown to affect stem growth and decrease

wheat yields when the crops were not capable of overcoming such stress (Whalley *et al.*, 2008).

Root growth, development and physiology are changed while overcoming the constraints imposed by a compact soil layer. The main axis extension growth rate in barley and wheat has been shown to slow by a half, and roots often become thicker. More resources are put into the main axis and thus less soil exploration occurs at depth (Materechera *et al.,* 1992, Bengough *et al.,* 2006, Tracy *et al.,* 2011). Barley lateral root branches may also be affected; however, lateral density (number of laterals per main axis cm) is not affected as much. Previous research has found that the main axis were reduced by a third whereas the density of the laterals of first order were only reduced about a 10% (Bingham and Bengough, 2003). This created a shallow root system mainly growing in the topsoil, hence reducing the possibility of nutrient uptake from the subsoil (White *et al.,* 2013, Lynch 2015).

Plant phenotyping focuses on how the genetics of a particular plant interact with the environment. Some plant varieties may be better adapted to soil compaction and have an improved ability to explore the soil, even under stress. Root phenotyping screens allow identification of variation in the way that root systems overcome soil constraints.

Phenotyping is often considered the bottleneck for genetic improvement (Richards *et al.,* 2010; Furbank and Tester, 2011), with much recent effort placed on developing new methods in both field and laboratory (McKenzie *et al.,* 2009; Richard *et al.,* 2015; Thomas *et al.,* 2016). Soil coring in the field is the method that better replicates natural conditions since it takes the roots directly from the field. However, the data is difficult to analyse due to the high variability found in such natural environments and root phenotypes may not appear as clear as they would in more controlled environments where roots are subjected to only one stress at a time (Trachsel *et al.*, 2011; Wasson *et al.*, 2014). Laboratory experiments include a range of techniques, from root washing in soil cores, transparent soil to minirhizotron and micro-Computed Tomography (μCT) (Heargraves *et al.,* 2009; Gregory *et al.,* 2009). Only 3D non-destructive visualization methods allow an assessment of root growth and architecture at different time points on the same plant specimen (Tracy *et al.,* 2012). Tracy *et al.,* (2012) and Helliwell *et al.*, (2013) both used a combination of techniques - root washing and μCT - to assess differences in root growth rate, visualize the full root system in 3D, and to investigate the way that roots of different branch order interacted with the soil. They found that with both methods root diameter increased and

length of the roots decreased which created changes in soil porosity in the compaction treatment.

This chapter aims to test two hypotheses. The first hypothesis is that barley genotypes have different abilities to explore soil, within low and high density soil creating differences in root growth extension rate. The second hypothesis is that the differences in root growth and interactions with the soil treatments will be maintained at later stages of growth and that the genotypic differences in the ability to explore the soil lead to different root system architectures.

## 4.2 Materials and Methods

### 4.2.1 Seed characterisation

The experiment was conducted with two-row spring barley cultivars collected from the experimental field at the James Hutton Institute, Invergowrie, Scotland, in summer 2014 and stored in the dark at 4$^{o}$C for eight months until the start of the experiment. The experimental field was a seed diversity trial where a wide range of cultivars were grown with no pesticides applied. 30 barley cultivars were selected taking into account the year they were released onto the market and the country of origin (Table 4-2) to provide a genetically broad and morphologically

distinct test population. Seed morphology of each cultivar was characterized by weighing 50 seeds individually and scanning them in a flatbed scanner. To avoid error due to differences within the images of every cultivar, three replicate scans with 12 seeds of seven cultivars were taken to be sure that the analysis of the seeds was consistent. The scanner used was an Epson Expression 836XL scanner (Regent Instruments Inc., Canada). Seeds were placed on a transparent PVC tray and covered with a plastic blue sheet. Scans were made at a resolution of 300 dpi and stored in format TIFF files. Scans were later analysed using Fiji ImageJ software (Schindenlin *et al.,* 2012). A segmentation of the first image was used to create a Simple Interactive Object Extraction (SIOX) using the imageJ plugin of the same name (Friedland, 2006) marking the seeds as the regions of interest or foreground and the blue colour as background. SIOX created a binary model image in which the seeds were differentiated from the background. This SIOX was applied to segment all the subsequent images. The segmented images were used for analysis of the particles to calculate the seed projected area, major: minor ratio, length and width (Figure 4-1).

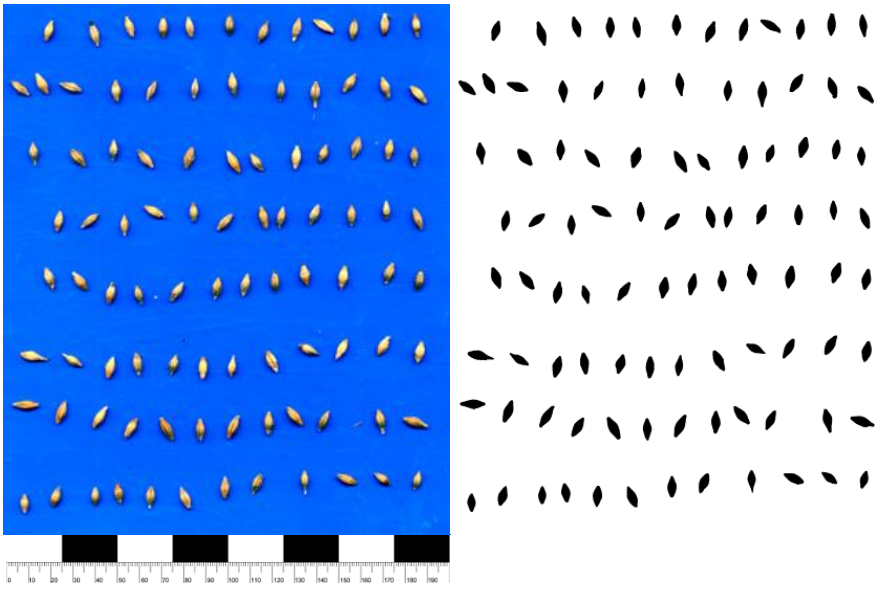

**Figure 4-1 Original (left) and segmented (right) images from the flatbed scanner.**

### 4.2.2 Soil column preparation

Soil was collected from the Boghall experimental farm, SRUC, Penicuik, Scotland (latitude 55.876, longitude -3.203) from the MacMerry series. The soil used was a Cambisol (FAO classification), a typical sandy clay loam. The soil was taken from the topsoil, air-dried and sieved to 2 mm. The gravimetric moisture content (gMC) of the soil was measured (Rowell, 1994) and raised to either 20% or 26% gravimetric moisture content (gMC) in Polythene plastic bags containing approximately 3 kg of soil. The sealed bags were stored overnight at 4°C to allow the moisture to distribute homogeneously through the soil. The soil was then packed into opaque PVC-columns of 5 cm (inner diameter) and 10.5 cm length (for the screening) and 20 cm length (for root architecture

characterization), with a polyester fabric mesh of 0.25 mm size holes covering the bottom of the column to prevent roots growing through, but allowing aeration of the soil. The soil columns were packed to provide two contrasting soil physical conditions (Figure 4-2). Each column was packed at a matric potential of -20 kPa, calculated in three replicates using tension tables (Ball and Hunter, 1988), and as either a loose soil treatment (1.1 g $cm^{-3}$ Bulk Density (BD), 26% gMC, 30% (w $w^{-1}$) air filled porosity (AFP), and 0.06 MPa soil strength) or a compacted soil treatment (1.5 g $cm^{-3}$ BD, 20% gMC, 13% AFP and 4.08 MPa soil strength). The columns were packed with a manual press in 2.5 cm layers. After packing each layer the surface was scarified (Lewis and Sjostrom, 2010) to minimise discontinuities between layers. Soil physical characteristics were based on the conditions tested in the previous experiment (3.3.3 Soil characteristics).

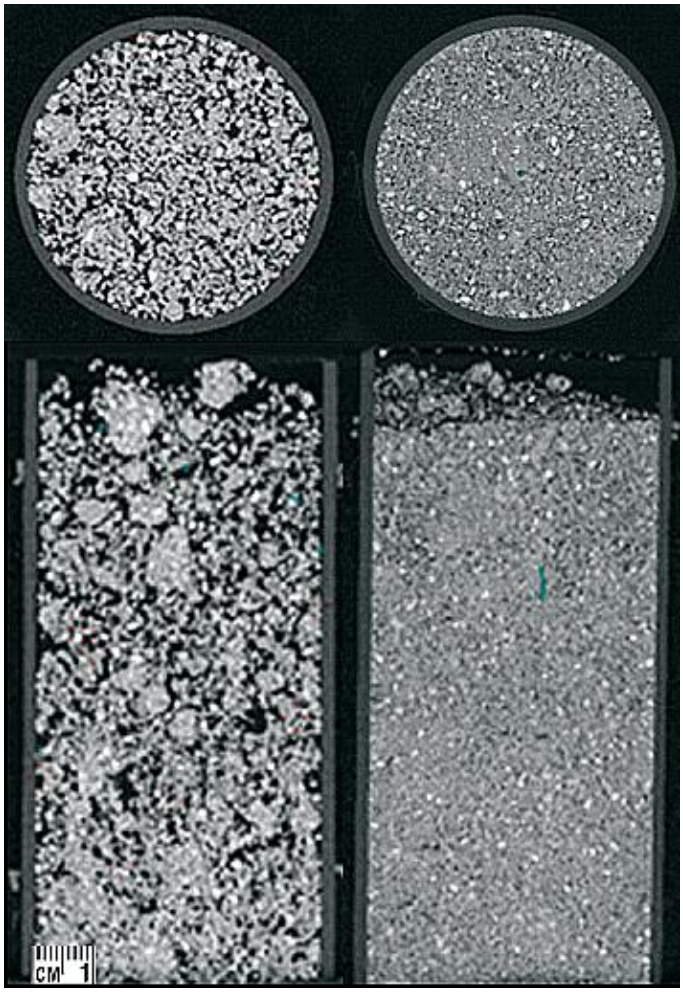


**Figure 4-2 X-ray μCT scan image of soil columns with two contrasting soil conditions. Images on the left are low BD soil (1.1 g $cm^{-3}$ and 26% MC, 30% AFP) and on the right high BD soil (1.5 g $cm^{-3}$ and 20% MC, 13% AFP). Top images show planar view and bottom images show axial view.**

### 4.2.3 Seeds germination and root growth

Seeds for each cultivar were germinated by placing seeds between two layers comprising four moistened layers of paper towels (Tork, Basic paper 1 Ply, dimensions 35 x 19.4 cm, SCA Hygiene Products, Dunstable, Bedfordshire). This was placed onto a layer of aluminium foil and rolled into a cylinder, then placed upright inside a 250 ml beaker with a 25 ml water reservoir; this set up allowed light to be excluded from the seed and developing roots and maintained the seeds in an upright position). The embryo of the seed was facing downwards to allow the root to grow in a vertical direction. The rolled paper towels were placed in a controlled environment cabinet at 15±1$^{o}$C with 16:8 h light:dark photoperiod. After germination and once the radicles reached a length between 15 and 20 mm (48 h of incubation) the main primary root was measured to the closest mm and two seedlings were transplanted into each soil column as previously described (3.2.1 Seed morphological characteristics and germination). Transplanted columns were fully randomised within the growth cabinet. Temperature and day length were maintained as described for germination. Light intensity at the height of columns was 121 μmol $m^{-2}$ $sec^{-1}$ photosynthetically active

radiation PAR supplied by 15 fluorescent lamps (Sylvania GRO-LUX®, OSRAM Sylvania, Westfield, USA).

### 4.2.4 Screening

After four days the columns were removed from the growth cabinet, roots were washed from the soil and the main primary root length was measured to the closest mm. Growth rate was calculated from the two measurements taken. The experiment was run ten times. Each run contained a fully randomised set of the varieties in the two soil treatments.

### 4.2.5 Root architecture characterisation

Four two-row spring barley cultivars, which showed contrasting root growth rate in the screening experiment, were chosen to evaluate growth responses to soil physical conditions at later growth stages (Figure 4-3): Ceylon, Welam, Digersano and Golf. For root architecture characterisation two sampling points were selected; the first was at eight days after transplanting the seedling, and the second at twelve days after transplanting. Two separate experiments were performed for each of the techniques presented below.

#### 4.2.5.1 Flatbed scanning

To characterise root system architecture in 2D soil was removed from the root system by washing. The samples were randomized and five replicates per variety and treatment were examined. Roots were washed, dispersed on a transparent PVC tray with a film of water and scanned with Epson Expression 836XL scanner (Regent Instruments Inc., Canada). Images were collected at a resolution of 300 dpi and stored as TIFF images. RootNav (Pound *et al.*, 2013) was used to measure total length of all primary axis and number of primary axis from the images. Root growth rates at day eight and day 12 after transplanting were calculated using the main primary root length at transplanting as the initial measurement and the length of the primary axis as measured with RootNav. Leaf length and width were measured to the closest mm, and the number of leaves counted. All plant matter (leaves and roots) were dried at 60$^{o}$C for 48 hours and weighed.

#### 4.2.5.2 Micro-CT (µCT) X-ray scanner

X-ray µCT was used for 3D root architecture characterization. All samples were scanned at day eight and day 12 after transplanting. The soil columns were scanned using a Phoenix Nanotom (GE Measurement and Control Solutions, Wuns-dorf, Germany) X-ray µCT. Each soil

column was scanned in three sections as a multiscan routine. The X-ray μCT scan set up for each of those sections was as follows: 140 kV and 200 μA, at a resolution of 50 μm, no filter was applied. Each section was scanned in 30 min (ie.1h 30 min for each column). For each section, 2161 image projections were captured. The full image stack size for each full column was approximately 50 GB. Once the image acquisition was finished, image reconstruction was undertaken with Phoenix Datos|x software package (GE Measurement and Control Solutions, Wunsford, Germany). First, all three sections were individually optimised to correct for any sample movement and an automatic beam hardening correction of 8 for different materials was applied. The full column was then reconstructed by joining the three sections with VGMax Studio 2.2 (Volume Graphics GmbH, Heidelberg). Root segmentation was performed with the same software. Roots were segmented using the region growing tool. Once the full root system was extracted as a region of interest, it was transformed into a new volume and exported as BMP image sequence. Rootrak (Mairhofer *et al.*, 2012) was not used for automatic root segmentation because of the high level of noise in the compacted treatment images and the length of time it took to complete a single stack due to the gavitropic and plagiotropic growth (more than ten

passes were needed to capture the full root system) of the lateral roots for each image stack (Mairhofer *et al.,* 2013).

Quantitative analysis of roots traits was performed with RooTrak, Rooth (Mairhofer *et al.*, 2013) and a combination of Fiji ImageJ software (Schindelin *et al.,* 2012) and R (R Development Core Team, 2016) procedures. Rootrak allowed the quantification of maximum root depth, root volume, convex hull (volume encapsulating the root system) and centre of mass. All these measurements quantify general root distribution across the soil profile. For example, centre of mass quantifies where most of the root mass was allocated; cultivars with a centre of mass allocated closer to the top of the column had more root mass in the upper layers of the soil, whereas cultivars with a greater centre of mass (further from the top of the column) had a root system more evenly distributed through the length of the soil column and had therefore explored the soil in more depth. Rooth was used to measure laterals of first order number, length and width. Fiji was used to erode the laterals and R to measure main seminal primary root length and number. Growth extension rates of primary roots were calculated from the subsequent scans and the initial root length. To compare the results from the screening with the subsequent growth patterns, the growth rate was calculated from the

subsequent scans using the length of the primary axis. In total four replicates were measured for each treatment and each cultivar.

### 4.2.6 Vermiculite root growth

Root extension rate was tracked daily in a low impedance media where roots could extend to their maximum capacity. Opaque PVC columns of 5 cm inner diameter and 20 cm length were filled with vermiculite to the top and watered with 200 ml of deionised water mixed with the equivalent of ¼ strength of the Hoagland Solution (Hoagland and Arnon, 1950) to provide the plants with the nutrients required for their growth. The top and bottom of the columns were covered in parafilm to avoid water loss. Seeds of cv. Digersano were pre-germinated and further planted and stored in controlled conditions following the same protocol as described previously (4.2.1 Seed characterisation). The experimental design included six replicates per sampling day and sampling occurred daily from day 5 to day 12 after planting into the columns. At every time point the samples were washed to remove the vermiculite and the root system was scanned following the same methodology as described previously (4.2.5.1 Flatbed scanning). Leaf length was measured by ruler to the closest mm and the number of leaves recorded. The root images were analysed with RootNav (Pound *et al.,* 2013) and main axis extension

rate calculated from the measurements obtained with the software and matching the number of replicate with that of the previous day to calculate the growth rate.

### 4.2.7 Statistical analysis

The number of replicates used for each experiment was based on a power analysis using the data from the previous experiments. The data was initially checked for normal distribution using the Shapiro-wilk test. If response variables were not normally distributed a test analysis of variance (ANOVA) model was generated using the response variate and random and fixed terms. A lambda value for yeo.johnson transformation (type of statistical transformation, a lambda value is calculated and the dataset is power-transformed to that value) of the response variable was generated using the model to be used and boxCox transformation approaches with the car package from R (Fox and Weisberg, 2011). One way ANOVA tests were performed to evaluate differences in seed weight, seed projected area and seed major: minor ratio between the cultivars. Two-way ANOVA tests were performed to assess differences in root growth rate between cultivars and the soil treatments in the initial seed screening experiment, using the initial primary root length as covariate and experimental runs as replicate blocks. Fisher´s protected

LSD test was used to choose the cultivars with the most distinct root extension rate pattern for further root architecture analysis.

The analysis of the root architecture traits was done using a restricted maximum likelihood (REML) approach in order to account for the maximum possible variation of the data and to include the two sampling days in the model. The fixed effects used were sampling day, BD and cultivar. Random effects were the individual sample over the two sampling points (for the CT scans) and the replicates. After the best model was fitted, backwards stepwise simplification of the model was performed using the MASS package (Venables and Ripley, 2002) using the Akaike information criterium (AIC) values for selection. REML was also used for the analysis of the vermiculite experiment with day and BD as the fixed effects and replicate the random effect. The statistical software used for the analysis was R (R Development Core Team, 2016) with packages lmerTest (Kuznetsova *et al.,* 2016) and ggplot2 (Wickham and Francois, 2015) packages.

## 4.3 Results

### 4.3.1 Screening

Cultivars showed different morphological seed characteristics. Cultivars differed in their seed weight, projected area and length: width ratio (Table 4-2). Furthermore, the range of cultivars differed in their main primary root extension rate after four days of growth in the soil columns. Correlation analysis was performed to test if differences in seed morphology were associated with the variation observed between cultivars in root growth rate after four days of growth in soil pots; however, no correlation ($P > 0.05$) was observed.

The results from the screening showed that the soil treatments had an effect on root growth rate and initial primary root length had a covariate effect. Soil was the factor which caused the greatest variation between the groups according to the mean squared error (s.s., Table 4-1). Roots in compacted soils grew at about half the rate they did in loose soil (Table 4-1, Figure 4-3). There was also a significant interaction between soil treatment and cultivar showing that cultivars differed in their response when they experienced physical constraints on the soil profile. The differences between the cultivar and the interaction had a low s.s. that

became significant due to the high number of different cultivars tested, however the total error within groups was bigger than that showing unexplained variations between the samples. Some cultivars (e.g. Digersano) showed a higher than average growth rate in both loose (0.43 mm $h^{-1}$ and std. 0.14) and compacted soil (0.87 mm $h^{-1}$ and std. 0.12), whilst others showed a lower than average rate in both soil conditions (Ceylon, average growth rates for loose and compacted soil respectively of 0.28 mm $h^{-1}$ and std. 0.13, 0.79 mm $h^{-1}$ and std. 0.18). However, some cultivars (e.g. Golf) showed a relatively slow growth rate under compacted soil (0.41 mm $h^{-1}$ and std. 0.14), but a higher than average rate under loose soil (0.738 mm $h^{-1}$ and std. 0.172), whilst others (Welam, average growth rates for loose and compacted soil respectively of 0.29 mm $h^{-1}$ and std. 0.13, 0.90 mm $h^{-1}$ and std. 0.14) behaved the opposite way. Cultivars that represented the four extremes and that were ranked as different in Tukey's test (highlighted in Figure 4-3) were selected for further investigation to determine whether the observed differences in root growth persisted over time and whether the differences were associated with differences in other potentially useful architectural traits.

Hence the percentage reduction in root elongation rate caused by the high bulk density treatment in comparison to the low-density treatment

was: Welam (67.08%), Ceylon (62.72%), Digersano (52.03%), Golf (44.63%).

**Table 4-1 ANOVA table of the results of the screening. The sources of variation analysed were: Soils are loose soil (1.1 g $cm^{-3}$ BD and 26% gMC) and compacted soil (1.5 g $cm^{-3}$ BD and 20% MC), cultivars are the one characterised in Table 4-2 and covariate is the initial root length of each sample measured. Number of replicates per treatment=10.**

| Source of variation | d.f. | (m.v.) | s.s. | m.s. | F | Covariance effect | P |
|---|---|---|---|---|---|---|---|
| Soil | 1 | | 62.11 | 62.11 | 2603.19 | 0.99 | <.001 |
| Cultivar | 29 | | 1.35 | 0.05 | 1.96 | 1 | 0.002 |
| Soil * Cultivar | 29 | | 1.28 | 0.04 | 1.85 | 1 | 0.004 |
| Covariate | 1 | | 2.05 | 2.05 | 86.09 | | <.001 |
| Residual | 1089 | (41) | 25.98 | 0.02 | | 1.08 | |
| Total | 1158 | (41) | 90.64 | | | | |

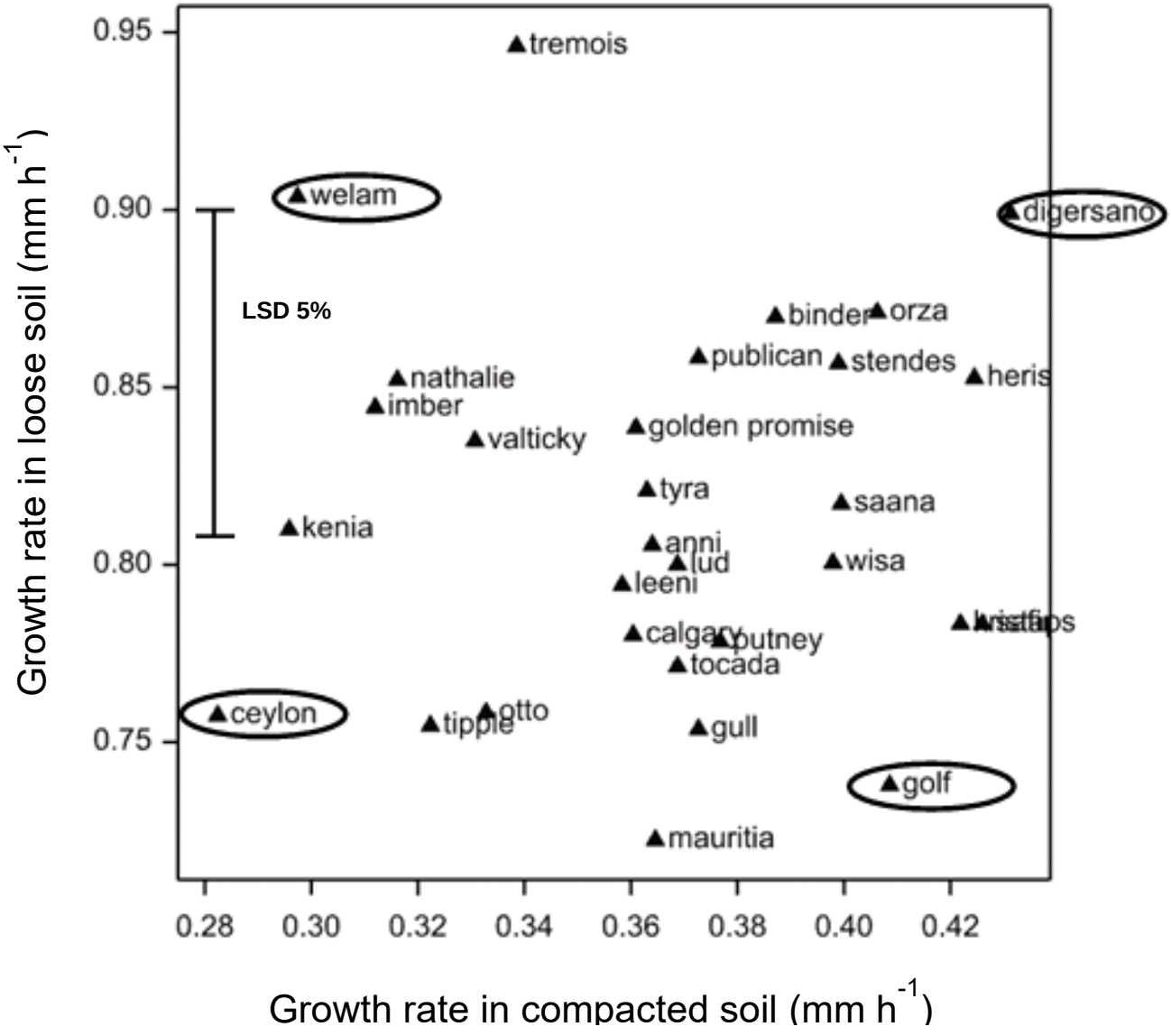


**Figure 4-3 Root growth rates for 30 spring barley cultivars under two contrasting soil conditions: low BD (BD 1.1 g cm$^{-3}$ and 26% gMC); and high BD (BD 1.5 g cm$^{-3}$ and 20% gMC). LSD 5% bar refers to the interaction between cultivar and soil treatment.N=10 samples**

**Table 4-2 Seed characterisation and origin of two-row spring barley cultivars. Seed of each cultivar was multiplied at the same UK site in harvest year 2014. P-value and LSD (5%) calculated for one-way ANOVA taking cultivar as factor.**

| Cultivar | Weight of fresh seed (mg) | Area ($cm^2$) | Major: minor ratio | Year | Country of origin | Pedigree | Breeding institution |
|---|---|---|---|---|---|---|---|
| Anni | 44.61 | 0.23 | 2.701 | 1980 | Estonia | Lola x Liisa | Unknown |
| Binder | 50.4 | 0.26 | 2.67 | 1916 | Denmark | Selection from Hann/a | Jogeva Plant Breeding Institute |
| Calgary | 45.85 | 0.23 | 2.58 | 2002 | France | (Dominique x Blenheim) x (Barleta x Chapka) | Abed |
| Ceylon | 43.91 | 0.23 | 2.78 | 2003 | Netherlands | Portia x Amber | Serasem |
| Digersano | 46.68 | 0.24 | 2.46 | 1991 | Italy | Mari-Coho x Sul-Mackta | Cebeco |
| Golden Promise | 47.03 | 0.24 | 2.53 | 1968 | United Kingdom | Maythorpe Gamma-Ray Mutant | Cermis/ENEA |
| Golf | 45.31 | 0.24 | 2.57 | 1983 | United Kingdom | (Armelle x Lud) x Luke | Miln Marsters |
| Gull | 44.61 | 0.22 | 2.47 | 1915 | Sweden | Landrace selection from Gotland | Nickerson |
| Heris | 42.08 | 0.28 | 2.73 | 1998 | Czech Republic | HE 4436 x CE 431 | Svalof |
| Imber | 49.07 | 0.25 | 2.40 | 1970 | United Kingdom | Elsa x Proctor | Hrubcice |
| Kenia | 47.44 | 0.23 | 2.53 | 1931 | n/a | n/a | Guinness |
| Kristaps | 43.15 | 0.29 | 2.58 | 2002 | Latvia | CF 79502 x STN9023 | n/a |
| Leeni | 40.68 | 0.28 | 2.60 | 2006 | Estonia | (Ingrid x Golden Promise) x Decor | Stende Plant Breeding Station |
| Lud | 47.79 | 0.23 | 2.39 | 1976 | United Kingdom | (Vada x Zephyr) x RMGH 59114 | Jogeva Plant Breeding Institute |
| Mauritia | 44.83 | 0.24 | 2.79 | 2004 | Germany | Madras x LP 217.96 | Nickerson |
| Nathalie | 45.99 | 0.23 | 2.59 | 2006 | Denmark | Alexandra x (Power x Recept) | Lochow-Petkus |

| Cultivar | Weight of fresh seed (mg) | Area ($cm^2$) | Major: minor ratio | Year | Country of origin | Pedigree | Breeding institution |
|---|---|---|---|---|---|---|---|
| Orza | 55.80 | 0.78 | 2.78 | 1994 | Turkey | Tokak x Landrace 4857 | Sejet |
| Otto | 49.82 | 0.26 | 2.58 | 1972 | Italy | Genealogical selection from African population | Tarla Bitkileri Merkez Ars.Ens. Müd. |
| Publican | 45.76 | 0.24 | 2.64 | 2006 | United Kingdom | Drum x Sebastian | n/a |
| Putney | 47.14 | 0.24 | 2.67 | 2003 | n/a | n/a | Syngenta |
| Saana | 47.29 | 0.24 | 2.40 | 1996 | Finland | Kustaa x (Olli x Eero) | n/a |
| Safir | 49.06 | 0.29 | 2.73 | 1978 | Czech Republic | (Valticky x Kneifel) x (Diamant x arabische Zweizeilige) | Boreal Plant Breeding Ltd |
| Stendes | 48.91 | 0.23 | 2.713 | 1972 | Latvia | Drost x Maja | Hrubcice |
| Tipple | 48.06 | 0.25 | 2.69 | 2005 | United Kingdom | (NFC 497-12 x Cork) x Vortex | Stende Plant Breeding Station |
| Tocada | 53.32 | 0.27 | 2.64 | 2002 | n/a | n/a | Syngenta |
| Tremois | 45.38 | 0.24 | 2.81 | 1989 | France | (Dram x Aramir) x Berac | n/a |
| Tyra | 52.36 | 0.26 | 2.638 | 1975 | Denmark | (Algerian x Herta*8) x (Rika x Drost) | Verneuil |
| Valticky | 49.23 | 0.24 | 2.56 | 1950 | Czech Republic | Valticky B x Starnovsky Kneifel | Pajbjerg |
| Welam | 49.67 | 0.24 | 2.48 | 1976 | Sweden | ((Monte-Christo x Clara) x WW-5793*2) x WW-5853*3 | Valtice |
| Wisa | 48.14 | 0.22 | 2.35 | 1951 | Germany | Weihenstephaner Mehltauresistente 1 x Isaria | Weibull |
| **p-value** | <0.001 | <0.001 | <0.001 | | | | |
| **LSD(5%)** | 2.636 | 0.014 | 0.078 | | | | |

### 4.3.2 Root traits of developing root systems day eight to 12

With the exception of the number of primary roots, root width, root dry mass and laterals density, all other root traits showed an increment over time from the first time point to the second-time point (Table 4-3). The traits that specifically quantified the distribution of the root system in the soil column - convex hull and centre of mass - were cultivar-driven (Table 4-3). Welam was the cultivar with the highest values for convex hull (245 $cm^3$) (Figure 4-4 a) and centre of mass (6.09 cm from the top of the column) (Figure 4-4 c) and explored the bigger volume of soil (Figure 4-5 and Figure 4-6) and Golf (182.11 $cm^3$) the one with the smallest convex hull and Ceylon with the smallest centre of mass (4.90 cm from the top of the column), although the three cultivars with the smallest convex hull and the centre of mass closer to the top did not diverge statistically (Figure 4-4 a and Figure 4-4 c). Moreover, convex hull was also influenced by the BD of the soil (Table 4-3). Soil physical constraints made a difference in the way that roots explored the soil (average convex hull of 0.22 $cm^3$ for 1.1 g $cm^{-3}$ low BD and average of 0.19 $cm^3$ for 1.5 g $cm^{-3}$ high BD); roots growing under loose soil conditions explored the soil to a greater extent than those roots growing under high impedance soils. Soil physical characteristics also influenced the depth (Figure 4-4 b) that

the longest root reached in the soil column (167.4 cm and 156.4 for low BD soil and high BD soil respectively, Table 4-3). Other root traits measured were volume of the root system, area and width. Area and volume (Figure 4-7 c and Figure 4-7 b, Table 4-3) increased with time 20.07 $cm^2$ and 0.14 $cm^3$ respectively but root width remained constant over the time period examined.

Number of primary roots was dependant on the cultivar used and the BD (Table 4-3 and Figure 4-8 a and Figure 4-8 b). Those plants growing into physically constrained soils developed a lower number of primary roots than those growing in loose soils (average of 0.48 primary roots fewer were detected in the μCT measurements). The cultivars which developed a higher number of primary roots were Ceylon and Welam, with an average of 5.5 and 5.7 primary roots per plant respectively.

Total root length (Figure 4-9 a and Figure 4-9 b) and average length of the primary roots (Figure 4-8 c and Figure 4-8 d) was reduced due to the increase in the BD of the soil (Table 4-3). Roots growing in soil with a high density explored a smaller volume of soil. Total primary root length was more than 10 cm longer for Welam, with little difference between the other three cultivars. The patterns of growth were the same

regardless of the technique but the significance of the results was higher with the root washing technique.

The interactions of the root growth extension rate of the cultivars (in 3D data calculated from length after transplanting to day eight and day eight to day 12 and in 2D data calculated from length before transplanting to day eight and length before transplanting to day 12) with the soil physical characteristics was not exactly the same as that observed in the screening (4.3.1 Screening). It was expected that Digersano would have the maximum extension rate in both soil treatments (Figure 4-3). While Ceylon the minimum. Ceylon had the slowest extension rate (0.53 mm $h^{-1}$ measured in 3D and 2D) (Figure 4-9 c and Figure 4-9 d), it was not statistically different from Digersano and Golf. On the contrary Welam, which was the cultivar that had the fastest extension rate for loose soil but not for constrained soil, was the cultivar with the fastest root extension rate in both soil types (0.60 and 0.57 mm $h^{-1}$ in 3D and 0.58 and 0.50 mm $h^{-1}$ in 2D for compacted and loose soil respectively). Differences between cultivars were significant for 2D measurements and not for 3D (Table 4-3), although the ranking of growth rate for the cultivars was the same for both techniques. Nevertheless, there was a reduction of more than half of the growth extension rate from the day eight to day 12. The

cultivars with the fastest root extension rates at the first-time point were the ones which slowed down their growth rate more dramatically by the second-time point. This was particularly notable for Welam (reduction of 0.45 and 0.40 mm $h^{-1}$ from day eight to day 12 in 3D and 2D respectively).

Laterals of the first order were also quantified and measured over the course of the experiment with 3D X-ray μCT scanning. The main influence of laterals development across all the traits was time (Figure 4-5, Figure 4-6 and Table 4-3). The second factor influencing laterals development (average lateral length and sum of lateral length) was the soil BD but it did not influence laterals number and density. The number of laterals per cm of primary axis was constant (1.25 number of laterals: cm of primary root).

Those root systems growing in high BD soils had shorter laterals (6.58 mm in low BD soil compared to 4.47mm in high BD soil) (Figure 4-10 b), and the sum of total length of laterals was also 40% smaller (Figure 4-10). Lateral width was only influenced by the BD (Figure 4-10 c); roots growing in soils with high impedance developed thicker laterals than roots growing in loose soil, width did not increase with time.

In addition, number of laterals (Figure 4-10 a) also varied between cultivars; Ceylon developed a larger number of laterals (102.1 laterals) than the other cultivars (Digersano 78.9; Golf 76.4 and Welam 82.1) (Figure 4-10 a).

Root dry weight did not vary across the time period because of the high variation found between individual samples (18.1 mg). All the measured traits of the shoot were influenced by cultivar. Welam developed the largest shoot dry weight average across treatments (17.06 mg) (Figure 4-11 a) and longest shoots and leaves (15.27 cm and 12.67 cm) (Figure 4-11 b and Figure 4-11 c) whereas Ceylon had the smallest shoot dry weight and shortest shoot and leaf measurements (14.8 mg average shoot weight, 13.46 cm total shoot length and 10.71 cm of longest leaf length).

**Table 4-3 Final fitted model for each trait measured in the 3D and 2D root scans and the P-values associated with each model (df day=1, df cv.=3, df BD=1). Model nomenclature is as follows: fixed factors+ (1+covariate | random factors).**

| Root trait | Method | Final model | BoxCox ($\lambda$) | $P_{model\ check}$ | $P_{day}$ | $P_{bd}$ | $P_{cv.}$ |
|---|---|---|---|---|---|---|---|
| General Root Architecture | | | | | | | |
| Volume | 3D | day+(1 \| replicate) + (1 \| sample) | -0.05 | <0.001 | <0.001 | n/a | n/a |
| Convex Hull | 3D | day+cv+bd+(1 + day \| replicate) | 0.15 | 0.001 | 0.01283 | 0.05 | 0.01 |
| Area | 3D | day+(1 \| replicate) + (1 \| sample) | -0.05 | <0.001 | <0.001 | n/a | n/a |
| Centre of mass z | 3D | day+cv+(1 \| replicate) + (1 \| sample) | | <0.001 | <0.001 | n/a | <0.001 |
| Total depth | 3D | day+cv+(1 \| replicate) + (1 \| sample) | | <0.001 | <0.001 | 0.005 | n/a |
| Root width | 3D | (1 \| replicate) + (1 \| sample) | | n/a | n/a | n/a | n/a |
| Root dry mass | 2D | cv+ (1 \| replicate) | | 0.001 | n/a | n/a | 0.04 |
| Primary roots | | | | | | | |
| Number of primary axis | 3D | cv+bd + (1 \| replicate) | | 0.002 | n/a | 0.03 | 0.02 |
| | 2D | cv+bd+ (1 \| replicate) | | <0.001 | 0.0011 | <0.001 | n/a |
| Total root length | 3D | day + bd + (1 \| replicate) + (1 \| sample) | | <0.001 | <0.001 | <0.001 | n/a |
| | 2D | bd+(day.cv)+ (1 \| replicate) | | <0.001 | <0.001 | <0.001 | <0.001 |
| Average primary roots length | 3D | day+bd+(1 \| replicate) + (1 \| sample) | | 0.02 | <0.001 | 0.01 | n/a |

| | Root trait | Method | Final model | BoxCox ($\lambda$) | $P_{model\ check}$ | $P_{day}$ | $P_{bd}$ | $P_{cv.}$ |
|---|---|---|---|---|---|---|---|---|
| | Main primary root extension rate | 2D | day+cv+bd+ (1 \| replicate) | | <0.001 | <0.001 | <0.001 | <0.001 |
| | | 3D | day+(1 \| replicate) + (1 \| sample) | | <0.001 | <0.001 | n/a | n/a |
| | | 2D | day+cv+bd+ (1 \| replicate) | | 0.04 | <0.001 | <0.001 | <0.001 |
| Laterals | | | | | | | | |
| | Average laterals length | 3D | day+bd+(1 \| replicate) + (1 \| sample) | -0.15 | <0.001 | <0.001 | <0.001 | n/a |
| | Sum of laterals length | 3D | day+bd+(1 \| replicate) + (1 \| sample) | 0.35 | <0.001 | <0.001 | 0.003 | n/a |
| | Laterals number | 3D | day+cv.+ (1 \| sample) | | 0.02 | 0.001 | n/a | 0.05 |
| | Laterals density | 3D | (1 \| replicate) + (1 \| sample) | | NS | n/a | n/a | n/a |
| | laterals width | 3D | (1 \| replicate) + (1 \| sample) | | NS | n/a | n/a | n/a |
| | Sum lateral sum main | 3D | day+bd+(1 \| replicate) + (1 \| sample) | 0.15 | <0.001 | <0.001 | 0.02 | n/a |
| Upper | | | | | | | | |
| | Shoot dry weight | 2D | day+cv.+bd+ (1 \| replicate) | | <0.001 | <0.001 | 0.003 | 0.018 |
| | leaf length | 2D | day+cv.+ (1 \| replicate) | 1.57 | <0.001 | <0.001 | n/a | <0.001 |
| | stem length | 2D | day+cv.+ (1 \| replicate) | 0.56 | <0.001 | <0.001 | n/a | <0.001 |
| | root:shoot | 2D | day+bd+ (1 \| replicate) | | 0.002 | <0.001 | 0.01 | n/a |

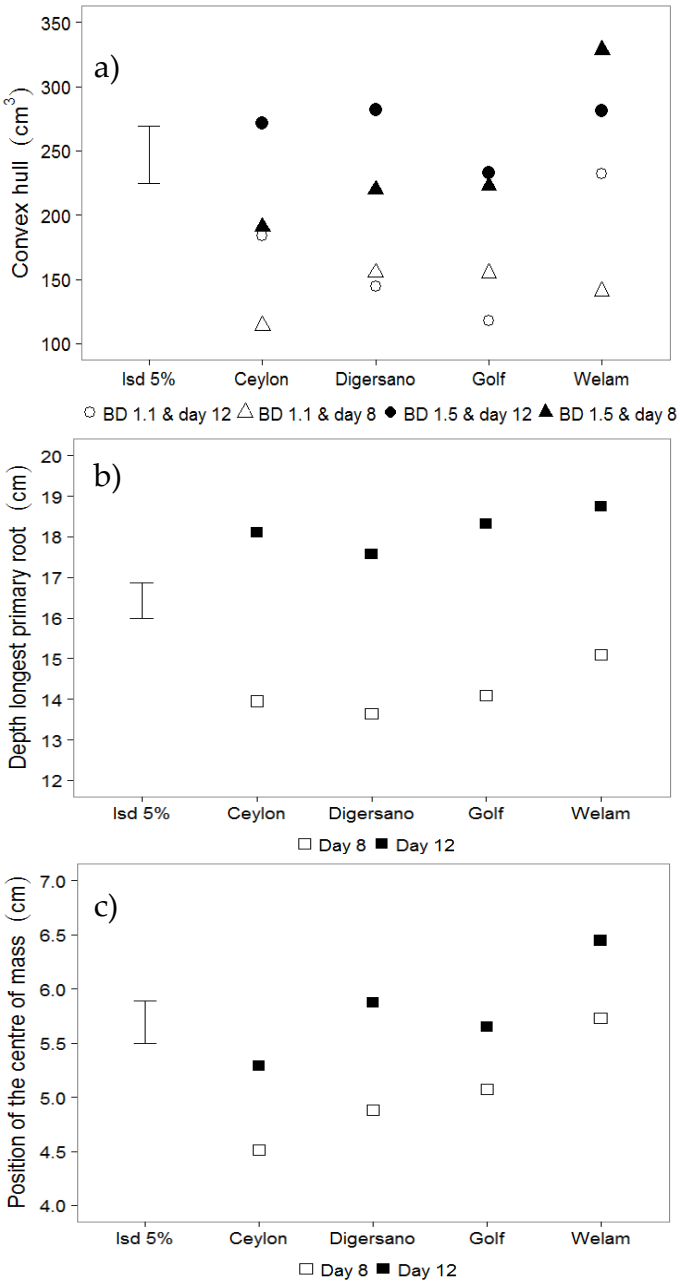


**Figure 4-4 Root architecture traits measured from the X-ray µCT scan experiment. Adjusted means and significant interactions are shown for a) convex hull ($cm^3$), b) depth of the longest primary root (cm), c) position of the centre of mass from the top of the column (cm). Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

**Figure 4-5 X-ray μCT images of roots growing in low BD soil conditions. a) to d) are images taken at the first time point and e) to h) the second time point. Scale is shown at the bottom of each set. Cultivars shown are a) and e) Digersano, b) and f) Ceylon, c) and g) Welam and d) and h) Golf. Scale bar represents 1 cm.**

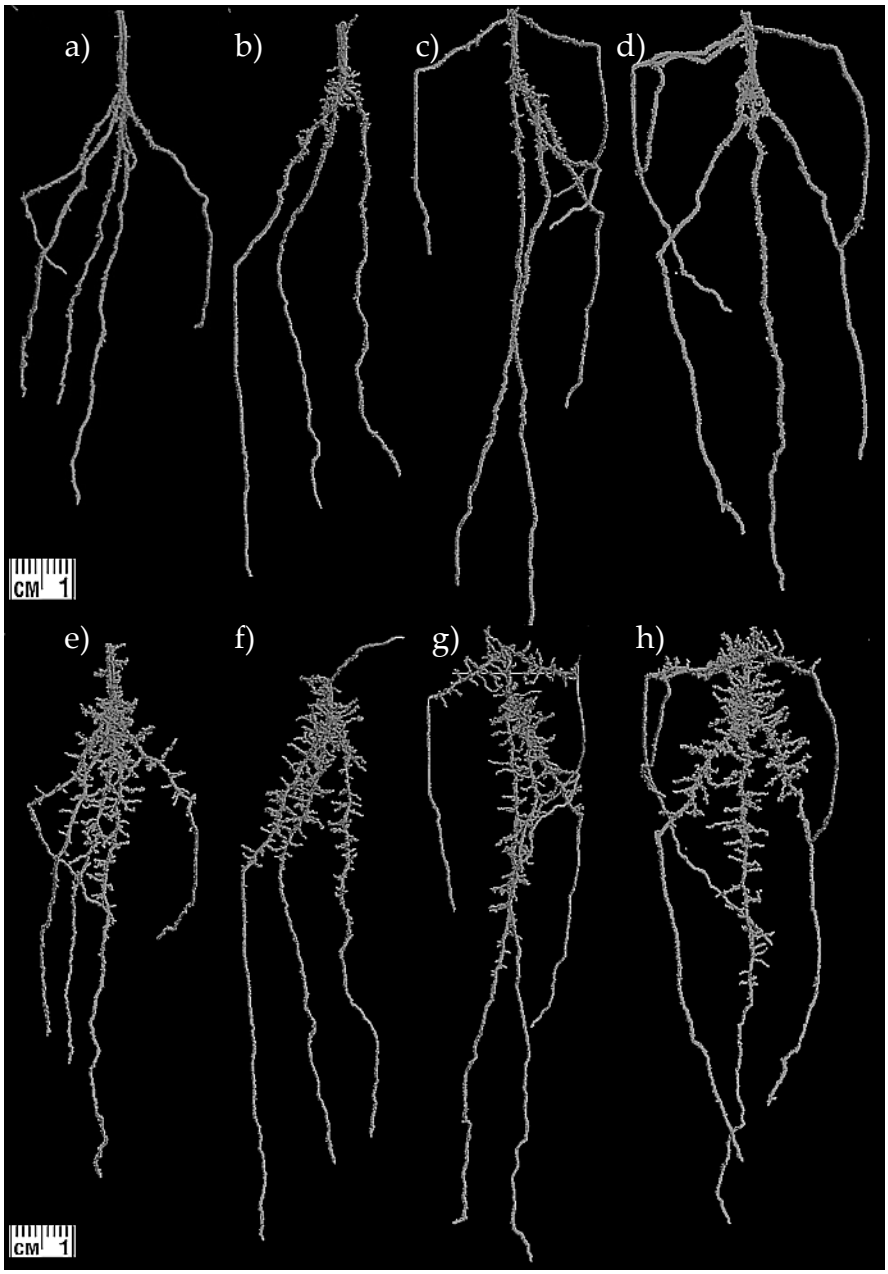


**Figure 4-6 X-ray μCT images of roots growing in high BD soil conditions. a) to d) are images taken at the first time point and e) to h) the second time point. Scale is shown at the bottom of each set. Cultivars shown are a) and e) Digersano, b) and f) Ceylon, c) and g) Welam and d) and h) Golf. Scale bar represents 1 cm.**

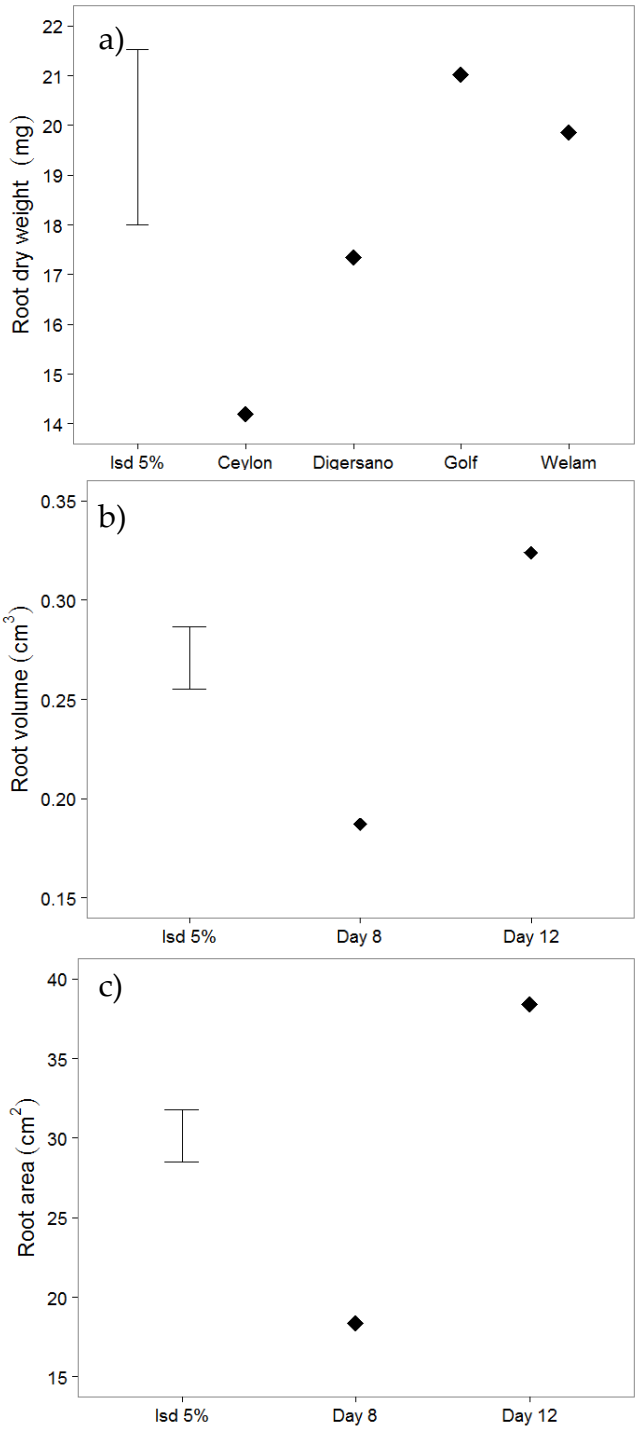


**Figure 4-7 Root architecture traits measured from the X-ray µCT scan experiment. Adjusted means and significant interactions are shown for a) root dry weight (mg), c) root volume ($cm^3$), b) root area ($cm^2$). Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

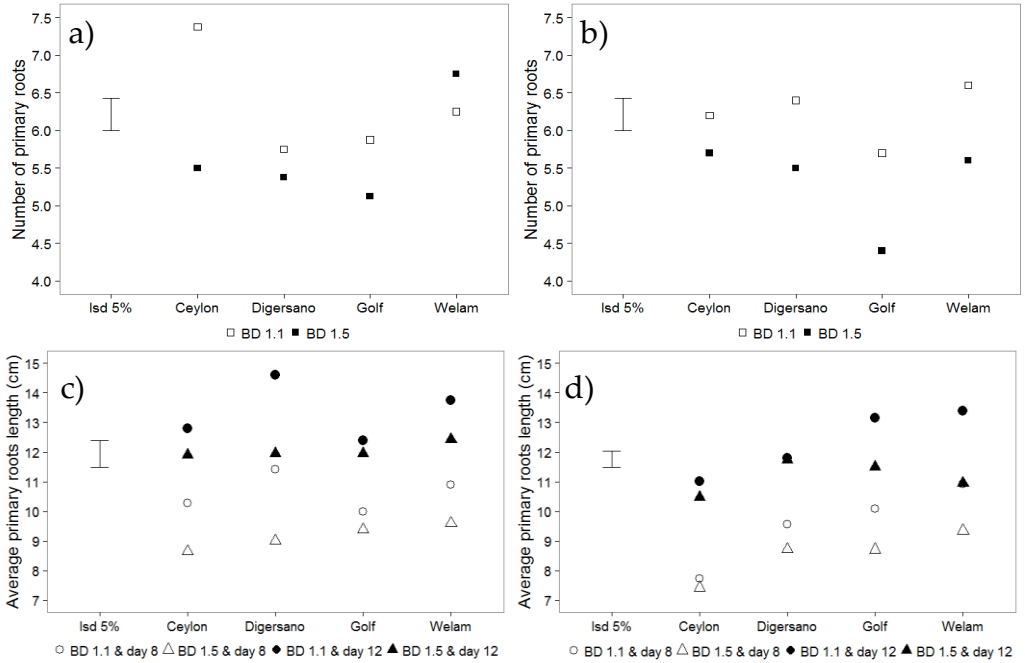


**Figure 4-8 Primary roots measurements. Graphs a), c) represent 3D X-ray µCT-scanning and b), d) graphs represent the 2D flatbed scanner. Adjusted means and significant interactions are shown for a) and b) Number of primary roots, c) and d) average of the primary roots length (cm). Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

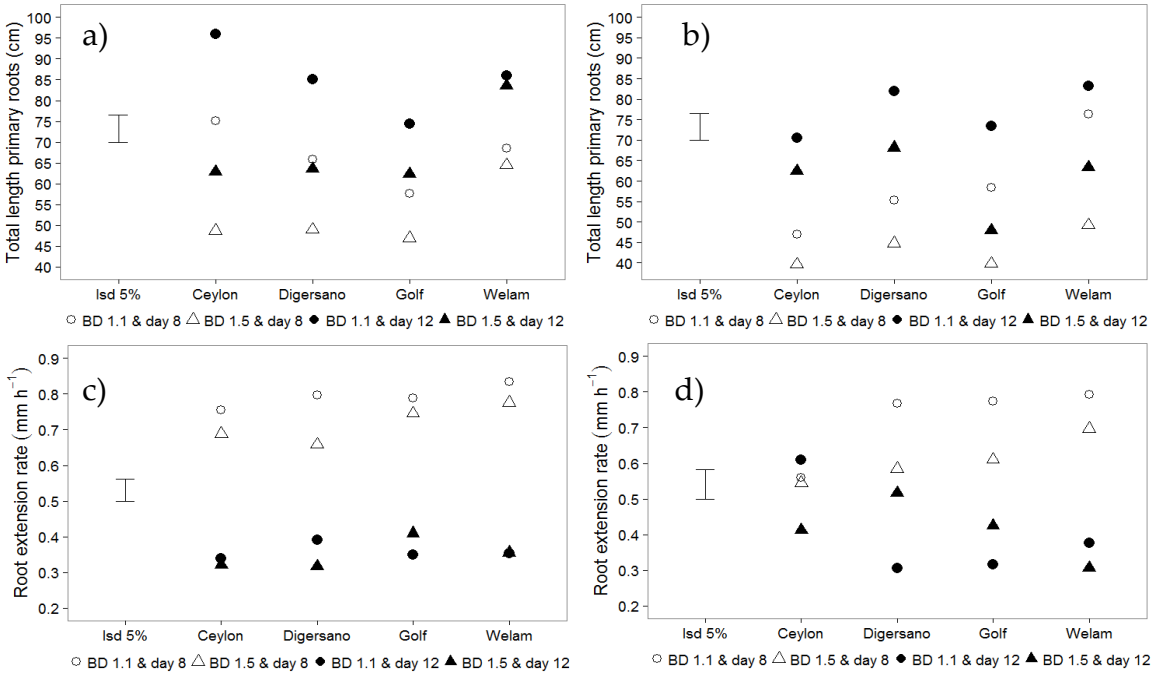


**Figure 4-9 Primary roots measurements. Graphs a) and c) represent 3D X-ray µCT-scanning and b) and d) represent the 2D flatbed scanner. Adjusted means and significant interactions are shown for a) and b) total length of primary roots (cm), c) main primary root extension rate (mm $h^{-1}$) calculated from length after transplanting to day eight and day eight to day 12 d) main primary root extension rate (mm $h^{-1}$) calculated from length before transplanting to day eight and length before transplanting to day 12. Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

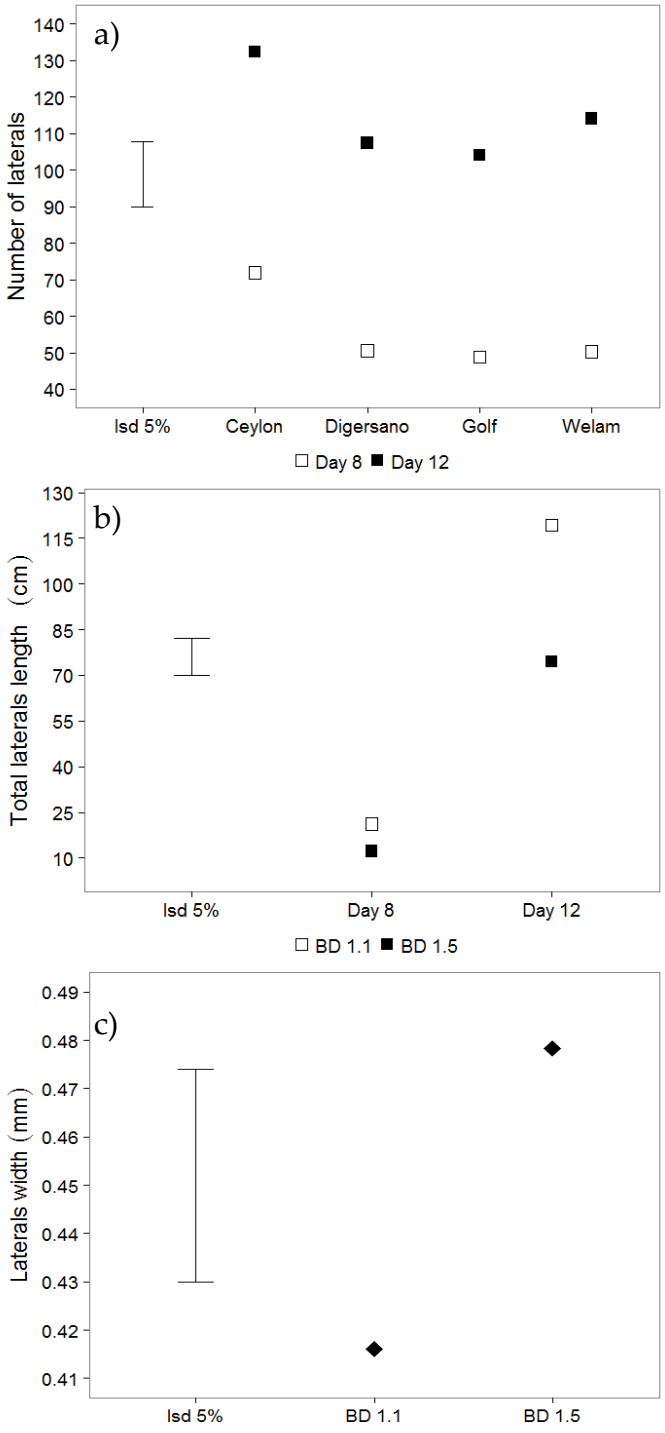


**Figure 4-10 First order lateral roots measurements. Adjusted means and significant interactions are shown for a) Number of laterals, b) total lateral length (cm), c) lateral width (mm). Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

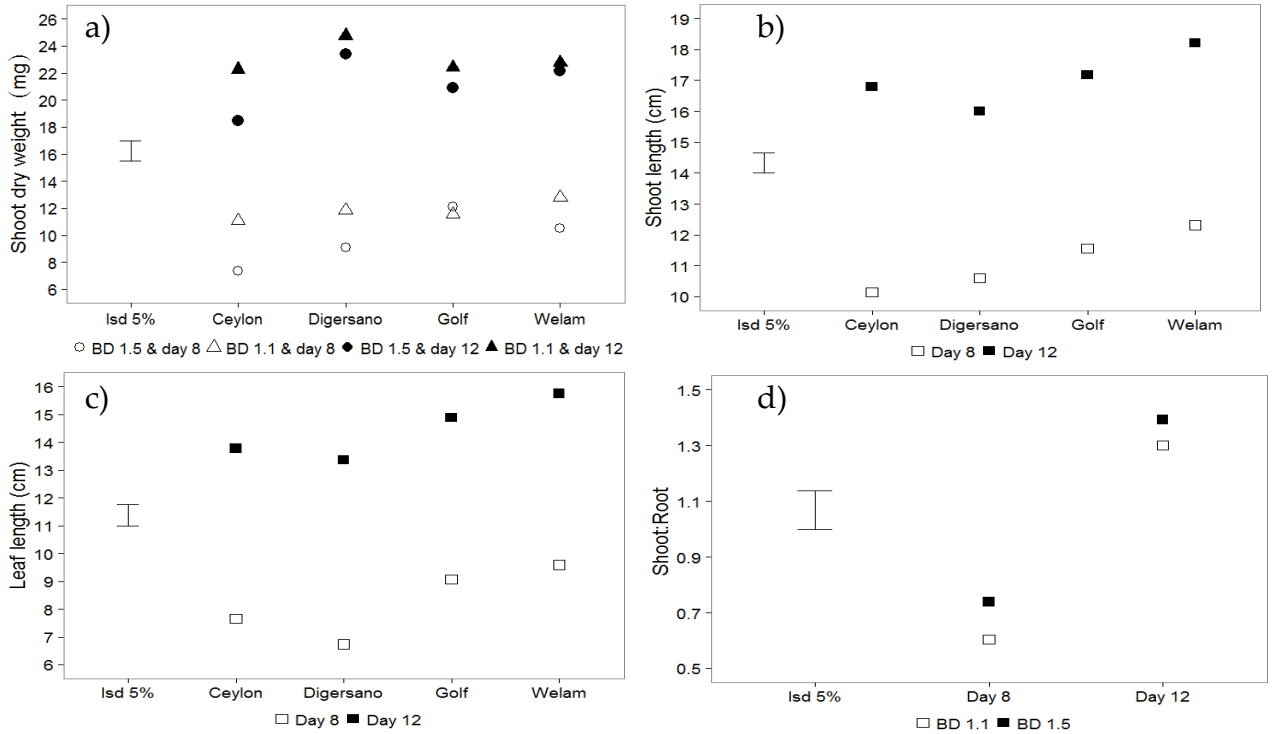


**Figure 4-11 Shoot measurements. Adjusted means and significant interactions are shown for a) Shoot dry weight (mg), b) shoot length (cm), c) leaf length (cm), d) shoot: root (mg: mg). Legend of the symbols is shown at the bottom of each graph. Error bar represents 5% LSD for the combination of factors represented.**

### 4.3.3 Vermiculite growth extension rate results

Root extension rate of Digersano in vermiculite followed a curve shape (Figure 4-12), being slower in the beginning and the end and having a peak in root extension between days seven and nine (df=7, F-value=2.278, P-value=0.046).

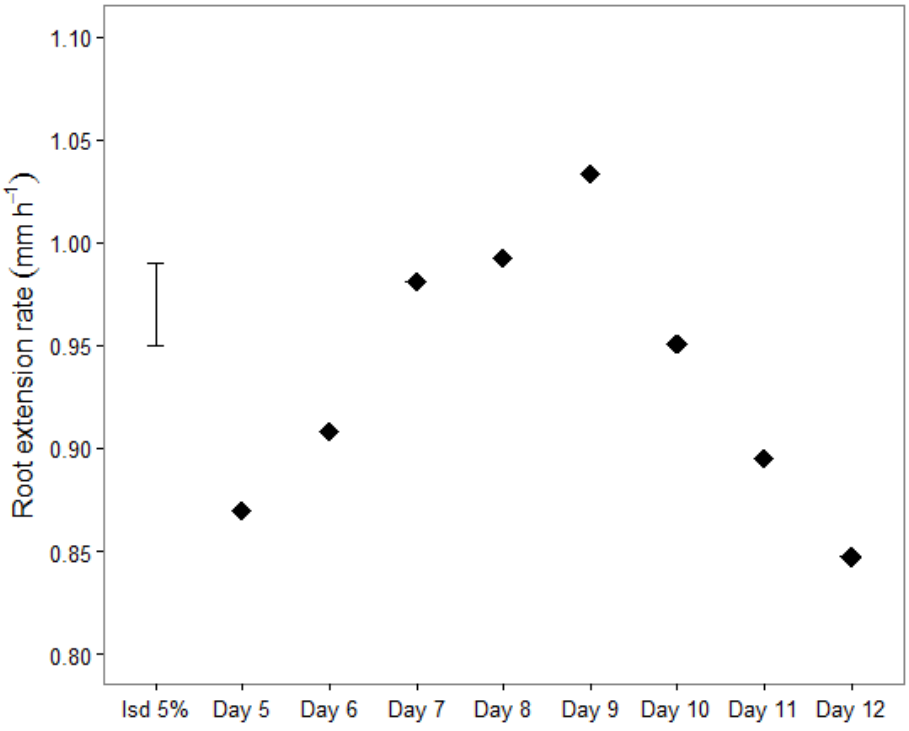


**Figure 4-12 Average of the longest primary root extension rate for plants growing in vermiculite. Day 5 shows the average growth rate between day 0 and day 5 whereas the rest of days show the growth rate between the previous day and the day shown. Error bar represent 5% LSD.**

## 4.4 Discussion

### 4.4.1 Validity of the screening

The initial focus was to find differences in the primary root extension rates between cultivars since longer roots earlier in the season can potentially lead to deeper root systems with a greater root length density in the subsoil. This could enable them to take advantage of the nitrogen and water from the deeper layer of the soil (Lilley and Kirkeegard, 2010; Wasson *et al.,* 2012; White *et al.,* 2013 Lynch, 2015). Four days after planting, the barley cultivars differed in their ability to overcome the soil physical characteristics.

Seedling screening of root traits was an effective way of undertaking medium-throughput phenotyping but care should be taken to translate the findings directly into the field since the traits found in artificial systems may not directly relate to the behaviour of the cultivar under field conditions (Clark *et al.,* 2002; Wasson *et al.,* 2014). Seedling screening was used as an initial tool to quantify differences between the cultivars and to assess the cultivars with more interesting traits. However, the results presented here showed that root phenotyping for root extension rate at seedling stage did not relate to the more mature plant root system.

At later stages of growth (days eight and 12 after planting), the four chosen cultivars differed in their ability to develop specific root traits, such as the convex hull, centre of mass, root dry mass, number of primary axis, total root length, primary root length, extension rate or number of laterals; however, the interaction between cultivar and soil treatments was not maintained at those later stages of growth.

One of the reasons for this was the low power of the analysis (0.63), due to their large variation between the samples it became increasingly difficult to detect genotypic differences at those later stages of growth. Other studies have shown a lack of correlation between seedling traits and plant maturity. For example, root angle measured at seedling stage in wheat did not relate to the plant behaviour on the field (Whalley *et al.*, 2013). Root systems are very variable (Wasson *et al.*, 2012; Thomas *et al.*, 2016) and individual plant variation has been shown to be considerable, so the degree of replication needed for root studies was high, and despite this study being undertaken under controlled environment conditions, a higher level of replication in this study would have been optimal. As plants matured, variation increased and the power of the analysis decreased suggesting that a higher number of replicates is needed. However, increasing replication is not always possible because of the

time it takes to analyse the samples or budget restrictions (Furbank and Tester, 2011; Herrera *et al.*, 2012).

### 4.4.2 Older plants root growth and further exploration of root traits

Soil BD did not influence convex hull or centroid of mass, but it influenced the length of the root systems and the number of primary roots that the cultivars were able to develop. Root systems growing under high impedance have been found to be more restricted axially than radially, with laterals development not being restricted to the same extent as extension rate of the main axis (Bingham *et al.*, 2003; Bengough *at al.*, 2006). This could lead to a similar convex hull or centre of mass in the different treatments. Number of primary root axes is a predetermined genotypic trait and the cultivars used in the experiment differed in the number of primary roots developed. However, the soil physical environment also played a role in whether all the primary roots developed or not. A smaller number of primary roots limit root exploration of the soil and influence the overall root architecture through a reduced number of laterals or changes in primary root extension rates. Root soil exploration in wheat was diminished for three cultivars when the roots were growing under high BD soils (Tracy *et al.*, 2011). There

were no significant differences in the way that the cultivars penetrated through the high impedance soil at days eight and 12 (Figure 4-13), all the cultivars slowed their growth to the same extent, in converse with that found in the initial screening. Whalley *et al.*, (2013) found similar results while studying the genotypic differences in the ability of wheat to penetrate through wax layers.

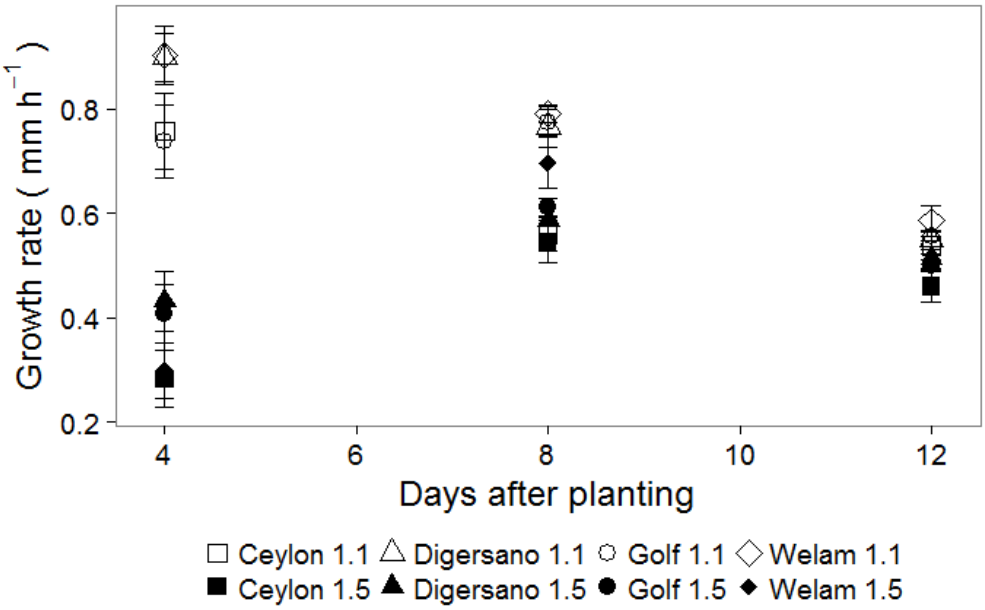


**Figure 4-13 Average growth rate (mm h-1) for the four selected genotypes (Ceylon, Digersano, Golf and Welam) at the three time point measured (4, 8 and 12 days after plating) growing in low buld density soil (1.1 g cm-3) or high bulk density soil (1.5 g cm-3). Error bars represent standard devation. n=6**

Key root architectural traits that could determine the size and distribution of the root system for both soil types, such as convex hull and centre of mass, were identified. The roots which had a faster growth extension rate at days eight and 12 after planting also had a greater convex hull and developed more primary roots and laterals. The morphology of root systems that explored a greater soil volume and had a faster development at early stages of growth in both soil types could lead to a root architecture which might be able to capture more water and N from the subsoil (Lynch, 2015).

Nevertheless, it is not known if the root systems which developed slower at the seedling stage achieve a similar root architecture to the one which develops faster at first, but over a shorter period of time. It needs to be taken into consideration that main axis extension rate slowed down to a greater extent in the variety with improved growth than in the slowest growing cultivar (Figure 4-9). The results from the vermiculite experiment (Figure 4-12) showed that the decrease in the root extension rate was caused by root physiology factors rather than soil conditions and it was expected that all cultivars will follow a similar root extension pattern over time, although the absolute values would be different. However, faster early root growth has been linked to improved nutrient

acquisition (Wang *et al.*, 2016) so the impact of the differences at that early stages should be studied with mature plants rather than only comparing the root systems at seedling stage.

Only the slower-growing cultivar in the screening (Ceylon) maintained a slower growth rate later in time. When differences in the root system architecture appeared between the cultivars it was only Welam which showed a better performance for both soil types. Nevertheless, Welam did not behave as predicted from the seedling screening since it was expected to have a fast extension rate in loose soil and amongst the slowest in extension rate for the high-density soil. The cultivar Welam, also had the heaviest seeds with a greater area compared to the other cultivars, which could have influenced the faster growing compared to the other cultivars since seed weight is associated with better performance of crops and higher yields (Ambika *et al.*, 2014). Although this only agrees with growth at days eight and 12.

In terms of shoot performance, Welam was also the cultivar with the greatest shoot dry weight and the longest shoot and leaves. This indicates that the cultivar which explored the soil to a greatest extent and faster was not allocating extra resources in the root system but the whole plant grew faster. It supports the idea of a better performing crop in general at

seedling stage which may lead to a better performing crop in loose and compacted soil since all the varieties were restricted to the same extent in high BD soil (Liao *et al.*, 2006; Rebolledo *et al.*, 2013; Ambika *et al.*, 2014; Kipp *et al.*, 2014; Thomas *et al.*, 2016). However, it has been shown in other studies where a positive relationship between root dry weight and shoot dry weight in wheat was not related with final yield because the irrigation affected it to a greatest extent than the genotype (Lipiec and Hatano, 2003; Ardvisson and Hakansson, 2014; Liu *et al.*, 2015).

From the results shown, plants exhibited genetic differences in their root system architecture and in their root extension rate, but it was difficult to predict whether these differences would persist over time. Variability between the samples increased over time and that caused some of the differences found at very early stages of growth to be non-significant. It was also found during the course of the experiment that variation in many of the traits measured increased over time. Every plant had an inherit capability to succeed in the medium they were grown in and some of them performed better than others due to the high plasticity of the root systems (Robinson 1994; Hodge 2004, Grossman and Rice, 2012), making it more difficult to draw conclusions because of the individual variation in the root system architecture.

## 4.5 Conclusions

The first hypothesis was supported with the data from the screening. The experiment showed how barley genotypes had different root extension rate when exploring soil with low and high densities. The interaction between soil conditions and cultivars demonstrated that some of the cultivars dealt better with certain conditions than other after four days of planting. In addition, the methodology developed allowed testing a medium range of cultivars. It can be a useful resource to reduce the number of cultivars for further analysis in laboratory conditions or to investigate their behaviour in a range of field conditions.

The second hypothesis was partially disregarded. The differences in root growth and interactions with the soil treatments were not maintained at later stages of growth. More mature plants did not behave as predicted from the screening partly because of the increasing level of individual variation found between older plants.

Although the interaction between soil physical characteristics and cultivars was lost, there were genotypic differences in root system architectures. Better performing cultivars were doing generally better, they had the longest root system, greater volume, convex hull and

amongst the greater number of laterals. High impedance soils decreased the growth on the whole but to a lesser extent for plants with faster developing roots. It reinforced the idea that fast early root growth may lead to better established crops.

# Chapter 5: Effects of crop genotype and soil physical conditions on nutrient capture from the subsoil.

## 5.1 Introduction

Nitrogen (N) fertiliser is an essential input to many agricultural systems in order to achieve the yields needed to satisfy future demands for food. However, currently the rate of input of those fertilisers in some production systems is so high that there can be a high risk of pollution into the wider environment, largely through nitrate leaching and greenhouse gas emissions, and the resultant economic cost is high (Dawson and Hilton, 2011).

To facilitate reductions in N fertiliser application, efforts are being made to increase the nutrient use efficiency of crops to maximise crop growth per unit of fertiliser added and to minimise losses from the system (Bishopp and Lynch, 2015; Thorup-Kristensen and Kirkegaard, 2016). A number of targets for improvement have been identified to help achieve this, ranging in scale from changes in nitrate transporter activity to modifications of root system architecture (Wirén *et al.*, 1997; Glass *et al.*, 2002; Garnett *et al.*, 2015; Paez-Garcia *et al.*, 2015). Root distribution in the soil profile and root system architecture are thought to play a key role in

efficient nutrient use, because a root system with the ability to grow extensively through the subsoil may be more effective at capturing soil resources (Foulkes *et al.*, 2009; Kell, 2011; Maeght *et al.,* 2013; Lynch, 2015). Root length density (RLD) in the subsoil is often considered to be insufficient for effective capture of nutrients and water.Modellingstudies have suggested that increasing RLD in the subsoil may increase water and N uptake, for the latter especially in nitrate leaching environments (Dunbabin *et al.,* 2003; King *et al.*, 2003). In addition, it has been argued there may be sufficient resources at depth to support plant growth and development and roots could be making better use of them (Salome *et al.*, 2010; Kautz *et al.*, 2013). Most breeding programs to date have however focused on improving desirable above-ground traits in order to increase yield. As the above- and below-ground traits are linked, breeding for increased yield may have inadvertently led to a smaller root system in wheat (White *et al.,* 2013). Crop improvement research is, therefore, focussed on quantifying variation in root system architecture and identifying the desired root traits and possible synergies between them so that yield improvements and the efficient use of soil resources can be combined (Kashiwagi *et al.*, 2005; Palta *et al.*, 2011; Herrera *et al.*, 2012; Acuna and Wade, 2013; Meister *et al.*, 2014; Ju *et al.*, 2015). To this end, crop genotypic variation has been found in root system length for

different cultivars of wheat, barley, *Brassica napus* and rice (Parzies *et al.*, 2000; Gahoonia and Nielsen, 2004; Shi *et al.*, 2013; Al-Shugeairy *et al.*, 2014; Canè *et al.*, 2014)

Although the mechanisms of nutrient capture are largely well described, along with traits such as root plasticity that allow plants to adapt to nutrient deficient conditions (Drew, 1975; López-Bucio *et al.*, 2003; Bingham *et al.*, 2010; Grossman and Rice, 2012; Smith and De Smet, 2012), fewer studies have focused on how roots make use of the resources in layered soils, for example in typical nutrient-rich topsoil with nutrient-poor subsoil (Barber, 1995). A point often overlooked when evaluating variation in root traits and in developing ideotypes for increasing nutrient use efficiency is the potential response of the root system to spatial variation in soil properties. Subsoils often have a lower organic matter content, lower nutrient bioavailability, higher bulk density and poorer soil structure than the overlying topsoil (Weier and MacRae, 1993). Morphological and physiology plasticity of root systems is widely documented and has been observed in response to a range of soil conditions including mineral nutrient availability and soil compaction (Drew, 1975; Robinson *et al.*, 1991; Robinson, 1994; Bingham and Bengough, 2003; Hodge, 2004). Thus, root systems can make compensatory adjustments by reducing growth in unfavourable soil

conditions and increasing growth in more favourable areas through the proliferation of lateral roots. In a similar way, roots are able to make physiological adjustments to increase rates of ion uptake in response to changes in concentrations at the root surface and internal cycling of nutrients within the plant (Robinson, 1994; Glass *et al.,* 2002; Garnet *et al.,* 2015). It is conceivable, therefore, that genotypes selected for deeper rooting in relatively uniform soil conditions, typical of many phenotypic screens, may not express the phenotype in field soils where topsoil and subsoil differ. Genotypes that are highly plastic may respond to relatively unfavourable conditions in the subsoil by preferentially locating roots in, and upregulating ion uptake, from the topsoil. By contrast those that are less plastic may grow and exploit resources from the subsoil more effectively. Few studies have focussed on how roots make use of resources in the subsoil when conditions in the topsoil are more favourable for growth. Different soil profiles have been shown to correlate with different matrix composition in the subsoil under field conditions (Appendix A; Lampurlanés *et al.,* 200;, Valentine *et al.,* 2012; Mangalassery *et al.,* 2014). In those subsoils where bulk density was high, roots were generally confined to the macropores, whereas in those soils with better structure, roots grew throughout the soil matrix and macropores. When maize was grown on stored soil water reserves in a

compacted soil, clustering of roots in cracks and biopores resulted in crop water stress even though potentially available water remained in the soil profile because of the inability of the roots to get out of the crack (Tardieu, 1994). In a similar way, the distribution of roots through the subsoil is also likely to influence the ability of the crop to access supplies of N. Thus, the extent to which a genotype might be able to exploit subsoil N would be expected to depend on both the relative plasticity of the genotype and also the structural conditions in the subsoil.

The overall aim of research in this chapter was to investigate the importance of root length density in the subsoil for nitrogen capture under realistic subsoil conditions and to determine the response of different genotypes to varying subsoil conditions when combined with a topsoil favourable for root growth. The first hypothesis tested was that soil structure influences root extension in the subsoil and consequently the ability of the root system to take up nitrogen from depth. The second hypothesis tested was that those cultivars with a greater rate of root extension and deeper root system identified in Chapter 4 will explore the soil matrix to a greater extent and uptake more N from the subsoil than the cultivars with a slower growing and shallower root system.

## 5.2 Materials and Methods

### 5.2.1 Soil preparation

Soil was collected from SRUC's Boghall farm (latitude 55.876°N, longitude 3.203°W) from an experimental site where no nutrient fertiliser and pesticides had been applied in the previous eight months. The soil belonged to the MacMerry series (Soil Information for Scottish Soils, http://sifss.hutton.ac.uk/SSKIB_Stats.php) and Cambisol (FAO classification). The previous crop grown in the field was spring barley. Topsoil was collected from the layer 0-20 cm from the soil surface and subsoil from the layer 30-50 cm. Topsoil was a sandy clay loam (the same as that used in 3.3.3 Soil characteristics and 4.2.2 Soil column preparation) and the subsoil was a sand soil. Topsoil and subsoil were sieved to a particle size < 5 mm. Soil mineral nitrogen (ammonium plus nitrate and nitrite) and pH were measured immediately after the soil was collected. Samples of soil (approximately 10 g fresh weight) were weighed to the nearest mg and extracted with 20 ml 2M KCl at room temperature for 2 h (Robertson *et al.*, 1999). The soil suspensions were agitated continuously during the process using an orbital shaker and finally centrifuged for 15 minutes at 7000 rpm to sink the soil particles. The concentrations of nitrate (plus nitrite) and ammonium in the extracts

was determined colourimetrically using a continuous flow auto-analyser (Skalar Analytical B.V., The Netherlands). Soil pH was measured using a CD620 digital pH meter (Wolf Laboratories Ltd., UK) on suspensions of 10 g of soil in 20 ml of deionised water. A further 15 g sample of fresh soil was oven dried at 103 $^{o}$C for 48 h for determination of gravimetric soil moisture content.

Dual labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) was added to the subsoil to obtain a 10% $^{15}N$ enrichment of the soil mineral N pool, $^{15}NH_4{}^{15}NO_3$ (98 atom % $^{15}N$, Sigma Aldrich Company Ltd, UK) was dissolved in deionised water to a concentration of 0.9 μg ml$^{-1}$ water and mixed thoroughly with the soil in 4 kg fresh weight soil batches to give an N addition of 4.8 μg N g$^{-1}$ dry soil equivalent. The soil was stored in plastic bags at 4$^{o}$C overnight to allow the moisture to equilibrate fully through the soil. Soil mineral N was quantified as described above to check that the mineral N content had risen 10% from the initial content.

The columns used for plant growth were PVC/plastic pipe of 7 cm inner diameter and cut to 60 cm length. An inner plastic sleeve was inserted in the column (Damp-Proof Membrane 1000ga – Screwfix, UK) to facilitate extracting the soil as an intact core at the end of the experiment.

Four base soil types were used in varying combinations: topsoil at low compaction (1.2 g $cm^{-3}$) and subsoil which was packed loose (1.2 g $cm^{-3}$) or compacted (1.5 g $cm^{-3}$) (Table 5-1). The later with or without macropores. The moisture content for the topsoil and subsoils were maintained at -10 kPa and -15 kPa respectively to determine the gravimetric water content required for each treatment and soil section. Additional PVC columns with 5 cm inner diameter and 10 cm length were packed at the loose and compacted subsoil densities and their matric potential adjusted using tension tables. Soil MC was then determined after oven drying the soil at 103 $^{o}$C for 48 h. The amount of topsoil and subsoil necessary for each column was calculated on a dry basis and then corrected for the MC of the soil and the MC content of the soil was adjusted prior to packing by the addition of water.

The packing comprised a 40 cm depth of subsoil overlaid with a 19 cm depth of topsoil. Subsoil was packed to achieve three different subsoil physical conditions (Table 5-1). Loose subsoil (dry bulk density 1.2 g $cm^{-3}$) was intended to act as minimal impeding subsoil where roots could grow without subsoil physical limitations. The other two subsoil treatments were designed to create impedance for root growth by means of high density soil (dry bulk density 1.5 g $cm^{-3}$). One of the treatments was a homogeneously compacted subsoil (compaction treatment) whilst

the other one was a compacted soil containing artificial biopores (macropore treatment). To fill the columns, soil was manually packed in 3 cm layers and the surface of each layer was slightly scarified to help the next layer to bind (Lewis and Sjostrom, 2010). The macropores were created after packing the subsoil, but before adding the topsoil, by inserting a 1.5 mm diameter wire through the full subsoil profile until it emerged from the bottom of the column. A template was created for macropores distribution. Macropores were created in a random distribution pattern on the surface of the subsoil such that the pores comprised 10% of the planar area of the column based on the macropore quantification published by Stewart et al., 1999 (Figure 5-1). The same template was used for each replicate of the macropore treatment. The edges of the soil columns were compacted to a higher bulk density (BD) than the target BD to minimise root growth down the edges of the column. The manual compactor had an inverted pyramidal shape with a base of 5 mm, the angle from the vertical was $90^{o}$ and the opposite angle $45^{o}$ (Figure 5-2). Topsoil was always packed as loose soil (1.2 g $cm^{3}$).

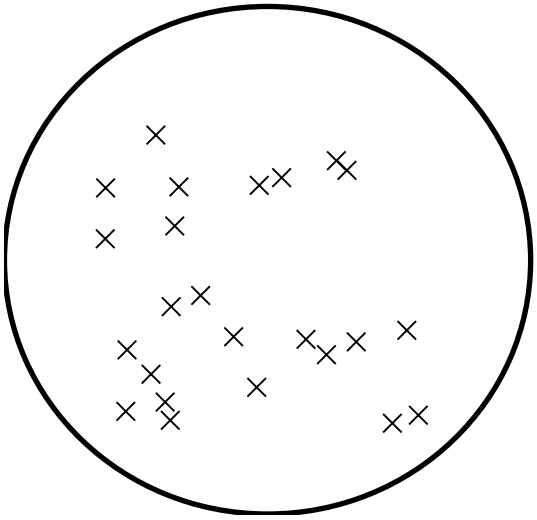

**Figure 5-1 Planar view showing the pattern of artificial macropores inserted into the compacted subsoil.**

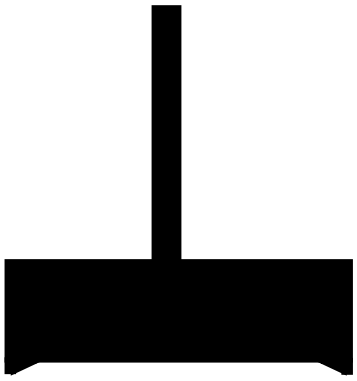

**Figure 5-2 Lateral view of the soil compactor.**

**Table 5-1 Selected soil physical properties of the soils examined in the experiment.**

| Layer | Treatment | Soil texture | Dry BD ($g\ cm^{-3}$) | MC ($v\ v^{-1}$) | Matric potential (kPa) |
|---|---|---|---|---|---|
| Topsoil | Loose | Sandy clay loam | 1.2 | 18.0 | -10 |
| Subsoil | Loose | Sand | 1.2 | 17.3 | -15 |
| | Compaction | Sand | 1.5 | 19.1 | -15 |
| | Macropores | Sand | 1.5 | 19.1 | -15 |

### 5.2.2 Plant preparation

Three different cereal cultivars were used for the experiment, spring oat (*Avena sativa*) cv. Firth, and spring barley (*Hordeum vulgare*) cvs. Welam and Ceylon. The barley cultivars were chosen because they showed the most contrasting root architectural traits in earlier experiments (Chapter 4). Oats were included to increase the possible contrast between genotypes as oats have a lower fertilizer N requirement than barley (Bingham, unpublished results).

Untreated seeds were pre-germinated prior to planting in the soil columns. Seeds were placed on tissue paper (Tork, Basic paper 1 Ply, dimensions 35 x 19.4 cm, SCA Hygiene Products, Dunstable, Bedfordshire) moistened with deionised water and then rolled into a

cylinder. The outer surface of the rolled paper was covered with aluminium foil to exclude light from the seed and developing root and to help to maintain the roll in an upright position. The cylinder roll was placed on one end in an upright position inside a 250 ml beaker with its lower-most end immersed in a 25 ml reservoir of water. The embryo of the seed was placed facing downwards to allow the root to grow in a vertical direction. They were germinated in a controlled environment chamber (Fitotron, Sanyo, Weiss Technik UK, UK) with 16:8 h photoperiod light: dark period with light supplied by fluorescent lamps at an intensity of 130 μmoles $m^{-2}$ $s^{-1}$ of photosynthetically active radiation and 60% relative humidity. Set point temperature was, 18:13$^{o}$C day: night. After three days the radicle reached between 1.5 and 2.0 cm in length. Seeds whose radicles were of approximately equal size were selected and the length of radicle measured to the nearest mm with a ruler. Germinated seeds were then planted into the soil columns with their radicles inserted in a 3 mm diameter hole and the seed covered with 1 cm depth of extra sieved topsoil.

### 5.2.3 Plant growth and nutrient treatments

The planted columns were incubated in a controlled environment chamber under the same conditions as the germination period. Soil

columns were weighed every 3 days and water was added from the top to compensate for water loss through transpiration and surface evaporation. N fertiliser in the form of $NH_4NO_3$ was applied on 3 occasions dissolved in distilled water at a concentration 0.6 mg $ml^{-1}$ over the first two weeks (day 5, 8 and 12 from planting) of the experiment to a final dose rate equivalent to 60 kg N $ha^{-1}$ for a plant population of 260 plants $m^{-2}$. This was lower than the recommended rate of 130 kg $ha^{-1}$ for field grown spring malting barley to 1) account for the lower growth potential in the controlled environment and 2) provide conditions of sub-optimal N supply so that the demand for subsoil N would be greater.

After 3 weeks of growth, the plants starting showing symptoms of manganese (Mn) deficiency (Figure 5-3). Plants were sprayed with manganese sulphate ($MnSO_4$) dissolved in deionised water to a concentration of 3.60 mg $ml^{-1}$ of Mn following the recommended Mn application in the field (YaraVita, Yara UK Ltd, UK). The application was made using a hand mister three times during the course of two weeks (day 21, 24 and 28 after planting) and the symptoms ceased increasing. After 3 weeks of growth a green polyester mesh was placed around every column to provide support to the plants and mimic the effects of neighbouring plants. The mesh had a height of 20 cm and the mesh size was 2 mm x 2 mm (Insect mesh, Sure Green, UK). The experimental

design had six replicate blocks of each individual soil treatment and cultivar combination. Treatment-cultivar combinations were fully randomised within each block.


**Figure 5-3 Manganese deficiency symptoms in a) oat leaves, b) barley cv. Welam and c) barley cv. Ceylon.**

### 5.2.4 Harvest

Plants were harvested 6 weeks after transplanting, when they were at the flag leaf emerged stage (growth stage 39, (Zadoks *et al.*, 1974)). Harvesting took place a block at a time over a period of 6 days. The leaves and stem of the plants were harvested. The shoot length was recorded (measured from the top of the seed to the tip of the longest leaf) and leaf and stem projected area determined with a Li-3100 Area Meter (LI-COR, Inc., USA). Number of tillers and number of leaves were counted. To assess the severity of damage caused by Mn deficiency, the symptoms were classified according to the area of the leaves affected at harvest and a weighted mean calculated where the total number of main shoot and tiller leaves was taken into account. The damage on the leaves was classified as: less than 25% damage, between 25-50% damage, more than 50% and dead leaf. The weight of each category corresponded to 0.1; 0.2; 0.3 and 0.4 respectively, it was multiplied for the number of leaves at each category and finally it was divided by the total number of leaves present in the plant. Plant tissue was subsequently dried at 60$^{o}$C for 48 h, weighed to the nearest 0.1 mg and then milled to < 100 μm particle size using a mixer mill MM 200 (Retsch GmbH, Germany).

The soil core was removed from the column by pulling out the plastic sleeve lining and laying the core horizontally on a bench. The core was cut into three sections, 0-20 cm (topsoil) 20-40 cm (subsoil 1) and 40-60 cm (subsoil 2). Digital photographs were taken of each cut surface for a record of root distribution. A sample of approximately 100 g of soil, free of visible roots, was taken evenly from the topsoil and a further combined sample from subsoil sections 1 and 2 prior to root washing. Samples were stored in plastic bags at 4$^{o}$C for up to 7 days prior to processing.

Roots were washed from the remainder of the soil in running water over a 2 mm sieve under. Roots were then placed in a film of water in a transparent Perspex tray, scanned in grey scale at 300 dpi resolution using an Epson Expression 836XL scanner (Regent Instruments Inc., Canada) and images saved in Tiff file format. Images were then analysed using WinRhizo software (Regent Instruments Inc., Canada) to give measurements of total root length, surface area, projected area, volume, diameter and the same parameters by root diameter class for each soil layer. After scanning, roots were gently blotted to remove surface water and oven dried at 60$^{o}$C for 48 h, weighed and milled to < 100 μm particle size.

The stored soil samples were thoroughly mixed before approximately 10 g samples were taken, weighed to the nearest mg and extracted in KCl for determination of $N0_3^-$ and $NH_4^+$ as described above. The rest of the sample was oven dried at 60ºC for 48 h and milled at 100 μm particle size for isotopic N analysis. Isotopic analysis of N in topsoil, subsoil, and root and shoot tissue was carried out by Iso-Analytical Ltd, UK, using an Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS).

### 5.2.5 Statistical analysis

Statistical analysis was performed using R software (R Development Core Team, 2016), packages lmer (Bates *et al.*, 2015), lmerTest (Kuznetsova *et al.*, 2016), dplyr (Wickham and Francois, 2015) and ggplot2 (Wickham, 2009). Data were checked for normality using the Shapiro-Wilk test and for homogeneity by visualising the boxplot diagram and when these conditions were not met data were transformed either using a logarithmic scale or boxCox transformation using the car package (Fox and Weisberg, 2011). Data were modelled using REML; fixed factors were the soil treatment and crop and, when applicable, soil layer. Random factors were the replicate and when soil layers were used also the column number to identify where the soil layers belonged to the same individual plant. After initial model was fitted, backwards

stepwise simplification of the model was performed using the MASS package (Venables and Ripley, 2002) relative to the AIC values. Variables that were transformed for analysis were back transformed for graphical representation.

## 5.3 Results

### 5.3.1 Plant growth

#### 5.3.1.1 Plant dry weight and leaf area

There were differences amongst the cultivars in overall plant dry weight (DW) and also in root and shoot DW (Table 5-2), but not between the soil treatments. Oat had the smallest DW for both root and shoot with a 35% lower root DW and a 22% lower shoot DW than Welam, the barley cultivar with the largest dry weight. Ceylon was intermediate between these two other cereals, but it was not significantly different from Welam (Table 5-2, Figure 5-4). Total plant DW was not affected by the soil treatment (Table 5-2) at approximately (1.73 g per plant) (Figure 5-4).

The percentage of total plant DW allocated to roots was similar for Welam and Ceylon, but 3% smaller for oat (Figure 5-5). In addition, leaf area was similar for Ceylon and Welam, but 50% smaller for oat (Table 5-2, Table 5-3, Figure 5-6). On average, oat produced a single main shoot,

while the barley cultivars produced a main shoot and 2 or more tillers each (Appendix B).

**Table 5-2 Linear mixed models calculated for each of the parameters measured along the experiment and the P-values attached to the model comparison with the null model ($P_{model}$) and the significance of all the experimental factors and the interactions when present. NS (not significant).**

| Parameter | Trans | Final model | $P_{model}$ | $P_{crop}$ | $P_{treatment}$ | $P_{layer}$ | $P_{interaction}$ |
|---|---|---|---|---|---|---|---|
| Plant dry weight | | crop + (1 \| replicate) | <0.001 | <0.001 | NS | | |
| Root dry weight | | crop + (1 \| replicate) | 0.01 | 0.01 | NS | | |
| Shoot dry weight | | crop + (1 \| replicate) | 0.024 | 0.024 | NS | | |
| Root percentage | | crop + (1 \| replicate) | 0.02 | 0.02 | NS | | |
| Leaf area | log | crop + (1 \| replicate) | <0.001 | <0.001 | NS | | |
| Mn deficiency | | treatment + (1 \| replicate) | 0.01 | NS | 0.01 | | |
| N in roots (mg) | | crop + (1 \| replicate) | <0.001 | <0.001 | NS | | |
| N in stem (mg) | | crop + (1 \| replicate) | <0.001 | NS | NS | | |
| Total plant N (mg) | | (1 \| replicate) | NS | NS | NS | | |
| N conc in roots | | (1 \| replicate) | NS | NS | NS | | |
| N conc in shoots | | crop + (1 \| replicate) | <0.001 | <0.001 | NS | | |
| $^{15}N$ in roots (mg) | | crop + (1 \| replicate) | <0.001 | <0.001 | NS | | |
| $^{15}N$ in stem (mg) | | (1 \| replicate) | NS | NS | NS | | |
| Total 15N | | crop + (1 \| replicate) | 0.03 | 0.03 | NS | | |

| Parameter | Trans | Final model | $P_{model}$ | $P_{crop}$ | $P_{treatment}$ | $P_{layer}$ | $P_{interaction}$ | |
|---|---|---|---|---|---|---|---|---|
| %$^{15}$N atom in roots from total N | | crop + treatment + (1 \| replicate) | <0.001 | <0.001 | 0.02 | | | |
| % $^{15}$N atom in shoots from total N | | crop * treatment + (1 \| replicate) | <0.001 | <0.001 | 0.01 | | | |
| $^{15}$N uptake per subsoil root length (mg cm$^{-1}$) | | crop + treatment + (1 \| replicate) | <0.001 | <0.001 | 0.003 | | | |
| Total root length | BoxCox ($\lambda$=0.45) | crop + treatment * layer + (1 \| replicate / column) | <0.001 | <0.001 | <0.001 | <0.001 | treatment* layer | 0.03 |
| Root surface area | BoxCox ($\lambda$=0.25) | crop + treatment * layer + crop : layer + (1 \| replicate / column) | <0.001 | <0.001 | 0.03 | <0.001 | crop*layer | 0.03 |
| Root volume | BoxCox ($\lambda$=-0.67) | crop * layer + (1 \|replicate / column) | <0.001 | <0.001 | NS | <0.001 | crop*layer | <0.001 |
| Root diameter | log | treatment + layer + (1 \| replicate / column) | <0.001 | NS | <0.001 | <0.001 | | |
| Soil ammonium conc | log | layer + (1 \| replicate / column) | <0.001 | NS | NS | <0.001 | | |

| Parameter | Trans | Final model | $P_{model}$ | $P_{crop}$ | $P_{treatment}$ | $P_{layer}$ | $P_{interaction}$ | |
|---|---|---|---|---|---|---|---|---|
| Soil nitrate conc | log | crop + treatment + (1 \| replicate / column) | <0.001 | <0.001 | 0.04 | | | |
| Soil total N conc | | treatment * layer + (1 \| replicate / column) | <0.001 | NS | 0.03 | <0.001 | treatment*layer | 0.001 |
| Soil $^{15}N$ conc | | crop * layer + (1 \| replicate / column) | <0.001 | 0.03 | NS | <0.001 | crop*layer | 0.01 |

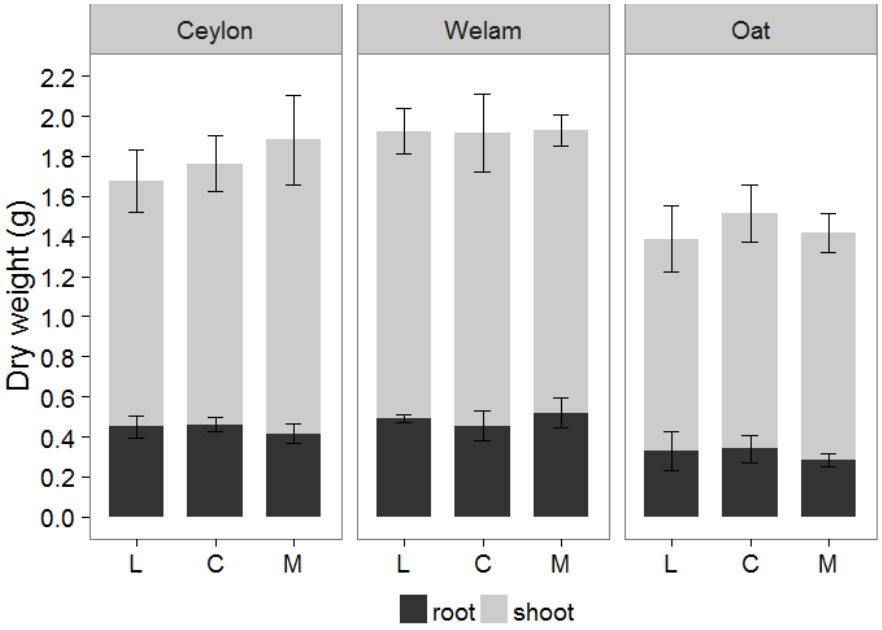


**Figure 5-4 Plant DW (g) for barley cultivars Ceylon and Welam and oat in three subsoil treatments: L (loose), C (compaction), M (macropores). Root DW is the black fill and shoot DW the light grey fill. Error bars show ±SEM for both the root DW and the shoot DW. Values are means of n=6 replicates.**

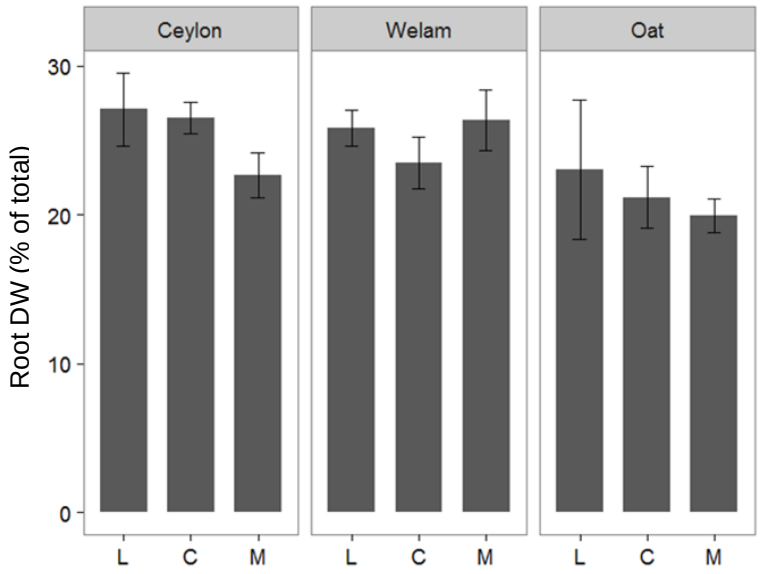


**Figure 5-5 Root dry weight as a percentage of total dry weight for barley cultivars Ceylon and Welam and oat in three subsoil treatments: L (loose), C (compaction), M (macropores). Error bars represent ±SEM. Values are means of n=6 replicates.**

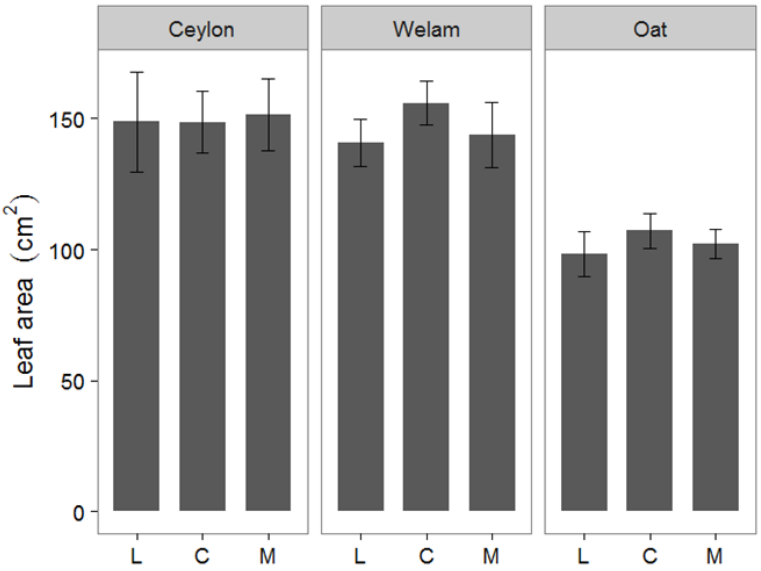


**Figure 5-6 Leaf area (cm²) per plant of barley cultivars Ceylon and Welam and oat for three subsoil treatments: L (loose), C (compaction), M (macropores). Error bars represent ±SEM. Values are means of n=6 replicates of the back transformed data.**

**Table 5-3 Main effect means of crops and subsoil treatments averaged over the other factors with the LSD values at 5% confidence levels. NS, means not significantly different (P-value>0.05)**

| Main effect | Root DW (g) | Shoot DW (g) | Root DW (% total) | Leaf area (cm2) | Mn deficiency (proportion of total leaf area) |
|---|---|---|---|---|---|
| Crop | | | | | |
| Ceylon | 0.44 | 1.33 | 25.43 | 149.6 | 0.14 |
| Welam | 0.49 | 1.44 | 25.23 | 102.6 | 0.13 |
| Oat | 0.32 | 1.12 | 21.28 | 146.8 | 0.14 |
| LSD 5% crop | 0.082 | 0.192 | 3.311 | 15.81 | NS |
| Soil treatment | | | | | |
| Loose | 0.42 | 1.24 | 25.32 | 129.2 | 0.16 |
| Compaction | 0.42 | 1.31 | 23.73 | 137.2 | 0.14 |
| Macropores | 0.41 | 1.34 | 23.98 | 132.5 | 0.10 |
| LSD 5% treatment | NS | NS | NS | NS | 0.0326 |

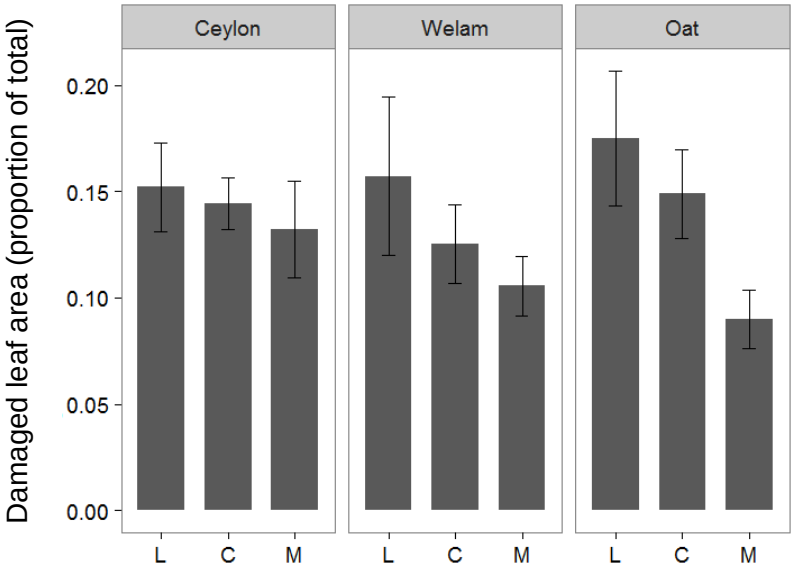


**Figure 5-7 Manganese deficiency symptoms, calculated as the weighted mean proportion of leaf area affected for barley cultivars Ceylon and Welam and oat in the three susoil treatments: L (loose), C (compaction), M (macropores). Error bars represent ±SEM. Values are means of n=6 replicates.**

Mn deficiency symptoms did not differ between crops, but plants growing in loose soil were more affected by Mn deficiency symptoms than plants growing in the compaction treatment; the least affected plants were those growing in the macropore treatment (Table 5-2 and Figure 5-7). There were no differences between the three crops and no interactions between the soil treatments and the crop.

#### 5.3.1.2 Nutrient uptake

Total N content in the plant (roots and shoots) did not differ significantly between soil treatments or crops (Table 5-7, Table 5-2) and nor did it for shoot N content. However, there were differences between crops in the

root N content. N quantity was smaller for oat compared to Ceylon and Welam (Table 5-4, Figure 5-8).

Shoot tissue N concentration (as % of DW) was highest in oat followed by Ceylon and Welam (Table 5-2, Table 5-4). Root tissue N concentration was not statistically significant among crops or soil treatments (Table 5-2).

Total $^{15}N$ uptake (roots and shoot combined, Figure 5-9) was lower in oat compared to the barley cultivars Welam and Ceylon. In roots, the $^{15}N$ atom % of total N was 0.5% greater in oat than Welam or Ceylon (Table 5-4). By contrast, in the shoots, Ceylon had the greatest $^{15}N$ atom %, followed by Welam and oat (Table 5-4).

There was a strong positive relationship between total N uptake and $^{15}N$ uptake (Figure 5-10), thus as N uptake increased the total uptake of $^{15}N$ increased also.

**Table 5-4 Significant main effect means of crop and soil treatments averaged over the other factors with the LSD values at 5% confidence levels.**

| Main effects | Root N content (mg) | % N in shoots from DW | Root $^{15}N$ content (mg) | Total plant $^{15}N$ content (mg) | %$^{15}N$ from atoms N roots | %$^{15}N$ from atoms N shoots |
|---|---|---|---|---|---|---|
| Crop | | | | | | |
| Ceylon | 8.42 | 2.84 | 0.33 | 1.83 | 3.96 | 4.23 |
| Welam | 8.75 | 2.67 | 0.32 | 1.69 | 3.58 | 3.91 |
| Oat | 5.30 | 3.37 | 0.22 | 1.62 | 4.08 | 3.54 |
| LSD 5% | 1.032 | 0.127 | 0.048 | 0.073 | 0.276 | 0.364 |
| Treatment | | | | | | |
| Loose | 7.99 | 2.95 | 0.28 | 1.65 | 3.52 | 3.75 |
| Compaction | 7.27 | 3.02 | 0.29 | 1.67 | 3.92 | 3.75 |
| Macropores | 7.22 | 2.90 | 0.29 | 1.82 | 4.19 | 4.17 |
| LSD 5% | NS | NS | NS | NS | 0.277 | 0.365 |

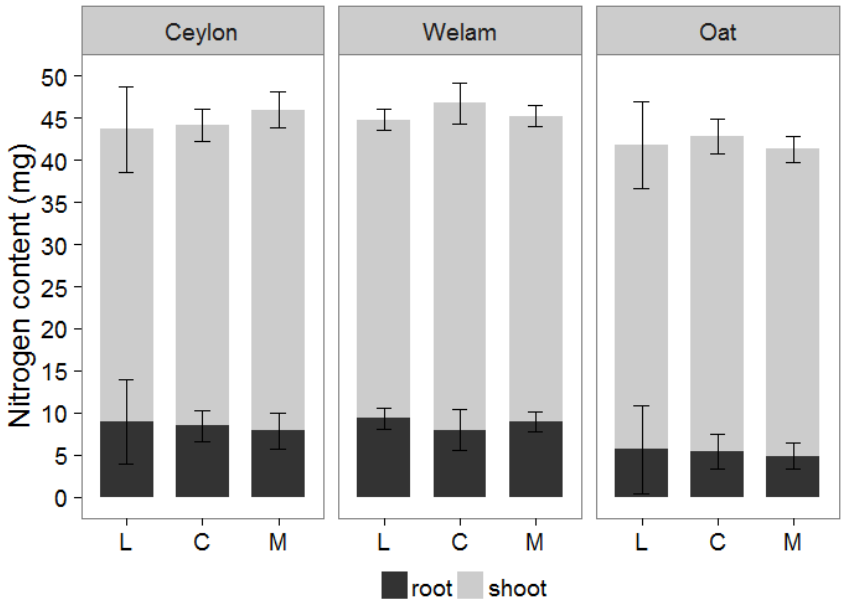


**Figure 5-8 Total N content (mg plant$^{-1}$) for barley cultivars Ceylon and Welam and oat and three soil treatments: L (loose), C (compaction), M (macropores). Root N content is the black fill and shoot N content the light grey fill. Error bars show the ±SEM for both the root N and shoot N content. Values are means of n=6 replicates.**

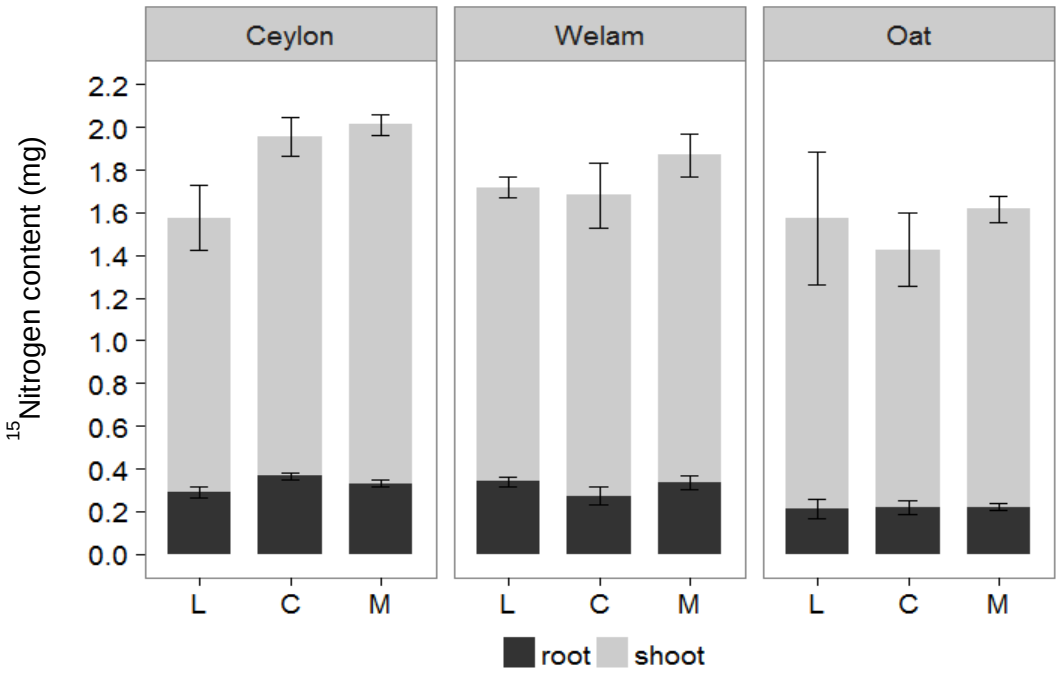


**Figure 5-9 Total $^{15}N$ content (mg plant$^{-1}$) for barley cultivars Ceylon and Welam and oat and three subsoil treatments: L (loose), C (compaction), M (macropores). Root $^{15}N$ content is the black fill and shoot $^{15}N$ content the light grey fill. Error bars show ±SEM for both the root and the shoot $^{15}N$ contents. Values are means of n=6 replicates.**

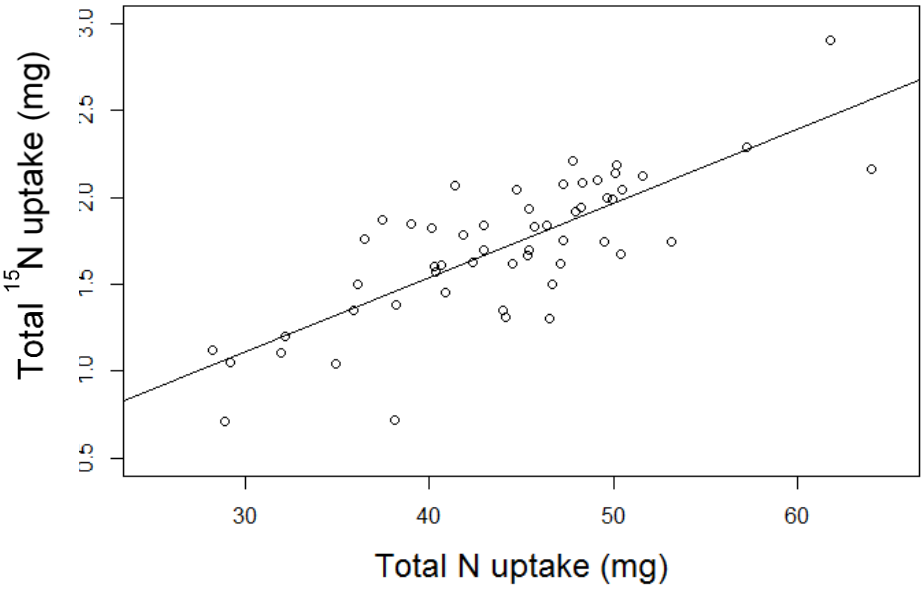


**Figure 5-10 Regression between total $^{15}$N uptake (mg) and N uptake (mg) for all the samples taken. Each point represents an individual replicate. (F-value= 80.54, df=1, P-value<0.001, $R^2$=0.61).**

### 5.3.2 Root distribution in the soil profile

Total root length differed significantly between crops, soil depth and soil treatment (Figure 5-11, Table 5-2). Welam had the greatest root length and oat the smallest with a total length 66% of that of Welam (Figure 5-11, Table 5-5). In addition, root length was 23% greater in the loose soil treatment compared to the compacted and macropore treatments (Figure 5-11, Table 5-5) which were almost equal. Averaged across crops, root length was approximately three times longer in the topsoil compared to any of the two subsoil layers (Figure 5-11, Table 5-5).

There was a significant interaction between soil treatment and soil layer (Table 5-2). Root length was similar in the topsoil for all the three

treatments regardless of the subsoil conditions, but the length in the subsoil (sum of each subsoil layer) was greatest in the loose treatment compared to the compacted and macropore treatment (Figure 5-11).

When averaged across crops, in the compaction and the macropores treatments, the overall root length was not significantly different. However, the root length distribution in the subsoil layers varied with soil treatment (Table 5-6).

Root surface area differed significantly between crops, treatments and soil layers (Table 5-2) and followed the same pattern as the root length (Figure 5-11 and Figure 5-13). Averaged over soil treatments, total root surface area was smallest in oat; 41% smaller compared to the largest cultivar Welam (Table 5-5). Root surface area was largest in the loose soil treatment whilst values for the compaction and macropores treatments were similar (Table 5-5). The largest root surface area was found in the topsoil, almost four times greater than the first layer of the subsoil (subsoil 1, 20-40 cm depth) which was the smallest (Figure 5-12).

A significant interaction between soil layer and crop was caused by the root surface area of oat being similar to the two barley cultivars in the subsoil 2 layer, but was smaller in all other layers (Table 5-6).

**Table 5-5 Significant main effect means of crop, soil treatments and soil layers averaged over the other factors with the LSD values at 5% confidence levels.**

| Main effect | Total root length cm | Root surface area $cm^2$ | Root volume $cm^3$ | Root diameter mm |
|---|---|---|---|---|
| Crop | | | | |
| Ceylon | 1091 | 144.2 | 1.55 | 0.42 |
| Welam | 1243 | 176.2 | 2.05 | 0.42 |
| Oat | 826 | 103.3 | 1.05 | 0.41 |
| LSD 5% | 107.9 | 16.95 | 0.242 | NS |
| Treatment | | | | |
| Loose | 1205 | 154 | 1.63 | 0.39 |
| Compaction | 988 | 135.7 | 1.55 | 0.44 |
| Macropores | 967 | 134 | 1.52 | 0.42 |
| LSD 5% | 107.9 | 16.95 | NS | 0.027 |
| Soil layer | | | | |
| Topsoil | 1893 | 269.9 | 3.12 | 0.46 |
| Subsoil 1 | 619 | 70.9 | 0.66 | 0.37 |
| Subsoil 2 | 648 | 82.9 | 0.87 | 0.42 |
| LSD 5% | 107.9 | 16.95 | 0.242 | 0.0268 |

**Table 5-6 Significant interaction means of crop, soil treatments or soil layers averaged over the other factors with the LSD values at 5% confidence levels.**

| | Topsoil | Subsoil 1 | Subsoil 2 |
|---|---|---|---|
| Total root length (cm) | | | |
| Compaction | 1835 | 575 | 555 |
| Loose | 1990 | 833 | 792 |
| Macropores | 1854 | 450 | 596 |
| LSD 5% treatment* soil layer | | | 168.8 |
| Root surface area ($cm^2$) | | | |
| Ceylon | 279.9 | 72.4 | 80.3 |
| Welam | 349.4 | 88.3 | 90.9 |
| Oat | 180.5 | 51.9 | 77.5 |
| LSD 5% crop* soil layer | | | 29.36 |
| Root volume ($cm^3$) | | | |
| Ceylon | 3.183 | 0.653 | 0.817 |
| Welam | 4.4 | 0.833 | 0.919 |
| Oat | 1.792 | 0.493 | 0.875 |
| LSD 5% crop*soil layer | | | 0.42 |

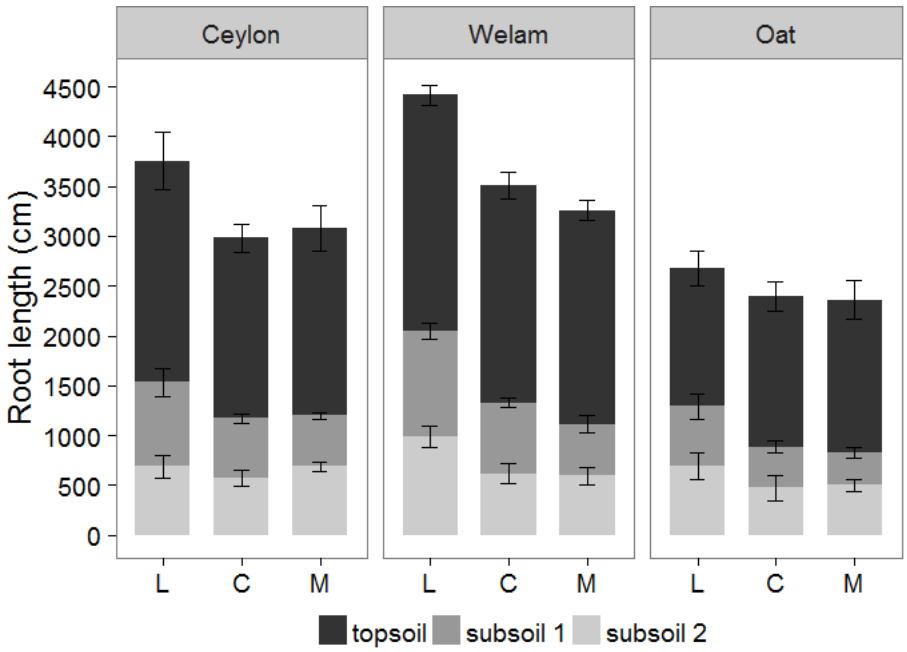


**Figure 5-11 Total root length (cm) for barley cultivars Ceylon and Welam and oat and three subsoil treatments: L (loose), C (compaction), M (macropores). Root length in the topsoil is the black fill, root length in the subsoil layer 1 (upper subsoil 20-40 cm depth) is medium grey and root length in the subsoil layer 2 (lowe subsoil 40-60 cm depth) is the light grey. Error bars show ±SEM for the root length of the cultivar in the soil treatment and soil layer. Values are means of n=6 replicates of the back transformed data.**

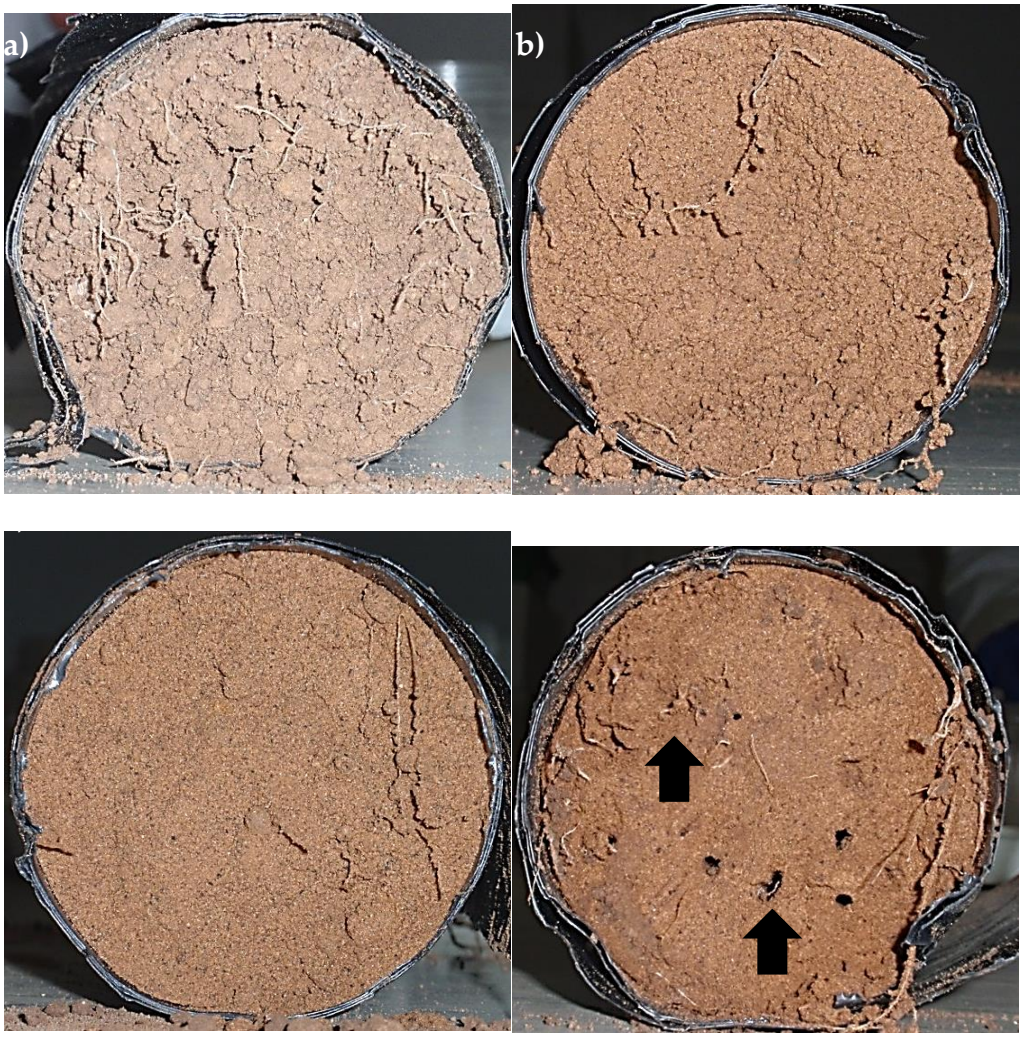


**Figure 5-12 Images of soil columns (diameter 7 cm) broken open to show a) loose topsoil with roots, b) loose subsoil with roots, c) compacted subsoil with fewer roots (note roots growing at the edge of the column), and d) macropores subsoil with most of the roots growing in the artificial macropores. Arrows in d) indicate roots growing through the macropores.**

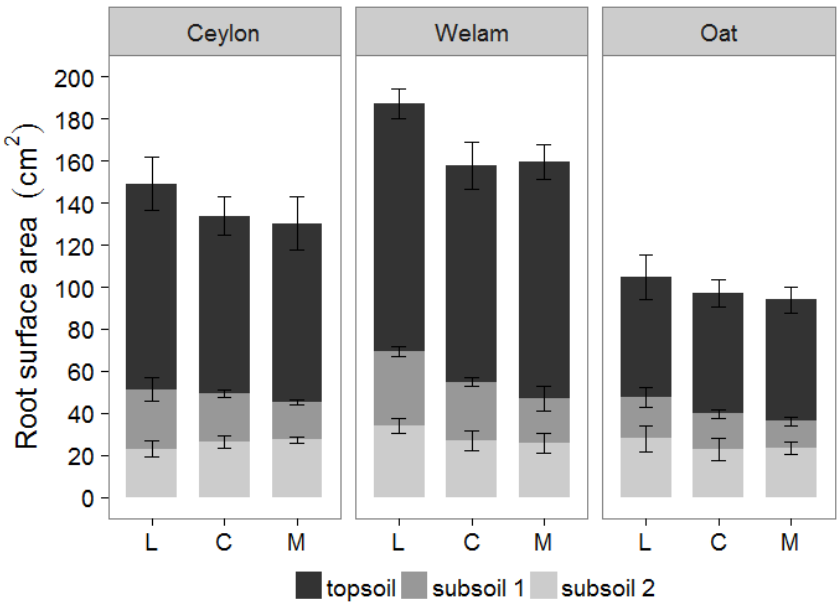


**Figure 5-13 Total root surface area ($cm^2$) for barley cultivars Ceylon and Welam and oat and three subsoil treatments: L (loose), C (compaction), M (macropores). Root surface area in the topsoil is the black fill, in the subsoil layer 1 (upper subsoil, 20-40 cm depth) is medium grey and in the subsoil layer 2 (lower subsoil, 40-60 cm) is the light grey. Error bars show ±SEM for the root surface area of the cultivar in the soil treatment and soil layer. Values are means of n=6 replicates of the back transformed data.**

There were no differences in root volume between the soil treatments, although the crops behaved differently (Table 5-2). Root volume of Welam was twice that of oat, with Ceylon intermediate (Figure 5-14, Table 5-5).

Root volume was largest in the topsoil and smallest in the subsoil 1 (Table 5-5). There was a significant interaction between soil layer and crop due to differences between varieties in their root volume in the lower subsoil layer compared to the upper subsoil and topsoil two

subsoil layers (Figure 5-14, Table 5-6). In subsoil 2 (lower subsoil), root volume was similar between the crops, while in the topsoil and in subsoil 1, root volume was largest for Welam and smallest for oat.

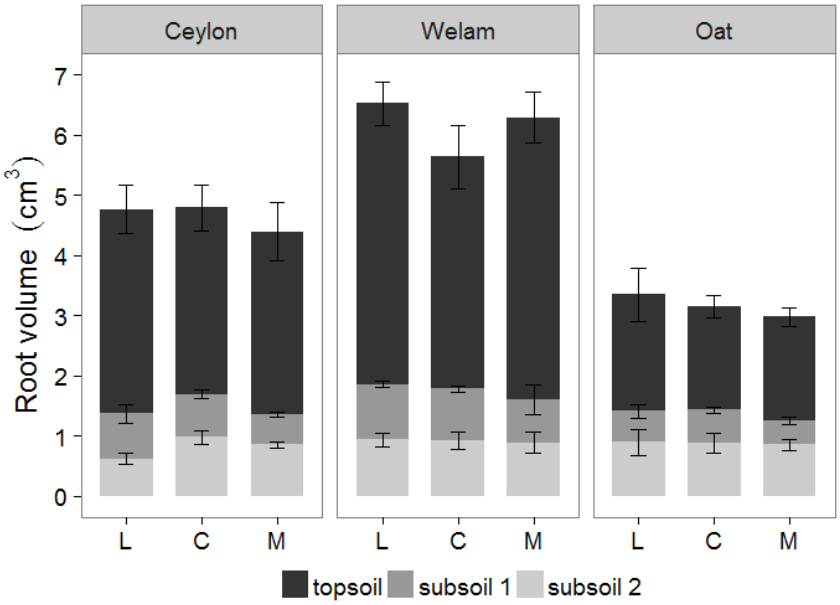


**Figure 5-14 Total root volume (cm3) for barley cultivars Ceylon and Welam and oat and three subsoil treatments: L (loose), C (compaction), M (macropores). Root volume in the topsoil is the black fill, in subsoil layer 1 (upper subsoil, 20-40 cm) is medium grey and in subsoil layer 2 (lower subsoil, 40-60 cm) is the light grey. Error bars show ±SEM for the root volume of the cultivar in the soil treatment and soil layer. Values are means of n=6 replicates of the back transformed data.**

Root diameter (Figure 5-15) did not differ between crops, but it did differ between soil treatments and soil layers (Table 5-2). Root diameters were smallest in the loose soil treatment and largest in the soil compaction treatment. Root diameter was largest in the topsoil and smallest in subsoil 1 layer (Table 5-5).

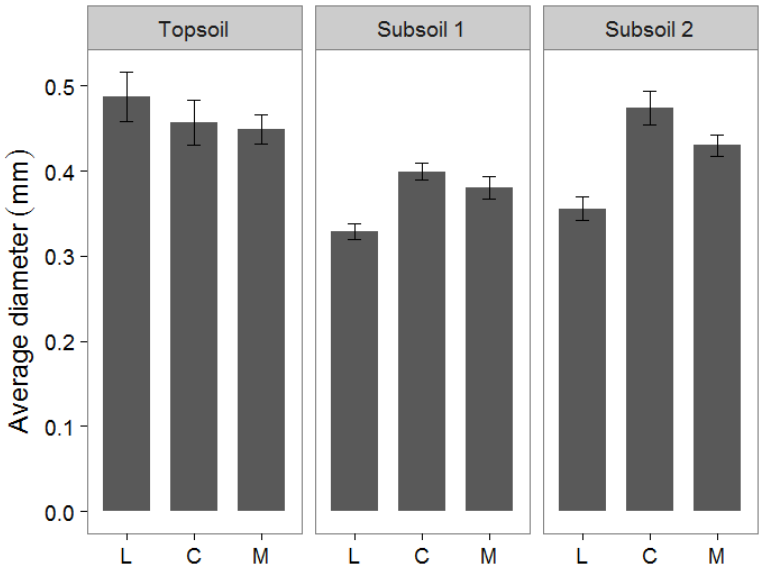


**Figure 5-15 Root diameter for each subsoil treatment: L (loose), C (compaction), M (macropores) and in each soil layer (topsoil, subsoil 1 and subsoil 2). Error bars are ±SEM. Values are means across cultivars of n=18 samples.**

### 5.3.3 Nutrient and root architecture relationships

Averaged across soil treatments there were differences (Table 5-2) between the crops in $^{15}N$ uptake per unit of subsoil root length, with lowest values in Welam and highest values in oat, while Ceylon was in the middle (LSD 5% for cultivars 48 μg $cm^{-1}$). Differences were also significant for the soil treatments when averaged across crops (Table 5-2). Loose soil was the treatment with the lowest $^{15}N$ uptake per unit root length in the subsoil (117.1 μg $cm^{-1}$) and there were no differences between the compaction and the macropores treatments (186.5 μg $cm^{-1}$and 185.8 μg $cm^{-1}$ respectively, LSD 5% for soil treatments 48 μg $cm^{-1}$).

Moreover, there was no interaction between crop and soil treatment indicating that crops responded to soil treatment in a similar way.

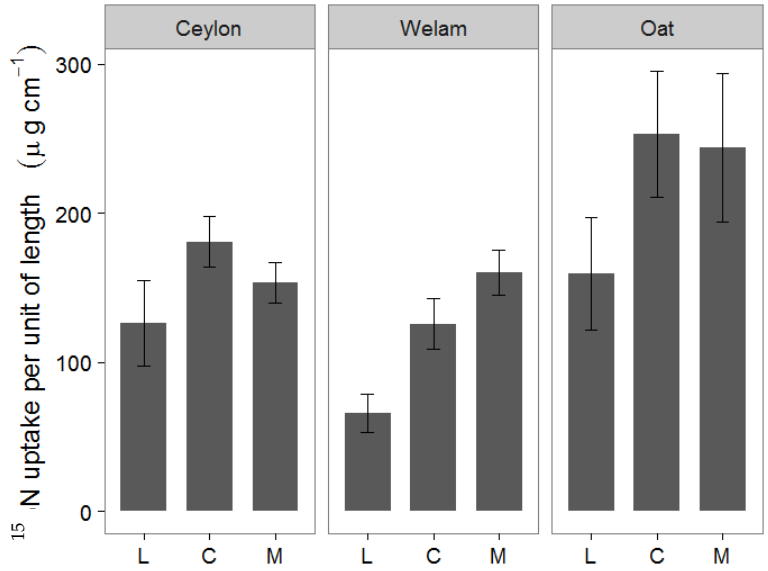


**Figure 5-16 $^{15}N$ uptake per unit of subsoil root length (µg $cm^{-1}$) of barley cultivars Ceylon and Welam and oat for three subsoil treatments: L (loose), C (compaction), M (macropores). Error bars represent ±SEM. Values are means of n=6 replicates.**

A linear model was fitted using individual replicate values of $^{15}N$ uptake by a plant and its root length in the subsoil (data not shown), however, no significant relationship was found, even when data were examined in subsets at the level of individual crops and treatments. However, a negative relation between root length in the subsoil and $^{15}N$ uptake was observed when using mean values for each crop in each soil treatment (Figure 5-17). A similar relation was observed for mean values of total N uptake and total root length or surface area.

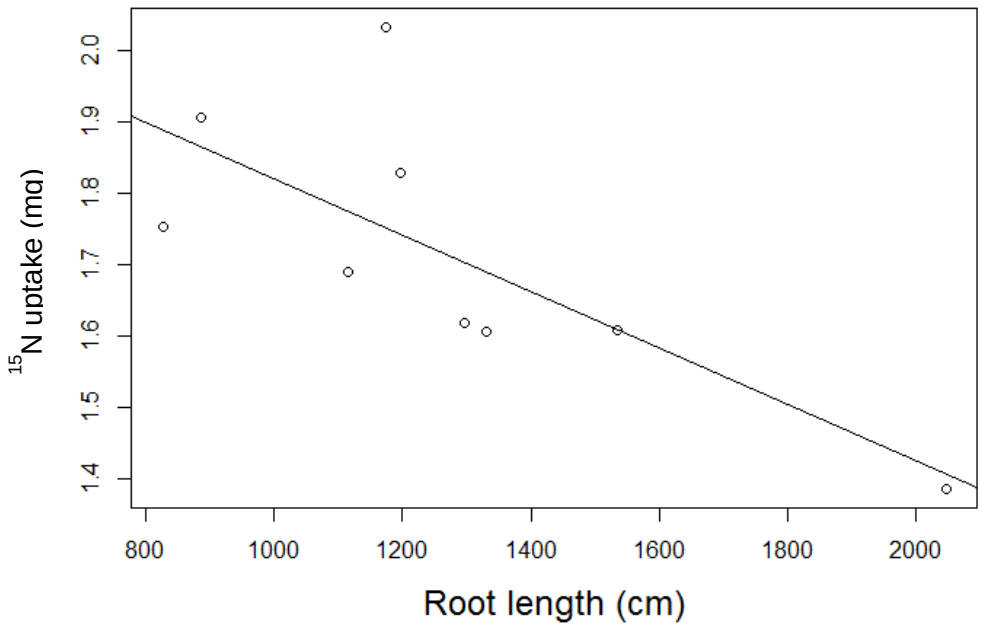


**Figure 5-17 Linear model representing $^{15}N$ uptake and root length in the subsoil. Data points are the averages of each cultivar within each treatment (n=6). (F-value=8.995, df=1, P-value =0.02, $R^2$=0.56)**

### 5.3.4 Final soil N concentrations

The major contribution to soil mineral N was from $NO_3^-$ present in the soil (3.07 μg $g^{-1}$ dry soil, average across soil treatments, crops and soil layers), $NH_4^+$ was present at a lower concentration (0.39 μg $g^{-1}$ dry soil, average across soil treatments, crops and soil layers). Soil $NO_3^-$ concentration at the end of the experiment was higher for oat compared to Welam and Ceylon (Table 5-2, Figure 5-19, Table 5-7). The soil treatment with the greatest $NO_3^-$ concentration remaining at the end of the experiment was the loose soil and it was caused by a high

concentration of $NO_3^-$ in the loose soil (Appendix C). The smallest concentration of $NO_3^-$ was in the macropore treatment. Soil $NH_4^+$ concentration was higher in the topsoil than in the subsoil for all cultivars and soil treatments.

Total soil N concentration at the end of the experiment did not differ for the three crops, but it was significantly different between soil layers and soil treatments (Table 5-2, Figure 5-17). Soil N concentration was lower in the subsoil compared to the topsoil (Figure 5-18). There was a significant interaction between soil treatment and soil layer (Table 5-2) these two factors resulting from the loose soil treatment having the lowest N concentration in the topsoil and the greatest together with the compaction treatment in the subsoil (Table 5-8).

The $^{15}N$ atom abundance in the soil at the end of the experiment was higher in the subsoil compared to the topsoil, with topsoil $^{15}N$ values close to natural abundance (0.366%) in soil (Table 5-2 and Figure 5-19). In addition, soil $^{15}N$ abundance was greatest for oat, and there was a significant interaction between cultivar and soil layer resulting from a higher $^{15}N$ abundance in the subsoil of oat than the two barley cultivars (Table 5-8, Figure 5-19).

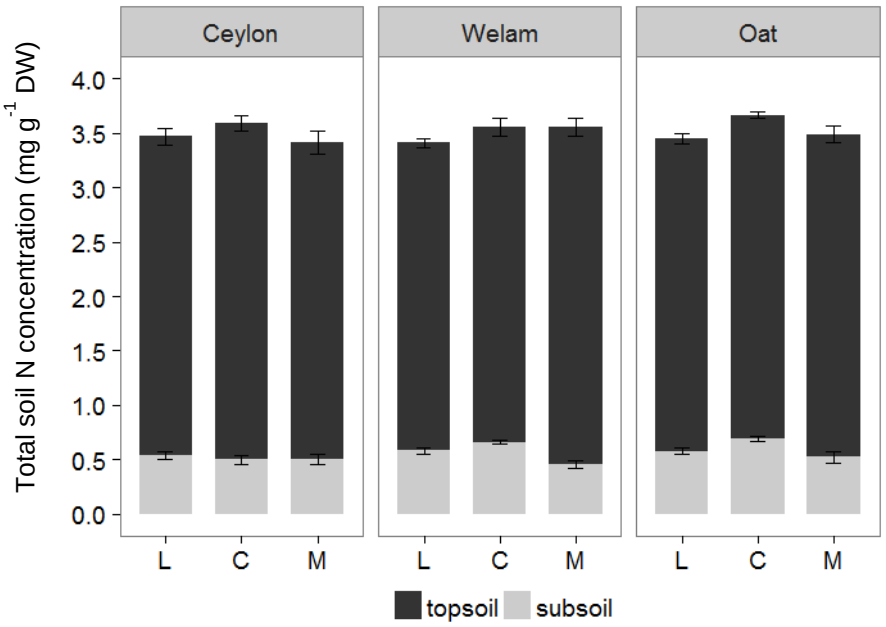


**Figure 5-18 Total soil N concentration (mg $g^{-1}$ DW) at the end of the experiment for barley cultivars Ceylon and Welam and oat by soil layers (t=topsoil and s=subsoil) and each soil treatment: L (loose), C (compaction), M (macropores). Error bars show ±SEM for each soil layer. Values are means of n=6 replicates.**

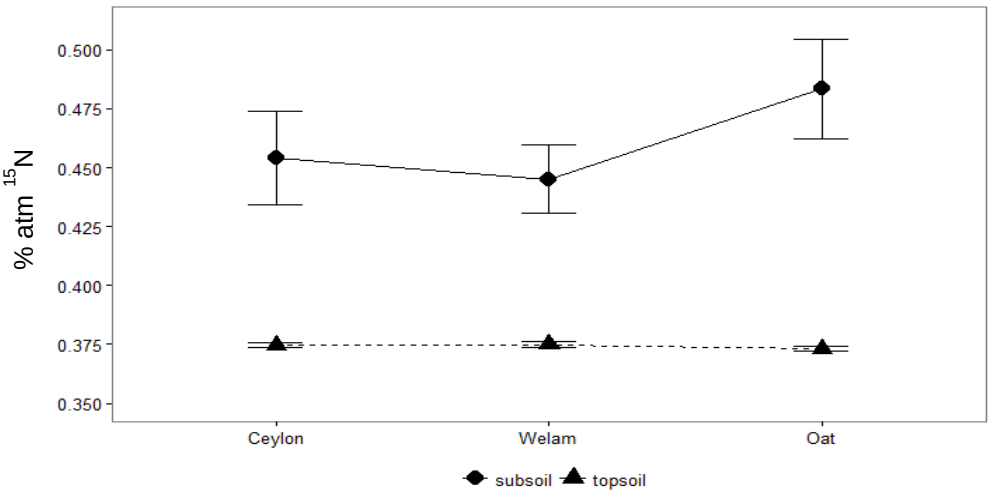


**Figure 5-19 Soil $^{15}N$ abundance (atomic % of total N) for two barley cultivars Ceylon and Welam and oat in the topsoil or subsoil. Values are means across soil treatments of n=18 samples and error bars are the SEM.**

**Table 5-7 Significant main effect of mean of crop, soil treatment and soil layers averaged and LSD values at 5% confidence level.**

| Main effect | Ammonium (μg $g^{-1}$ DW) | Nitrate (μg $g^{-1}$ DW) | Soil N (g $g^{-1}$ DW) | Soil $^{15}N$ (% atom of N) |
|---|---|---|---|---|
| Crop | | | | |
| Ceylon | 0.40 | 1.79 | 0.17 | 0.414 |
| Welam | 0.38 | 2.01 | 0.18 | 0.410 |
| Oat | 0.40 | 5.4 | 0.18 | 0.428 |
| LSD 5% | NS | 2.044 | NS | 0.0154 |
| Treatment | | | | |
| Loose | 0.39 | 4.2 | 0.17 | 0.41 |
| Compaction | 0.42 | 3.22 | 0.18 | 0.42 |
| Macropores | 0.38 | 1.79 | 0.17 | 0.42 |
| LSD 5% | NS | 2.044 | 0.006 | NS |
| Soil layer | | | | |
| topsoil | 0.544 | 3.42 | 0.3 | 0.374 |
| subsoil | 0.244 | 2.72 | 0.06 | 0.461 |
| LSD 5% | 0.0351 | NS | 0.005 | 0.0125 |

**Table 5-8 Significant interaction means of cultivar, soil treatments or soil layers averaged over the other factors with the LSD values at 5% confidence levels.**

| | Topsoil | Subsoil |
|---|---|---|
| Soil N concentration | | |
| Loose | 0.29 | 0,06 |
| Compaction | 0.30 | 0,06 |
| Macropores | 0.30 | 0,05 |
| LSD 5% treatment*soil layer | | 0.009 |
| Soil $^{15}$N concentration | | |
| Ceylon | 0.375 | 0,454 |
| Welam | 0.375 | 0,445 |
| Oat | 0.373 | 0,483 |
| LSD 5% cultivar*soil layer | | 0.0217 |

## 5.4 Discussion

### 5.4.1 Influence of subsoil structure on plant growth

Soil structure influenced root extension and morphology within the subsoil for each of the three crops measured. Root length and surface in the compacted subsoil, both with and without macropores, were reduced by 23% compared with that in loose subsoil whilst root diameter was increased and root volume was not different. This suggests that roots subjected to greater mechanical impedance were shorter but thicker, so that although overall length was influenced by subsoil characteristics, the total volume of root tissue was not however affected

by soil treatment. In addition, root dry weight in the subsoil was not affected by the soil treatments. These observations are consistent with effects of mechanical impedance on root growth measured at the cell and tissue scale. (Goss, 1976; Brereton *et al.*, 1986; Pfeifer *et al.*, 2014). Thicker roots are caused by an increased vascular cylinder area rather than a higher production of cells (Lipiec and Hatano, 2012, Pritchard, 1994; Croser *et al.*, 2000) which is thought to improve penetration in compacted soils because of a higher growth pressure but also reduces the risk of root axis buckling (Tomos and Pritchard, 1994; Bengough *et al.*, 1997; Croser *et al.*, 2000; Rosolem *et al.*, 2002; Lipiec *et al.*, 2012)

In the current study, the adjustments in subsoil root morphology had no effect on the overall growth of the plant. Total plant biomass and the partitioning of dry matter between shoot and root system was unaffected by soil treatment. Moreover, leaf area was also unchanged in spite of the reduction in root extension in compacted subsoil (with or without macropores). Leaf expansion has been reported to be particularly sensitive to the mechanical impedance of root growth, showing reduction from 21% to 54% for roots exposed to high impedance through soil or glass ballotini (Masle, 1992a; Young *et al.*, 1997; Monatgu *et al.*, 2001; Bingham *et al.*, 2010). There is evidence that the response might be regulated by chemical signalling between roots and shoots. Mainly

caused by an increased ABA and ethylene concentration which reduces stomatal conductance (Masle, 1992b, Passioura and Fry, 1992, Mullholland 1996; Jin *et al.,* 2013) and also causes inhibition in shoot growth (Sharp, 2002).

However, much of the evidence for the regulation of leaf expansion and stomatal conductance by soil physical properties has come from studies in which roots were subjected to uniform mechanical impedance or soil compaction. In the field soil physical properties vary spatially, both horizontally and with soil depth, and thus a more realistic experimental design is to use split-root systems or layered soil (as in the present work) to represent the heterogeneity in soil conditions (Monatgu *et al.,* 2001; Bingham and Bengough, 2003). In heterogeneous systems, compensatory adjustments in root growth can occur, so that root growth in favourable soil conditions is increased in response to a restriction in growth elsewhere in the root system (Monatgu *et al.,* 2001; Bingham and Bengough, 2003). Monatgu *et al.,* (2001) reported that reductions in leaf expansion were observed only when total root length was restricted. Thus, in layered soil compensatory adjustments in root growth in the favourable layer can offset the potentially negative effects on leaf expansion of soil compaction in other layers. In the current study, however, there was no evidence of any compensatory increase in root

length in the topsoil when root extension in the subsoil was restricted. It is possible that, despite the reduction in total root length when the subsoil was compacted (with or without macropores), signals arising from the impeded roots were below the threshold concentration or flux required to elicit a growth response in the shoot.

Soil at depth is usually at a higher density than the topsoil (Gilroy and Jones, 2000) and it may restrict root growth. Under those conditions it has been suggested that the main path for the roots to penetrate the subsoil is by growing through soil macropores (Kirkeegard and Lilley, 2010). Macropores are more likely to be used as a route into the subsoil when the conditions are limiting, for example in dry soils and when subsoil conditions are physically constraining growth. Conversely, the use of macropores by roots could be counterproductive since the roots may grow clustered in the macropores or cracks and not make enough use of the resources available at depth and in the bulk soil, sometimes even by becoming trapped in the macropores (Valentine *et al.,* 2012). Nevertheless, recent research suggests that roots in the subsoil grow through macropores only in those circumstances when the bulk soil is limiting for root growth. When there are no physical limitations the roots explore the bulk soil (Kautz *et al.,* 2013,Lilley and Kirkeegard, 2011).

In the present study, the presence of artificial biopores (the macropore treatment) had little effect on the total root length produced in the subsoil. With oat and the barley cv. Ceylon the total subsoil root length was almost identical between the compaction and macropore treatments, whilst for Welam it was actually less with the macropores. However, the presence of macropores appeared to influence the relative distribution of root length between the upper and lower subsoil layers. With macropores, the length in the upper subsoil was reduced relative to the lower layer. This suggests that when roots found a macropore they grew through it to reach the bottom of the column (Gao *et al.*, 2016), reducing the root length in the compacted bulk soil of the upper layer vs that in the lower layer. Given that overall plant growth was insensitive to subsoil compaction in this experiment, it is understandable that the relatively small effects of macropores on subsoil root distribution had no measurable effect on plant growth.

### 5.4.2 Genotypic differences in root growth and distribution

When young plants were grown in uniform topsoil packed to a high and low soil bulk density, the barley variety Welam had a greater rate of root extension, a lower centroid of mass and larger convex hull than Ceylon (4.3.2 Root traits of developing root systems day eight to 12). On the basis

of these results it was hypothesised that Welam would be able to develop a greater root length in the subsoil by the end of vegetative growth. Results from the current experiment support this hypothesis. When grown in a layered soil with topsoil and subsoil conditions, Welam produced a greater root length, root surface area and volume in both the top and subsoil compared to Ceylon. Moreover, this occurred across the range of subsoil treatments imposed.

The oat variety Firth provided a wider contrast in root growth and morphology than that of the two barley varieties. Oat produced the smallest root length in both topsoil and subsoil, and a smaller total root surface area and tissue volume. The differences in surface area and volume were most pronounced in the topsoil. Thus, the architecture of the root system differed considerably between oat and barley. Nevertheless, the response to soil physical conditions did not differ between the species studied. There was no significant interaction between crop and soil treatment for any of the plant growth characteristics measured and thus no evidence to support the suggestion that oat, as a species, is more susceptible to soil compaction than barley (Arvidsson and Hakansson, 2014).

### 5.4.3 Effects of genotype and soil structure on N capture from subsoil

Across a range of species, genotypes with a deeper root system have been positively linked to an increase in water capture, particularly in water stressed conditions (Lopes and Reynolds, 2010; Wasson *et al.*, 2014). Similarly, crop ideotypes designed to improve nitrogen use efficiency propose increasing the root length density in the subsoil. This is especially in leaching environments where nitrate concentrations in the subsoil may be appreciable (Foulkes *et al.*, 2009; Carvalho and Foulkes, 2013; White *et al.*, 2013; Lynch, 2015).

Results from the present study question the general applicability of that approach. This study found that, of the two barley varieties, Welam had a greater root length in the subsoil than Ceylon, but captured less $^{15}N$ and thus exploited less of the available subsoil N. Similarly, soil compaction and compaction with macropores reduced root extension in the subsoil, but had no effect on $^{15}N$ uptake. Thus, when data for genotypes and soil treatments were combined there was a negative relationship between root length in the subsoil and $^{15}N$ uptake. These findings highlight the importance of variation in the physiological capacity for N uptake (uptake per unit root length) in determining N capture from the soil.

Consideration must, therefore, be given to genotypic differences in uptake capacity as well as root growth in order to maximise N uptake. This is consistent with recent observations on the effects of breeding on N capture by wheat. The greater N uptake of modern varieties was not associated with a larger root system, but with an increased N uptake efficiency for nine varieties released in a 50 years' time period covering from 1958 to 2009. The results also show good correlation between controlled conditions in the laboratory and the field (Aziz *et al.*, 2016).

Based on theoretical and empirical evidence it has been suggested that the critical root length density for efficient uptake of available water and mobile ions such as nitrate is in the range 1-2 cm $cm^{-3}$ (King *et al.*, 2003). In the current experiment the root length density in the topsoil ranged from 3.01 to 2.78 cm $cm^{-3}$, whilst that in the subsoil ranged from 1.27 to 0.70 cm $cm^{-3}$. Thus, root length densities in the subsoil were close to the optimum. Had the experiment been conducted in deeper soil layers, where root length densities tend to be significantly lower, it is possible that a stronger positive correlation between root length and N uptake may have been found. Wiesler and Horst (1994) reported positive correlations between root length of maize and nitrate uptake from the subsoil, but not the topsoil where RLDs are typically greater than 1-2 cm $cm^{-3}$.

Increases in $^{15}N$ uptake per unit root length were able to offset the restrictions to root extension caused by subsoil compaction, thereby maintaining uptake of subsoil N. These adjustments were observed in all the genotypes studied, both barley and oat. Plasticity in the physiological capacity for N uptake has been reported previously and is an important mechanism for the rapid adjustment to variation in N availability, roots develop in N rich patches with an increased proliferation of lateral roots (Drew, 1973; Robinson, 1999). N uptake is regulated by nitrate and ammonium transporters and closely related to N plant status (Garnett *et al.*, 2009).

The increases observed here in response to subsoil compaction might be related to the increase in root diameter and volume of the cortex. It is possible that the increase in root volume is accompanied by a greater surface of cortical cell plasma membrane and associated nitrate and ammonium transporters (Masclaux-Daubresse *et al.*, 2010).

## 5.5 Conclusions

Poor subsoil structure (compacted soil with or without macropores) reduced root extension in that layer (supporting hypothesis 1). However, the hypothesis is not supported in full, because the restricted root

extension did not reduce N uptake from the subsoil. Compensatory adjustments were made to the uptake of N per unit root length, thus N uptake and accumulation in the shoot were maintained in spite of the adverse subsoil conditions and the shorter root length. Hypothesis 2 those cultivars with a greater rate of root extension and deeper root system will explore the soil matrix to a greater extent and uptake more N from the subsoil than the cultivars with a slower growing and shallower root system is also only partially supported. Welam, the barley genotype identified in Chapter 4 as having the greatest rate of root extension and deepest root system in young plants, generated the largest root length in both topsoil and subsoil. However, the greater root length of Welam compared to Ceylon did not enable it to exploit subsoil N reserves any more effectively because it had a lower rate of uptake per unit root length. The results show that the physiological capacity of root systems for N uptake and not just their length must be considered when selecting genotypes for more efficient N uptake from deep soil layers. Both traits in synergy may increase the plant capacity to exploit the N at depth and allow a lower input of N in the topsoil.

# Chapter 6: General discussion and conclusions

Increasing the ability of roots to grow and exploit water and mineral nutrients in the subsoil is an ambition of many research groups in the world (McCallum *et al.*, 2004; Hassan *et al.*, 2007; Kirkegaard *et al.*, 2007; McDonald *et al.*, 2013; Lynch and Wojciechowski, 2015). Since subsoil conditions are difficult to manage directly most attention is being focussed on the genetic improvement of rooting depth. Ideally a number of steps must be taken before a breeding programme can be established to improve root growth. Firstly potential traits that may lead to greater root depth must be identified. Secondly, the extent of variation in those phenotypic traits within the breeding population needs to be determined and a rapid and reliable method for selecting them developed. Thirdly, it must be demonstrated that the increased root growth achieves the desired outcome in terms of resource capture. The objectives of this thesis, therefore, were to i) investigate the importance of seed quality in root phenotypic screens, ii) quantify the genetic variability of barley roots to penetrate dense soil at early growth stages, iii) to determine whether differences between genotypes in the size and architecture of root systems measured during early plant growth in loose and compacted soil

are associated with differences in their ability to grow and explore the subsoil iv) quantify the benefit of deeper rooting for nitrogen (N) capture.

## 6.1 Key points about the reliability of information derived from seedling screens

Root seedling screens are a good approach for investigating the genetic variability in key traits that might be critical for plant establishment (Herrera *et al.,* 2012). They have the added advantage of taking less time and requiring fewer resources to complete compared to screens of mature plants. The first objective of this work was to develop a suitable high-throughput phenotyping method that would allow the genetic differences in barley roots to extend through dense soil to be tested at the seedling stage. It was found in chapter 3 that in root seedling screens, seed quality plays a crucial role. Thus if genetic differences are to be found, the seeds should be of the best quality possible or the results may be confounded by effects resulting from the loss of quality and loss of vigour of the roots. To make valid comparisons between genotypes, seeds need to be produced under the same maternal environmental, stored for a short period of time and under good conditions (low MC and cool temperature) to minimise deterioration.

The second objective was accomplished when genetic variation in root extension rate was found after four days of growth in loose and compacted soils on 6 days old seedlings (Chapter 4). However, the results showed that these genotypic differences did not persist to 12 days after planting. In contrast, genotypic differences in traits such as root length density or root surface area and volume established at that later stage of seedling growth (eight and 12 days) were consistent with those observed at the flag leaf stage for the genotypes under study (Chapter 5). How well results from root phenotypic screens in seedlings relate to mature plants is an issue under discussion and many studies do not link the phenotypic screens with plant behaviour of more mature plants or the implications for crop yield (Manschadi *et al.*, 2008; Thomas *et al.*, 2016).

The number, extension rate and initial growth angle of primary roots are traits studied heavily (Liu *et al.*, 2008; Hargreaves *et al.*, 2009; Wasson *et al.*, 2012; Kuijken *et al.*, 2015). They are linked, theoretically, with the ability of the root system to penetrate deeper in the soil at later stages of growth on the basis that seminal roots with a high extension rate, a good initial establishment followed by gravitropism will make the root system grows deeper in the soil profile (Manschadi *et al.*, 2008; Canè *et al.*, 2014). However, variation in root traits observed at the seedling stage do not

always correlate well with variation observed in growth of mature root systems. Genotypic differences have been found for root length at the seedling stage for wheat; the same lines correlated well with root depth of young plants in the field, but they were not well correlated with plant performance at flowering time (Watt *et al.*, 2013). In contrast, for *Brassica napus* the correlation between seedling root length and yield was positive (Thomas *et al.*, 2016). Botwright Acuña et al., (2012) reported a positive correlation between the ability of rice roots to grow to depth through wax layers and plant performance in the field. Root angle is also a controversial trait, while some studies made on wheat have been able to link initial growth angle with plant performance (Manschadi *et al.*, 2006), others have not (McDonald *et al.*, 2013). One reason for divergence in the results may be that seedling age is not standardized and different studies used different seedling ages (Pierret *et al.*, 2005). As seen in the current study, the relative differences between genotypes in some traits (root extension rate in this case) can change over a period of a few days after germination. Some progress has been made in unifying the methods and screening techniques (Lobet *et al.*, 2015) to ensure more consistent outcomes and trying to unify the phenotyping efforts such as creating data bases where meta-data can be stored.

Additional sources of variation within studies are the screening techniques used and plant root growth stage. Hargreaves *et al.,* (2009) used three different techniques to study root traits in barley. Those were gel chambers, soil columns for X-ray μCT and soil pots for 2D analysis. Results showed variation in the ranking of the traits measured (root length, root angle and vertical spread) between the two soil methods and the gel chamber. For the two soil methods, despite differences in the measurements the ranking of the cultivars was the same. In addition, high variability between samples has been reported for high-throughput phenotyping when using a 'pouch and wick' method (Thomas *et al.,* 2016). Phenotypes at the seedling stage may change in more mature plants because genotypic expression, the mechanisms regulating the trait(s) might change during plant development and changes in root structure as the plant develops (Gregory *et al.,* 2007) and because the trait variation might increase with the genotypic by environment interactions in field conditions (Thomas *et al.,* 2016). It is important to define and understand the desired traits and their development in the plant and how environmental constraints influence the genotypes.

Another potential issue is that interesting traits could be lost if the traits of interest change as the plant grows and the screening for the trait is performed at the seedling stage. As an example, Welam was chosen for

further investigation because of its contrasting ability to grow in loose and compacted soil during the first four days after transplanting. It had amongst the fastest extension rate in loose soil whereas it was amongst the slowest in compacted soil in the first screening (four days of growth in soil). However, by day eight and 12 after transplanting, it was the cultivar that showed the greatest extension rate for both loose and compacted soil and produced the greatest root length density at the flag leaf stage. These changes in the phenotypic trait expression may have also happened for some of the discarded cultivars. They could have shown faster growth or improved soil exploration at later stages, contrasting with their performance at the seedling screening.

The third objective was to determine whether differences between genotypes in the size and architecture of root systems measured during early plant growth in loose and compacted soil are associated with differences in their ability to grow and explore the subsoil. Welam, the cultivar with the greatest absolute root length, higher centre of mass and greatest convex hull and volume at days eight and 12 (which meant good early root growth) also had greatest root length density at depth, whether the subsoil was compacted or not. Good early plant establishment will allow the roots to develop faster. Faster root development added to strong gravitropism will make roots penetrate deep in the soil and have

access to more the resources by a greater exploration of the soil (Palta *et al.,* 2011). Herrera *et al.,* (2012) reviewed the link between N uptake and found that for wheat early vigour allowed roots to reach a higher root length density because the primary roots grew longer due to the extended period of time they had to grow prior to developmental slowdown in growth. Despite the variation in seedling screens some studies show how general trends or ranking of the cultivars at seedling stage link with growth under field conditions. They have in common that the best performing cultivar in controlled conditions was the best performing cultivar in the field too or when compared in different field environments (Rich *et al.*, 2016; Thomas *et al.*, 2016). Seedling screens, if validated with plant behaviour in the field or at maturity, can be a powerful tool to investigate root traits faster since they are faster and cheaper to perform. This study has given insightful information about how root traits have evolved from four days after planting to flag leaf stage. At flag leaf stage, the longest rooting cultivar was the same as after 12 days of growth in soil columns for both loose and compacted soil. The same relation did not occur between the plants grown for four days after planting and the plants at flag leaf stage. In this instance, the screening would have more relevant if plants had been grown 12 days in the soil columns.

Nevertheless, selection of the desired traits is only the first step towards crop improvement. The next step is to confirm that the desired traits are functional and fulfil the purpose they were investigated for, i.e. more root length in the subsoil may help to increase N uptake. Finally, the aim would be to isolate the QTLs and perform introgression genetics (Paez-Garcia *et al.*, 2015). Many efforts have been made to identify QTLs for root traits and some of them are very close or the same as some regulating yields in wheat, maize and rice (Price *et al.*, 2002; Talamè *et al.*, 2004; Zhu *et al.*, 2005; Kubo *et al.*, 2007; Christopher *et al.*, 2013; Pestsova *et al.*, 2016). Introgression genetics have successfully worked for root length and thickness in rice improving the ability of the cultivar to adapt to drought in India (Steele *et al.*, 2006).

## 6.2 The value of deeper rooting

Deeper rooting is believed to have two potential benefits for crop growth: improved water uptake and improved capture of N and other nutrients available at depth (Kautz *et al.*, 2013; Lynch, 2013; White *et al.*, 2013). The fourth objective of this work was to quantify the N uptake from the subsoil by trying to reproduce realistic subsoil conditions. The result was that N uptake could not be related to root length density in the subsoil.

The cultivar Welam, which produced the greatest root length density at depth had also the greatest surface area and volume. However, the results from chapter 5 suggest that the N capture from the subsoil was not directly linked to genotypic differences in root length density in the subsoil or total root length. The longer root system of Welam did not take more N from the subsoil than the other barley cultivar (Ceylon). In addition, the extension rate was greatest in loose than in compacted soil but differences in uptake were not correlated to the soil physical properties. This suggests differences in N uptake between genotypes and soil conditions result from physiological and not just morphological differences between root systems.

In addition, when roots grow unconstrained in the topsoil layers, plants may not make full use of the resources at depth, possibly because they are at a lower concentration in the subsoil and roots grow preferentially in the more N rich topsoil areas (Drew, 1975; Robinson *et al.*, 1999; Hodge, 2004). In agricultural soils, most nutrients are placed on or in the topsoil - up to 20-50 cm depth (Kautz *et al.*, 2013). N presence at depth is dependent on environmental factors such as leaching of nitrate in sandy soils (Shepherd and Lord, 1996). In other types of environment there will be very little leaching and most of the N present in the subsoil will be derived from fixed N, previous crop residues and any N imported by the

subsoil faunal community (Kuhlmann *et al.*, 1989; Weier and MacRae, 1993). The subsoil is nutrient poor and has less biotic activity (Yunusa and Newton, 2003). In the UK, where typical agricultural soils have rates of N leaching below the average of other European soils (Leip *et al.*, 2008), deep rooting may not be a beneficial trait. On the other hand, although subsoil is poorer in nutrients and has less biotic activity compared to the topsoil, it has generally a greater volume. The calculated theoretical amount of soil mineral N present in the subsoil from the field (Chapter 6) is *c.* two times greater than in the topsoil (125.05 kgN $ha^{-1}$ in the subsoil calculated from 80 cm depth and soil BD 1.5 g $cm^{-3}$ and 61 kgN $ha^{-1}$ in the topsoil calculated from 30 cm depth and soil BD 1.3 g $cm^{-3}$). If the aim is to reduce the amount of N fertilisers applied to the system, deeper rooting and greater uptake capacity in the subsoil will be more important and efficient. To make roots more efficient for N uptake further investigations are needed to understand the underlying mechanisms of N uptake from depth and from the lower N concentrations that occur there. Although progress has been made in identifying the nitrate and ammonium uptake transporters and genes regulating their expression, regulation of N uptake itself, particularly in constrained growing conditions is still not fully understood (Kiba and Krapp, 2016). More work is needed to understand the best way to optimise resource uptake

and the different ways of improving nutrient uptake from deep layers of the soil and from lower N concentrations. This will allow a reduction in the amount of fertilisers applied to the system. It seems a possible path since it has been shown that root systems adapt to N concentration in the soil and some genotypes have better abilities to adapt and forage for resources when competing with others (Robinson *et al.,* 1999).

In addition, subsoil nutrient dynamics should be investigated further and methods to enhance the nutrient availability for plant capture. There is disagreement about the scope for utilising resources from the subsoil other than N (Kautz *et al.,* 2013, Berisso *et al.,* 2012). For example, it is believed that some phosphate in the subsoil could potentially be accessed by plants (Oehl *et al.*, 2002). Nevertheless, better structured soil will potentially enhance root growth at depth easier as it was found in the VESS (Appendix A). Agronomic practices may have a better output in the short term since they are implemented in the systems faster than breeding programs (Thorup-kristensen and Kirkegaard, 2016). If enough nutrients were available at depth some benefits of deeper rooting may be enhanced by soil management techniques such as minimum tillage or reducing the number of passes by machinery that will create a better soil structure (Mangalassery *et al.*, 2014). More macropores in terms of number and more stable macropores and together with a greater

biodiversity at depth may lead to a greater soil exploration of the soil matrix by the roots (Stewart *et al.*, 1999; Uteau *et al.*, 2013).

## 6.3 Future research directions

Full determination of how the desired traits will improve the root system is necessary and it is probably the first step towards better root systems. It is important to describe the issues in a theoretical way, but also to combine modelling and experiments to help bring together the trait into reality. Understanding the synergies between the different constraints that the root systems may encounter is not an easy task. However, interactions are likely to happen, particularly in certain environments such as dry climates where roots may be growing under drought conditions but soil strength may be high and N may be deficient (Gregory, 2009; Lynch, 2015). Unravelling how the roots will overcome soil constraints and grow deeper and how they will benefit from it is fundamental, while also understanding the further implications this will have for above ground organs and yields.

Working in laboratory or controlled conditions is useful for trying to unravel mechanisms, but to get full conclusions and make them valuable for breeders working at field scale is necessary. Although more tedious, it is more realistic and helps to bring together all the abiotic and biotic

stresses that the plants suffer during their growth. A good understanding of the synergies between the root traits and the costs versus benefits of certain traits for the full plant is necessary too (Fiorani and Schurr, 2013; Kuijken et al., 2015; Paez-Garcia et al., 2015) i.e. it is important to consider whether the plant is allocating many resources to grow into the subsoil at the expense of growth in the topsoil as that may decrease the amount of nutrients captured because there are fewer nutrients or at a lower concentration in the subsoil. In this way a root system might not be making full use of what is available on the top layers of the soil.

This project has considered growth from seedling to close to flowering, but it would be interesting to do a final assessment of the consequences that the determined traits have for final yield under a range of climatic and environmental conditions to determine if the root traits undermine the shoot growth or the subsequent grain filling period. Although up to the point measured shoot performance was not predicted by root performance.

The use of modelling tools will help to better understand how plant traits might behave in different environments (King *et al.*, 2003; Hirel *et al.*, 2007; Pedersen *et al.*, 2010; Granier and Vile, 2014). They will also allow defining new possibilities and testing the defined root architectural traits

under a full range of environments. Modelling can help to realize the potential under different conditions without spending resources in field experiments (Dupuy *et al.*, 2010; Bingham and Wu, 2011; Dunbabin *et al.*, 2013; Meister *et al.*, 2014; Elazab *et al.*, 2016). For example, more vigorous roots were more likely to succeed, but how this might translate into different systems or how those cultivars might grow and yield under a wider range of soil and atmospheric environments is unknown. Modelling tools could help elucidate this. After the environments with physical and chemical limitations for growth have been identified and the focus traits identified, it would be useful to use model simulations to test how the 'improved cultivars' perform under these conditions and establish the improved phenotypes.

To date most attention has focussed on the constitutive expression of deeper root phenotypes and the potential benefits they might have for crop growth (Lynch, 2013). However, a different approach would be to develop or exploit a more plastic root system with the capacity to adapt to as many environments as possible and secure the yields for a broad range of circumstances. Some environmental circumstances change yearly (i.e. rainfall in dry areas) and it is important that the roots systems can adapt to them. It is more challenging to breed for plasticity because it is a more difficult trait to measure. Plasticity of the root system is a

significant trait to allow root systems to overcome stress (Miner *et al.*, 2005; Grossman and Rice, 2012; Koevoets *et al.*, 2016). In addition, it is also fundamental to get a deeper understanding of the subsoil environment, its possibilities and the dynamics between roots and subsoil, not only through physical limitations, but also chemical. If the aim is to decrease the input rate of N fertilisers, then more experimentation is needed to better understand the dynamics not only for plant growth but also to assess how nutrient distribution may relocate under this new system. It will help to understand how to foster subsoil resources and the limits it may have and how roots behave in a system where N concentration in the topsoil is lower than in the current systems.

## 6.4 Summary of key conclusions

Root traits that improve the exploration of the soil at seedling stage were not fully maintained for mature plants and they did not improve the nutrient acquisition from the deeper layer of the soil, making better use of the resources available.

1. Seed quality is an important factor to control when screening genotypes for rates of seedling root extension.

2. Genotypic variation exists within barley germplasm for seedling root extension rate in loose and dense soil.

3. Differences between genotypes in root extension rate in loose and dense soil depend on the age of the seedling. Variation observed in very young seedlings (four days after transplanting) is not a reliable indication of differences in older plants.

4. Differences between genotypes in root system characteristics observed eight and 12 days after transplanting were consistent with differences observed just prior to flowering when plants were grown in layered soil. Thus, the most vigorous genotype at 12 days after transplanting was the one with the largest root system in all soil conditions prior to flowering.

5. Differences between genotypes in root length density in the subsoil did not lead to differences in N uptake from the subsoil. Variations in uptake capacity per unit root length offset the differences in root length.

6. Subsoil compaction reduced root extension in that soil layer, but not N uptake or plant growth. Increases in uptake capacity

per unit root length offset the reduced root extension in the subsoil.

7. Both root growth and the physiological capacity for N uptake need to be taken into account when designing root systems for improved N capture from the subsoil.

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# Appendix A Root distribution in the subsoil under different field conditions.

## A.1 Aim of the study

As background for further experimental work, a preliminary study was conducted to determine subsoil conditions representative of those experienced by plants growing in field conditions through visual assessment of the subsoil characteristics and using μCT analysis to capture the root distribution in the subsoil under field conditions.

## A.2 Materials and Methods

### A.2.1 Soil sampling

Soil columns were extracted from two field sites. The first site was Boghall farm, Penicuik, Scotland. The samples were harvested from a commercial farm growing spring barley cv. Westminster under conventional tillage (Inversion tillage with a plough depth of 20 cm). The soil type was sandy clay loam of MacMerry series (Soil Information for Scottish Soils, http://sifss.hutton.ac.uk/SSKIB_Stats.php), Cambisol (FAO classification). The second site was Pilmore experimental farm, Invergrowie, Scotland. The samples from this site were collected from plots of spring barley cv. Optic grown under two different tillage

systems, conventional tillage (Inversion tillage with a plough depth of 20 cm) and minimum tillage (5 cm non-inversion tillage). Pilmore's soil type was a Cambisol (FAO classification), Carpow soil series (Soil Information for Scottish Soils, http://sifss.hutton.ac.uk/SSKIB_Stats.php), sandy loam with a sand bed at approximately 60 cm depth. At the time of sampling plants were at booting stage (Tottman *et al.*, 1979).

To collect the samples, a pit was dug to about 50 cm depth on a 2mx1m area. PVC columns of 5 cm inner diameter and 10 cm depth were hammered into the soil at random positions over the pit surface to extract columns filled with undisturbed soil. Finally a visual subsoil assessment was performed using the VESS approach (Ball *et al.*, 2015). Eight samples per field or experimental plot were collected in total; at Pilmore samples were collected from only one pit per tillage system, while at Boghall samples were collected from two different pits.

### A.2.2. Soil analysis

The soil columns were stored in the cold room at 4ºC and scanned by X-ray μCT two days after harvest to minimise the likelihood of root decay. The samples were scanned using a Phoenix Nanotom (GE Measurement and Control Solutions, Wunsford, Germany) X-ray μCT. The X-ray μCT scan set up for each column was as follows: 140 kV and 200 μA, at a

resolution of 50 μm, no filter was applied. The time taken to complete a scan of a column was 24 min. For each column, 3600 image projections were captured for each sample. The image stack for each full column was approximately 10 GB. Once the image acquisition was finished, image reconstruction was undertaken with Phoenix Datos|x software package (GE Measurement and Control Solutions, Wunsford, Germany). First, the samples were optimised to obtain a smooth surface over the whole column (in case the column moved during the image acquisition) and an automatic beam hardening correction algorithm was applied. Root segmentation was performed with VGMax Studio 2.2 (Volume Graphics GmbH, Heidelberg). Roots were selected manually and via semi-automated tools using the growing tool from VGMax Studio 2.2.

Once the full root system was extracted as a region of interest, it was transformed into a new volume and exported as a bmp image sequence. The porosity of the column was automatically segmented using the automatic material algorithm tool which allowed the distinction between soil and air in the soil columns. The root system was expanded radially 0.5 mm to quantify the air filled porosity and soil around the root system (Figure A-1; this method was adapted from Schmidt *et al.*, 2012 where they used a smaller surface but since the aim was to quantify macroporosity a greater surface was used in this experiment). This new

region showing soil/air space around the root system (Figure A-1) was transformed into a new volume and exported as bmp sequence.

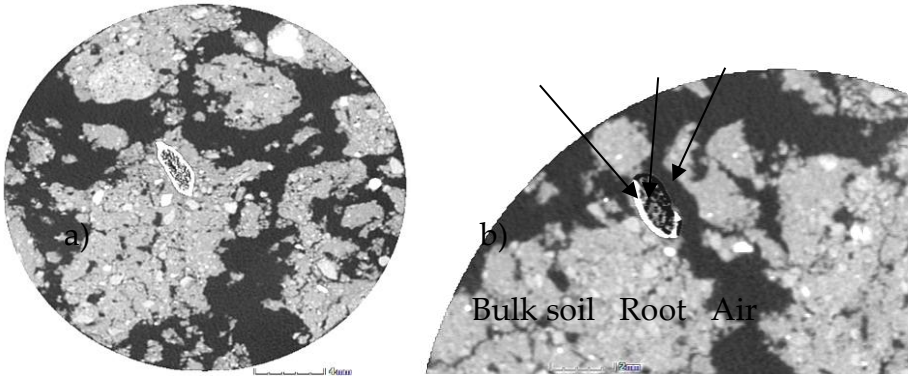


**Figure A-1 μCT images of the undisturbed soil columns (5 cm inner diameter), segmented roots and area around them (0.5 mm expansion from root area). a) An expanded root area of 0.5 mm from the root surface is shown (white enclosed surface) enclosing the segmented root (grey area inside the expanded area). b) Magnification of the column focused on the area around of a segmented root, white area around the roots is soil and the black area around the root is the air filled porosity.**

Also the same area filled in black was exported to allow quantification of the full perimeter around the root. Finally, a bmp image stack of the column was exported to measure the amount of total air filled space in the column (Figure A-2). The particles of the bmp image stacks were analysed using Fiji ImageJ (Schindelin *et al.*, 2012) particle analyser. Total root area per sample was also quantified following the same method. After the μCT scanning, soil physical parameters were measured, including penetration resistance, moisture content calculated by

comparison of wet and dry soil mass (MC) at the time of sampling, bulk density (BD) of the samples and water release curves for each of the columns. Penetration resistance was determined using the penetrometer 5966 Dual Column for Mechanical Testing (Instron, Illinois Tool Works Inc. ITW, UK.) to define the mechanical impedance to root growth at the sampling MC. The penetrometer tip was 0.95mm, the tip length was 1.7 mm, the cone angle was 32.45$^{o}$ and the shaft diameter anterior to the cone was 0.75 mm. The probe was inserted into the soil to a maximum depth of 15 mm in a period of 4 minutes. Three individual measures per column were performed.

Water release curves of packed columns were determined using tension tables and pressure plates following the method stated by (Ball and Hunter, 1988) at matric potentials of 0, -1, -6, -10, -20, -100, -200 and -1500 kPa. The water release curves were fitted using the Mualem equation (Mualem, 1976) in RECT (van Genuchten *et al.*, 1991).

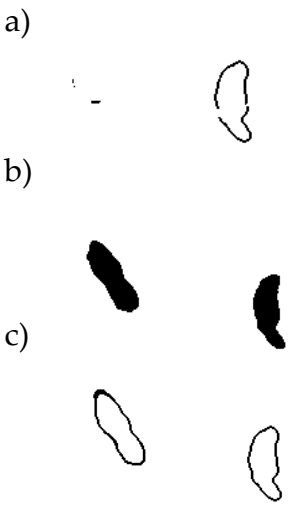


**Figure A-2 Images of the process of analysis of root area, area around the roots and porosity measurements. a) Segmented root, black area shows the area of the root in planar view. b) Expanded area (0.5 mm from the root surface) around the root, which corresponds to the white area around the root in Figure A-1 a). c) Area around the root comprising air filled porosity (black surface); the deleted part was classified as bulk soil.**

Available water and air filled porosity at each matric potential were calculated from the water release curves according to Ball and Hunter (1988). The size of the pores at each matric potential was calculated as 300/matric potential (Marshall and Holmes, 1988). For the measured matric potential, the calculated pore diameters were >300μm, 300-60 μm, 60-30μm, 30-15μm, 15-6μm, 6-0.3μm, 0.3-0.15μm, <0.15μm.

## A.2.3 Statistical analysis

Statistical analysis was performed using R (R Development Core Team, 2016) and lme4 (Bates *et al.*, 2015) and ggplot2 (Wickham, 2009) packages. The data were checked for normal distribution using the residuals and

the Shapiro-wilk test. One way analysis of variance (ANOVA) tests were conducted to evaluate differences in root area, soil porosity around the roots, total soil porosity, soil strength, moisture content and BD of the soil. Linear models were fitted to relevant parameters such as soil porosity around the root and total soil porosity and soil porosity around the root and penetrometer resistance.

## A.3 Results

### A.3.1 Visual soil assessment

The soil at all the sites was classified as soil with some compaction (Table A-1) (Ball *et al.,* 2015). Roots growing in the subsoil in Boghall were mainly doing so through the macropores and cracks and often had distorted and flattened shapes (Figure A-4 a and Figure A-4 c). However, in Pilmore, roots in both of the sampled treatments (conventional and minimum tillage) grew through the macropores and through the bulk soil (Figure A-5 b and Figure A-5 c and Figure A-6 b and Figure A-6 c). Further, roots were not distorted, although in the topsoil (0-25 cm depth) of the conventional tillage treatment the roots were often seen in macropores (Figure A-5 b).

**Table A-1 Visual subsoil assessment (VSSA) following the protocol stated in Ball et al., 2015 for each sampling point.**

**Boghall pit 1**

| Topsoil | Layer depth (cm) | Score | Quality | | | | |
|---|---|---|---|---|---|---|---|
| Layer 1 | 0-10 cm | 2 | Intact | | | | |
| Layer 2 | 10-20 cm | 3 | Intact | | | | |
| Subsoil | Layer depth (cm) | Mottling | Strength | Roots | Porosity | Aggregate size/shape | Structure quality |
| Layer 1 | 25-39 | 1a-3a | 3b | 3c/4c | 3d | 3e | 3 |
| Layer 2 | 39-70 | 1a/3a | 3b | 3c/4c | 2d/3d | 2e | 2*3 |
| Comments: Good structure, roots not very distorted and few stones. | | | | | | **overall score =** | **3** |

**Boghall pit 2**

| Topsoil | Layer depth (cm) | Score | Quality | | | | |
|---|---|---|---|---|---|---|---|
| Layer 1 | 0-10 cm | 2 | Intact | | | | |
| Layer 2 | 10-20 cm | 3 | Firm | | | | |
| Subsoil | Layer depth (cm) | Mottling | Strength | Roots | Porosity | Aggregate size/shape | Structure quality |
| Layer 1 | 29-39 | 1a/3a | 3b | 3c | 3d | 2e/3e | 3 |
| Layer 2 | 39-60 | 1a/3a | 3b | 4c | 3d/4d | 3e | 3*4 |

Comments: Layers change colour at 39 cm. There were no anaerobic signs. It doesn't break into clean aggregates.
There were occasional roots on the macropores but they grew mainly on the soil profile.
The roots were distorted and grew through the cracks, there were flattened roots. The soil was compact and the roots grew in the weakness zones.

**overall score=3**

**Pilmore conventional tillage**

| Topsoil | Layer depth (cm) | Score | Quality | | | | |
|---|---|---|---|---|---|---|---|
| Layer 1 | 0-15 | 3 | Firm | | | | |
| Layer 2 | 15-25 | 2 | Intact | | | | |
| Subsoil | Layer depth (cm) | Mottling | Strength | Roots | Porosity | Aggregate size/shape | Structure quality |
| Layer 1 | 25-38 | 1 | 3b | 3c | 2d | 3e | 3 |
| Layer 2 | 38 | 2a | 3b | 3c | 2d | 3e | 4 |

Comments: Barley cultivar Optic growing in conventional tillage. Lots of macropores on the upper layers and roots growing through them. **overall score= 3**

**Pilmore minimum tillage**

| Topsoil | Layer depth (cm) | Score | Quality | | | | |
|---|---|---|---|---|---|---|---|
| Layer 1 | 0-10 cm | 2 | Intact | | | | |
| Layer 2 | 10-20 cm | 2 | intact | | | | |
| Subsoil | Layer depth (cm) | Mottling | Strength | Roots | Porosity | Aggregate size/shape | Structure quality |
| Layer 1 | 48cm | 1a/3a | 2b/3b | 2c/3c | 2d | 3e | 2*3 |

Comments: No differences in colour between topsoil and subsoil. Sandy loam type. There was a sand layer at about 60 cm and the samples were loose on the bottom. **overall score= 3/2**

At Boghall, there were almost no roots from the subsoil downwards (Figure A-4 b). Subsoil and topsoil differed in colour, although there were no signs of anoxic conditions (Table A-1), and subsoil appeared more compact than the other soil layers. Similar features were observed for soil sampled from Pilmore conventional tillage treatment (Figure A-5 a). However, Pilmore minimum tillage treatment showed no colour distinction between the topsoil and the subsoil; visually it had the appearance of a well-structured soil, with roots distributed throughout the soil profile at depth (Figure A-6 a).

### A.3.2 Soil physical parameters

Penetrometer resistance differed for the three sampled field sites ($df=2$ and 18, $F=4.53$, $P=0.026$), as did the MC ($df=2$ and 18, $F=25.95$, $P<0.001$) and the BD ($df=2$ and 18, $F=4.25$, $P=0.028$). Pilmore minimum tillage had the best soil structure with the lowest penetrometer resistance and the highest moisture content and intermediate soil density (Table A-2). The pore size distribution as determined by the water release curves differed between the sites (Figure A-3). Soils from Pilmore conventional tillage system had slightly more of the largest pores (>300 μm) which are considered macropores and fewer of the smallest pores (<0.15 μm), and

therefore drained more quickly (Figure A-3), whereas the minimum tillage soil had a greater proportion of small pores (Table A-3).

**Table A-2 Average and standard deviation (in brackets) of soil physical measurements for each field sampled at field conditions (number of samples per field =8).**

| | BD (g $cm^{-3}$) | MC (%) | Penetrometer resistance (MPa) |
|---|---|---|---|
| Boghall | 1.36 (0.12) | 21.13 (0.04) | 1.71 (0.47) |
| Pilmore conv | 1.22 (0.13) | 18.53 (0.02) | 0.93 (0.55) |
| Pilmore min | 1.29 (0.08) | 29.90 (0.05) | 0.73 (0.50) |

**Table A-3 Field pore volume distribution by diameter of the pores.**

| | Boghall | Pilmore conv | Pilmore min |
|---|---|---|---|
| >300μm | 1.39 | 6.93 | 1.78 |
| 300-60 μm | 0.32 | 0.35 | 0.99 |
| 60-30μm | 2.30 | 2.33 | 2.52 |
| 30-15μm | 1.58 | 2.14 | 1.18 |
| 15-6μm | 20.07 | 18.87 | 20.54 |
| 6-0.3μm | 1.07 | 1.18 | 0.70 |
| 0.3-0.15μm | 1.69 | 5.82 | 1.78 |
| <0.15μm | 9.59 | 7.14 | 11.60 |

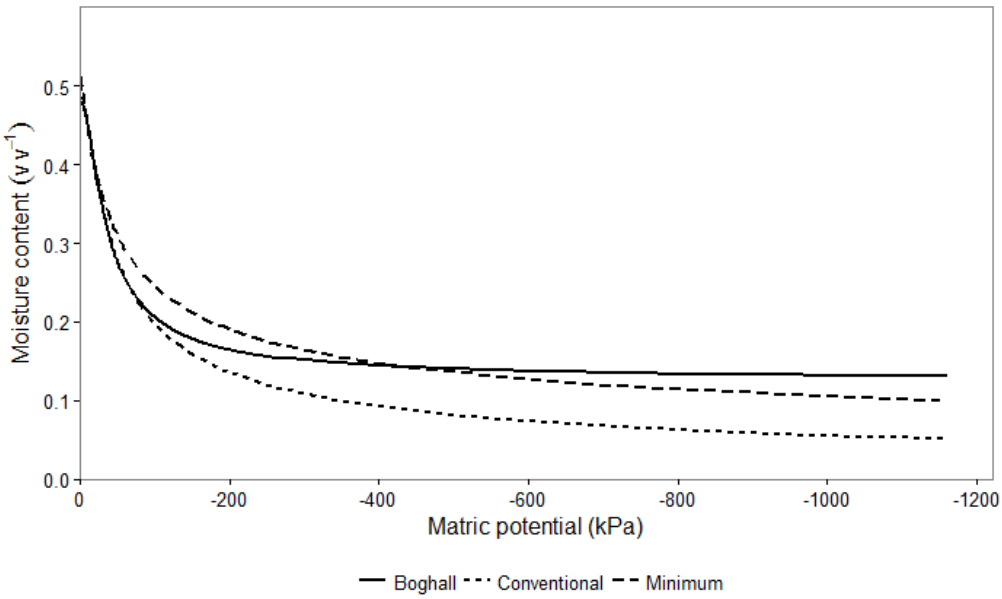


**Figure A-3 Fitted curve of water retention data for the average of the data using the Mualem equation for each plot sampled.**


**Figure A-4 Samples from Boghall field. a) Roots growing through a large macropore. b) Soil profile, the blue mark indicates the start of the subsoil. c) Roots flattened from growing through a crack.**


**Figure A-5 Pilmore conventional tillage a) Soil profile, the blue mark indicates the start of the subsoil. Roots growing through b) a large macropore and c) through the bulk soil.**


**Figure A-6 Pilmore minimum tillage. a) Soil profile. Roots growing through b) a large macropore, and c) the bulk soil.**

## A.3.3 Root distribution in the soil profile

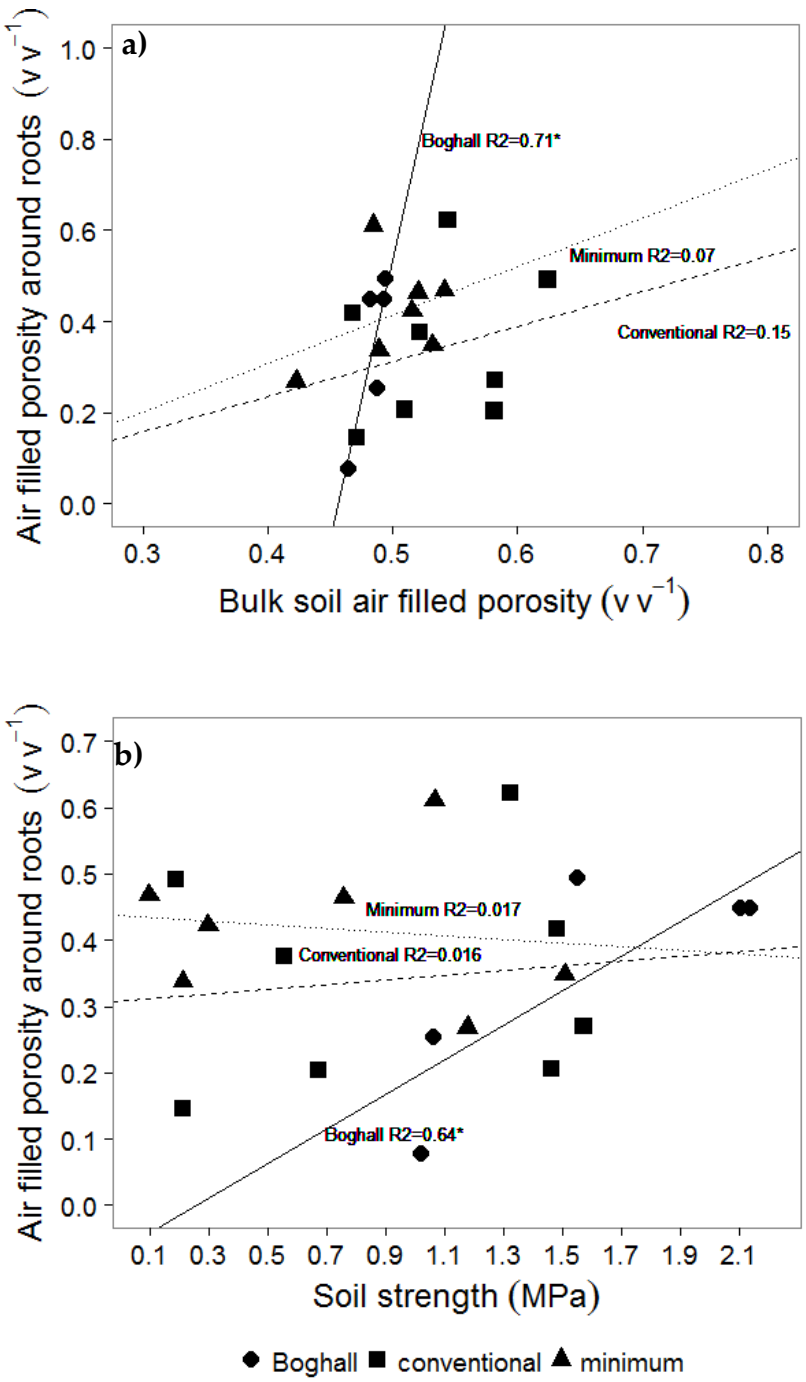


**Figure A-7 Relation between the proportion of air filled porosity around the roots and either a) proportion of air filled porosity in the bulk soil, or b) soil strength for each field site sampled. Lines show the linear regression fitted for each field site with the $R^2$ value for each of them (*P-value<0.1).**

Analysis of μCT data showed that there were no significant differences between the treatments in root area per sample due to large variation in the amount of roots present in each column (df=2 and 1, F=0.31, P>0.05). Air filled porosity around the roots was not statistically different for the three sites (P >0.05). However, in Boghall three of the soil columns did not contain roots, and at Pilmore the minimum tillage system did not have roots in one of the columns while the conventional tillage system had roots in all the columns. The regression between air filled porosity of the soil around the roots and the total soil porosity was significant for the Boghall site (df=1 and 3, F=7.32) but not for the two Pilmore sites (df=1 and 6, $F_{Conventinal}$=0.44, $F_{Minimum}$=0.85) (Figure A-7 a). Similar relationships were observed between air filled porosity around the root system and the soil strength of the soil, with a significant relation for the Boghall site alone (df=1 and 3, F=9.54) (Figure A-7 b).

## A.4 Summary

Subsoil conditions changed between the two sampling fields. Subsoil physical conditions at Pilmore presented low compaction levels and low penetration resistance. Root growth at depth was not limited and the roots were found indistinctively in the bulk soil and in the macropores. In contrast, Boghall presented soil physical limitation for root growth

due to a higher penetration resistance (Table A-1) and a higher level of soil compaction. In this case, less columns had roots. It was identified in the VESS that roots were growing distorted and through the macropores mainly. The presence of macropores under such conditions seemed as the best way for root penetration at depth.

## A.5 References


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# Appendix B

**Table B-1 Average and SEM (standard error of the mean) for the three cultivars tested in chapter 5. Number of samples =18.**

| | | Length shoot (cm) | Length longest leaf (cm) | Number of leaves main shoot | Number tillers | Leaves in tillers |
|---|---|---|---|---|---|---|
| Ceylon | Mean | 29.71 | 60.88 | 6.35 | 2.29 | 9.35 |
| | SEM | 2.34 | 2.83 | 0.23 | 0.11 | 0.51 |
| Welam | Mean | 38.24 | 65.92 | 6.67 | 2.28 | 9.12 |
| | SEM | 1.80 | 0.99 | 0.31 | 0.16 | 0.60 |
| Oat | Mean | 53.90 | 81.55 | 4.83 | 1.31 | 4.44 |
| | SEM | 2.75 | 2.30 | 0.28 | 0.12 | 0.44 |

# Appendix C

**Table C- 1 Average and standar deviation of the factors grouped by cultivar and soil treatment in the topsoil and subsoil. Number of samples = 18**

| | | Ammonium (μg $g^{-1}$ DW) | | Nitrate (μg $g^{-1}$ DW) | |
|---|---|---|---|---|---|
| | | Topsoil | Subsoil | Topsoil | Subsoil |
| Ceylon | Compaction | 0.706 | 0.176 | 2.26 | 1.63 |
| | Loose | 0.419 | 0.285 | 2.27 | 1.95 |
| | Macrores | 0.533 | 0.272 | 1.45 | 1.21 |
| Welam | Compaction | 0.533 | 0.226 | 2.15 | 3.32 |
| | Loose | 0.432 | 0.328 | 2.05 | 1.38 |
| | Macropores | 0.646 | 0.117 | 1.53 | 1.66 |
| Oat | Compaction | 0.683 | 0.170 | 4.36 | 5.58 |
| | Loose | 0.417 | 0.434 | 12.77 | 4.79 |
| | Macropores | 0.525 | 0.187 | 1.96 | 2.95 |
| | s.e.d | 0.071 | 0.051 | 1.90 | 0.69 |