DEVELOPMENT OF A ROOT IMAGING TECHNIQUE TO QUANTIFY ROOT RESPONCE TO WATERLOGGING

By

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Abstract

Waterlogging is a common stress for crops globally which can result in yield losses of up to 60%. Developing waterlogging tolerance in crops such as barley which is the ninth largest crop globally will have significant benefits in feeding future populations.

This thesis set out to develop a method of quantifying root response to waterlogging using an existing technique. All of the experiments in this thesis were carried out using seedlings from an f1 hybrid tomato *Solanum lycopersicum*, mutant barley *Hordeum Vulgare* L: MC 169 and its parental genotype MC 182. A series of images were generated which displayed the roots elongation at 3 hour intervals before, during and after waterlogging. Results showed that it is possible to quantify root response to waterlogging using this technique. Improvement in the sealing process of the plates allowed the plates to be sealed without effecting root elongation and plant health. By analysing the images generated it was possible to quantify the response of seminal roots to waterlogging over a five day period. Differences in the root elongation rate were quantified above and below the waterlogging level allowing root elongation rate of seminal roots above the waterlogging level to be compared with seminal roots below the waterlogging level.

Results from the experiments carried out have shown that during waterlogging seminal root elongation rate above a waterlogging level increases as seminal roots elongation rates below the waterlogging level decreases, there are varietal differences within species in their response to waterlogging and a mutant line of barley that was identified by Martinez et al (2003) having significantly straighter and faster elongating roots when compared to its parental type while using hydroponics. Using this technique it was found that the significant differences found by Martinez et al (2003) were not consistent when the lines were grown in compost. This suggests that if the mutant line is to be used as experimental as suggested by Martinez et al (2003) consideration on how root environmental conditions affect the level in which the mutant lines traits are exhibited.

Acknowledgments

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1 Literature Review

The Oxford dictionary defines waterlogged as “saturated with or full of water” this occurs when water enters soil faster than it can drain away under gravitational force in the case of this thesis the focus will be on developing a efficient way of quantifying root response to waterlogging. Waterlogging has the ability to seriously reduce crop yields by putting a crop under stress. It is estimated that worldwide approximately 10% of all irrigated farmland suffers from frequent waterlogging resulting in a decrease in crop productivity or around 20% (Jackson 2005). It is also estimated that 10 million ha of land is waterlogged in developing countries every year. In waterlogging tolerant genotypes of wheat ear dry weight can be reduced by approximately 20% after 3 days of waterlogging and if the water level rises 10mm above the soil surface ear weight can be reduced by as much as 60% (Davies & Hillman 1988).

1.1 Adverse effects of waterlogging

The physiological responses to waterlogging are well reported in literature. From reducing the diameter of the metaxylem and protoxylem vessels of the nodal roots (Huang et al. 1994) to the decreases in wheat yields of 37-45% due to waterlogging have been observed (Musgrave, 1994; Wu et al. 1992; Cai et al. 1994; van Ginkel et al. 1992; Boru. 1996).

1.2 Plant response to waterlogging

The main objective of a plant during waterlogging is to preserve root tips and maintain nutrient supply to the plant. Plants differ in the way they do this but the fundamental principles are the same generally the development of aerenchyma and there is a shift from elongating the roots deeper into the soil profile to increasing roots structures above

the flood line. The development of aerenchyma normally occurs in one of two ways to form lysigenous or schizogenous aerenchyma. Lysigenous aerenchyma are formed by cell differentiation and collapse, and schizogenous aerenchyma are formed by cell separation without collapse see figure 1.2 either way they assist in gas exchange within the waterlogged root system. Roots have two main responses to waterlogging the formation of aerenchyma and the elongation of roots below the flood line is stopped and root elongation is focused above the flood line.

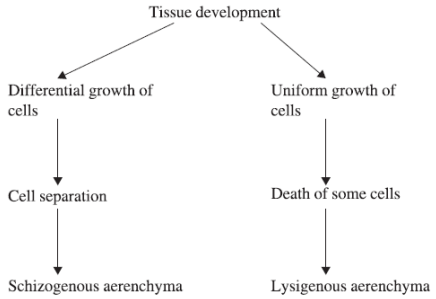


Figure. 1.2-Aerenhyma formation, the processes of lysigenous and shizogenous aerenchyma formation.. Evans 2003

Either way this leads to the formation of large continuous air spaces that allow diffusion of oxygen from shoot to the root. This process works especially well if a plant is able to elongate its shoots during flooding. This process of elongation is believed to be triggered by the build up of ethylene at the roots. Some of the oxygen that passes through the aerenchyma to the root tip leaks out of the pores of the root and into the surrounding soil known at the rhizosphere. This results in a small area of oxygenated soil around the plants roots which is referred to as an oxidized rhizosphere. This oxidized rhizosphere allows normal root metabolism to occur even when the soil is flooded. Failure to do this causes death of root tissue and will seriously effect plant health and consequently crop yields. It has been shown that a period of partial oxygen shortage caused by waterlogging can change the way in which genes are expressed and

make roots more tolerant to subsequent anaerobiosis caused by waterlogging (saglio et al 1988)

During waterlogging roots tend to stop elongating as aerenchyma form (Palta et al 2007). It has been shown that after waterlogging existing roots do not always recommence growth instead new roots develop three days after waterlogging (Palta et al 2007). These new roots are thicker than the existing roots and emerged from the first node. The development of these roots represented a considerable carbon and nitrogen cost to the plant. What this means is that there will be less carbon and nitrogen in which the plant can use to form tillers and for plant maintenance which ultimately will lead to a reduction in ear numbers and grain yields.

During waterlogging the ability of gases to diffuse through soil is strongly inhibited which leads to a situation where a slowing of oxygen influx causes injury to the roots in a condition known as hypoxia (Vartapetian and Jackson 1997). There is only 3% of the normal available oxygen available to a root during logging per unit of area (Arshad and Frankenberger 1990). Most this oxygen is used up by micro organisms in the soil. Although the absence of oxygen will normally result in the death of growing root tips and seminal roots as little as 0.006-0.01 mol m^{-3} of oxygen in solution is enough to keep roots alive (Huang and Johnson 1995). To put that in prospective water at 25^{o}C contains approximately 0.25 mol m^{-3}. Waterlogging also stops release of and oxidative breakdown of ethylene and carbon dioxide (Arshad and Frankenberger 1990). Although the accumulation of ethylene will only slow root extension a build up of carbon dioxide will severely damage roots resulting in poor plant health by reducing essential root up take.

1.3 climate change

In an age where there is an ever increasing population the demand for staple foods continues to rise on a decreasing landmass. It is believed the world climate is changing resulting in more extreme weather conditions (Mikhail 2008). Most models suggest that climates will have an increase wind, hotter summers, colder winters and higher levels of rain. The Met office are predicting that there will be a 10% increases in rain fall by 2050 (Maraun *et al.,* 2008). Some of these areas are within the 10 million hectares of wheat that already experience medium to serious waterlogging so additional rainfall will make crop production in these areas very difficult (Boru et al 2001). In the UK around 40% of the cereal growing area has waterlogging problems (Cannell et al 1980). There is genotypic variation within crop species with regard to tolerance to waterlogging (Davies, M.S., and G.S. Hillman. 1988). Davies et al 1988 in their paper entitled "Effect of soil flooding on growth and grain yield of tetraploid and hexaploid species of wheat" showed that *T. macha* was much more flooding-tolerant than the other hexaploid species and the Pontus population of the emmer wheat, *T. dicoccum*, was more tolerant than the Bordeaux population in the southwest of France of this species and than *T. spelta* and *T. Aestivum*. Economic modelling suggests that wheat could achieve similar economic returns to wool in high rainfall areas of Western Australia if constraints to waterlogging could be overcome when currently severe yield penalties can occur from waterlogging (Paterson 2007).

Projections have suggested a 40% increase in demand for food within the next 20 years (Rajaram 2001). Barley is the ninth biggest crop grown in the world with over 154 million tonnes being produced every year to supply both the animal and the human food chain (FAOStat 2007). As the world population is expected to increase by 2.5 billion by 2050

the demand on food production will increase (UN 2007). During the green revolution between 1943 and the late 1970s the development of high-yielding varieties of cereal grains, the expansion of irrigation infrastructure and the distribution of hybridized seeds, synthetic fertilizers, and pesticides to farmers allowed food production to more than double in developing nations between the years 1961–1985. During this time there was little focus on manipulating crop response to stress and things were more focused on increasing yields through response to fertilizer and controlling pest and pathogen attack through artificial chemical applications. There is now a call for a second green revolution to again increase yields once again. This time the focus will be more on the use of developing crop lines to be more efficient in their surroundings and to be more resistant to environmental and pest attack.

1.4 Genetics behind waterlogging tolerance

There is some evidence for genetic control of waterlogging resistance. Alcohol fermentation genes such as Adh found in wheat, barley and rice are associated with waterlogging tolerance. Mujer et al in 1993 showed evidence that a few genes controlling hypoxia tolerance in grasses and Boru et al in 2001 suggested that tolerance to waterlogging is controlled by a small number of genes in spring wheat.

There has been some progress in molecular studies to identify QTL association with waterlogging tolerance but it is still not known if these traits are heritable (Collaku et al 2005). Since there is no reliable marker for waterlogging tolerance different morphological and physiological traits have been used in genetic studies of waterlogging tolerance. This is not always an accurate way of assessing such a characteristic as they are mostly quantitative traits and expression of these traits can be heavily affected by environmental influences.

The degree of stress on cereal crops during waterlogging depends on its growth stage, and the duration of waterlogging. Generally crops are more tolerant to waterlogging in later growth stages than in early ones meaning yield is affected less (Meyer and Barrs, 1988). Wheat that has been waterlogged for six days can result in grain yield reductions of between 39% and 47%. In 1992 Van Ginkel et al identified fourteen spring wheat lines tolerant to flooding when he screened a total of 1344 lines under extended waterlogging conditions in the field. Four genotypes produced more than 2t ha^{-1} after five weeks of waterlogging and seemed to perform well when waterlogged at various growth stages.

Some studies suggest that waterlogging tolerance as a trait is highly heritable (Cao et all. 1995) while others demonstrated there is little variability for waterlogging tolerance among wheat lines (Tesemma et all. 1991) some scientists have found that a single trait can be controlled by a single gene (Cao et al. 1992) while others maintain that all traits are polygenic (Hamachi et al. 1989)

Boru et al (2001) found that the presence of four tolerance genes Wt1, Wt2, Wt3 and Wt4 and that the presence of the Wt1 gene and one of the other three tolerance genes provided a high level of tolerance to waterlogging. Boru did this by measuring the percentage of leaf chlorosis under field conditions in North West Mexico. Boru concluded that all three tolerant genotypes in his study carried different genes, although they all possess one Wt1 gene and that these different genes may be related to different mechanisms of waterlogging tolerance and so combining them may result in an increases level of tolerance.

1.5 Soil structure

Approximately 40-60% of soil volume is made of solid material that is permeated by spaces or otherwise known as pores filled with gas, water, roots and other living organisms. The type of soil which is dictated by the particle size also determines how much water is held by the soil and how easily water drains through the profile.

It is generally understood that sand based soils are better drained and aerated than clay based soils which means they respond better to waterlogging. Interconnecting pores of a diameter greater than 50μm can generally drain under gravitational force, pores between the sizes of 50-0.5μm can hold water against the force of gravity but are still weak enough for roots to access to extract the water, pores of 0.2μm hold water so strongly that nether gravity or roots can extract the water (Jackson 2005). Organic matter reduces the risks of pan formation and allows for optimum rooting conditions (Singh et al 1992). Soil organic matter levels can be improved by the application of green manures, straw and animal manures.

1.6 Recovery from waterlogging

Not only is it important to consider root response to waterlogging but also how well it recovers after waterlogging. It has been found that an application of nitrogen after waterlogging can significantly help a plant to recover. 30-42% of the nitrogen applied at sowing was lost from the soil during waterlogging (Palta 2007). when lost nitrogen is reapplied one day after waterlogging only 10% of the nitrogen is used by plants but when the same amount was applied three weeks after waterlogging up to 55% was used to generate new shoot growth (Palta 2007).

There is evidence to suggest that there are varietal differences within plant lines. Work at CSIRO in Australia have shown that after waterlogging root growth of CICM89 was faster than Vigour 18 and Wyalkatchem particularly in the top 200-400mm of the soil profile where root branching increased. Tillering was reduced by 37% in CICM89, 40% in Vigor 18 and 60% in Wyalkatchem. Although synthetic wheat have poor agronomic characters by crossing them with normal breed wheat many of these poor characteristics can be overcome within F2 and F3 generations.

Many agronomic practices can affect waterlogging stress by simply adjusting sowing dates to coincide with reduced rainfall patterns is one way to avoid waterlogging however this is not always possible. Maintaining good soil organic matter through the application of green and livestock manures not only increased the availability of iron and magnesium several fold under flooded conditions it also improved soil physical factors (Dickson 2001 Personal communication). What this means is that there is less risk of soil surface crusting and pan formation. Crops which had been direct drilled were more sensitive to waterlogging between germination and emergence than crops sown in ploughed land (Rasmussen 1988). As winter sown crops are longer maturing they are more tolerant to waterlogging than earlier maturing spring sown crops (Gardner and Flood 1993). An application of a nitrogen fertilizer after waterlogging has been shown to reduce the negative effects caused by waterlogging by assisting the plants recovery to water stress (swarup & Sharma 1993).

1.7 Potential experimental methods for studying root response to waterlogging

One way in which root elongation has been monitored is by digging a trench through a soil profile within a field (Kucke et al 1995). This makes it possible to observe and

measure root length through soil horizons within a field. Another is to extract a plant from a profile and then after the soil has been washed off, measure the roots (muhlich et al 2008). Although this is the preferred technique by many researchers it is prone to environmental influences and results cannot be easily replicated.

There are a few laboratory techniques that have been developed to map and phenotype roots. One such method is the use of x ray micro tomography which allows roots grown in soil media to be scanned without disturbing or causing any damage to root systems. Although this is still a new and developing technique it does offer 3D modelling of root systems by scanning at intervals of 200μm which can be combined to form a 3D model (Asseng et al 2000). This method does have limitations, it is expensive and is subject to small sample sizes.

A more simple technique is to simply grow seeds on damp paper with in a Petri dish and photographing after a few days. Although this technique is useful for some applications it would not be appropriate for monitoring root responses to waterlogging or anything longer than two weeks. Although it does have the advantage that large sample sizes can be scanned quickly and simply.

The use of aeroponics, hydroponics and agar plate systems make it possible to monitor plant roots in later growth stages. Hydroponics was developed in the 19th century when it was discovered that plants absorb essential mineral nutrients as inorganic ions in water. Hydroponics does have many advantages such as no soil is needed, the water stays in the system and can be reused thus, lower water costs, it is possible to control the nutrition levels in their entirety thus, lower nutrition costs, pests and diseases are easier to get rid of than in soil because of the container's mobility. But due to the nature of the propagation process it is not suitable for mapping root response to waterlogging in soil or compost.

A technique that has been developed at SCRI is the use of gel chambers in which roots are grown in a narrow gap between layers of agar. The chambers were then inserted into flatbed scanners which were set to scan every 3 hours. Bengough et al (2004) and Dresbøll (Personal communication 2010) have used such chambers to successfully demonstrate the evolution of the barley root system during domestication and subsequent breeding. Dresbøll (Personal communication 2010) used the same plates but instead of using agar she used compost and angled the chambers at 80^{o}with the clear side of the chamber facing downwards so that the roots grew towards and down the front of the clear side. Dresbøll (Personal communication 2010) successfully used this technique to monitor tomato *Solanum lycopersicum* roots during and after waterlogging. There is one problem with her technique and that is the use of a silicone based sealant to seal the chambers during waterlogging. This significantly affects plant health and consequently has an effect on data gained by this method.

1.8 My thesis

The aim of this thesis is to develop a method of sealing the plates used in the technique used by Dresbøll (Personal communication 2010) and quantify root response to waterlogging in tomato and two comparable lines of barley. If successful this technique could prove to be useful in developing and selecting plant lines which are tolerant to waterlogging and allow a simple method of quantifying root response to waterlogging.

Tomato was selected for initial experiments due to its sensitivity to stress, fast root elongation from a tap root. Tomatoes are used as a model plant over other model plants on occasions because they have features such as fleshy fruit, a sympodial shoot, and compound leaves which other model plants do not have.

Barley lines MC 169 which is a mutant and its wild parental line MC182 were selected for further experiments. This was based on documented evidence (Martinez et al 2003) that showed significant differences in the two lines when grown using hydroponics. Not only was the mutant line noted of having a higher root elongation rate but it also showed a tendency to grow root tips in straighter lines when compared with its parental line.

Compost was used as a suitable growth medium as it is commonly used in research and horticulture and may give a comparable growth to natural growing conditions. Compost can be easily broken down into a texture that can be easily spread between the two plates without compaction occurring due to its high level of organic matter which assists structure.

2 Method

For the experiments in this thesis the technique as used by Dresbøll (Personal communication 2010) was adopted. Following the same protocol compost was prepared in the same manner and used to fill the plates which were later placed into Cannon Canoscan 5600F scanners and controlled by scanning software called archiScan_multi. All images generated by archiScan_multi were processed using Image J and analyzed using MS Excel.

There were two main areas that had to be covered by the experiments in this thesis one was to establish a method of sealing the plates that had no effect on plant growth and was able to maintain a secure seal throughout the experiment and secondly to use the developed technique to quantify root response to waterlogging.

2.1 Material

2.1.1 Seed

First of all a F1 hybrid tomato *Solanum lycopersicum* variety was used sourced from Dr Dorte Bodin Dresbøll of Aarhus University in Denmark. This seed was used to quantify the effect the previous silicone based sealant had on plant germination and growth and in the development/testing of new sealing techniques.

Secondly two different lines of barley *Hordeum Vulgare L* seed were used a mutant line MC 169 and its parental wild type MC 182 these were sourced from Instituto Nacional de tecnologia Agropecuaria in Argentina. This was used in the quantifying of root response to waterlogging and using the technique to compare two different lines within a species.

All seed was stored 4^{o}C in sealed containers. Tomato seeds were planted 10mm from the top of the tray directly below the openings. Barley seed was planted 20mm from the top of the tray again directly below the openings.

2.1.2 Sealant

Four materials were used in sealing the plates

1. Silicone sold in 310ml tube applicator batch number 646763. This product is manufactured and distributed by Wickes Building supplies Limited, 120 – 138 Station Road, Harrow HA1 2QB, UK. This sealant was only used in germination tests to quantify the effect on the germination and growth of the tomato seeds.
2. 10,000mm x 1,000mm x 2mm polythene foam batch number 365732. This product is manufactured and distributed by B & Q plc, Chandlers Ford, Hants, SO53 3YX, UK. This was selected because of its low odour and its ability to be easily cut to shape.
3. 7,000mm x 75mm x 1mm MS Polymer based Fixofol pond liner tape batch number EM3166/01/3 – 10/08. This product is manufactured by OASE GmbH, Tecklenburger Str 161, 48477 Horstel, Germany and distributed in the UK by **OASE(UK) Ltd,** 3 Telford Gate, Andover, SP10 3SF. This product was selected as it was designed to be used in plant ecosystems and could be cut into shape and easily applied.
4. MS Polymer Uni fix + pond liner sealant sold in 230ml tube applicator batch number 0335810. This product is manufactured by OASE GmbH, Tecklenburger Str 161, 48477 Horstel, Germany and distributed in the UK by **OASE(UK) Ltd,** 3 Telford Gate, Andover, SP10 3SF. This product was selected as it was designed to be used in plant ecosystems and could be applied without any joints.

2.1.3 Growth medium

All seeds were grown in Pindstrup faerdigblanding substrate peat compost with a ph value of 6.5, which can be sourced from Bulrush Horticulture ltd, Newferry Road, Bellaghy, County Londonderry, BT45 8ND, Northern Ireland. Compost was selected to be used as an alternative growth medium due to its common usage throughout plant propagation.

2.1.4 Plates

The plates are constructed from Perspex and measure 300mm x 210mm with a 5mm border around the inside edge, three 20mm wide gaps are located along the top edge of the plates at a spacing interval of 40mm . One plate is clear so that the roots can be displayed and the other side is black to reduce background light from interfering with generated images (Figure 2.1). The two plates are held together with plastic clamps constructed from sections of plastic binding which is normally used to hold sheets of paper together in booklets.

Black Perspex plate

Clear Perspex plate

Figure 2.1-Examples of both the clear and black Perspex plate construction. These plates were clipped together once they had been prepared with compost using plastic clips.

2.1.5 Scanner & Camera

The scanners used to generate the root images were Cannon Canoscan 5600F which were of the following specification:

- 4800x9600 dpi resolution
- 48-bit colour
- 300dpi A4 scans in 11 seconds
- Zero warm-up time

This scanner was selected because it was able to produce a high quality image through the Perspex plate and scanned in a short period of time meaning the scanned roots were subjected to the minimum possible amount of light during scanning.

The camera used was a Nikon D5000 with an 18-55mm lenses the specification of this camera are as follows:

- 12.9 megapixel 4,288 x 2,848 DX-format CMOS sensor (effective pixels: 12.3 million)
- IS0 200-3200 range (100-6400 expanded)
- Expeed 12 bit image processing engine
- 11 AF points (with 3D tracking)

This camera was selected due to its ability to have full manual control of shutter speed, aperture, and ISO settings which were required when using an Infrared filter.

Opteka 52mm High definition, multi coated, Infrared filter >720nm. This filter was selected due to the high quality of the filter and filters of 850nm and 950 nm would produce images which were of a lower quality in the available lighting conditions.

2.1.6 Growing Conditions

All plants were grown in a Sanyo growth cabinet at 21.5^{o}C with an 18 hour day length. This growth cabinet was selected because of its large cabinet area which was required for all six scanners.

2.1.7 Software

ArchiScan_multi was used to control the scanners so that they would perform routine scans at 3 hour intervals. ArchiScan_multi is a piece of software developed by MultiMediait. Archiscan_multi is designed to be run on a PC to allow multiple routine scanning and to save and organise scanned images on a preselected hard drive. For the experiments in this thesis a three hour scanning interval was selected as this would give a sufficient amount of images over the scanning period to allow accurate analysis of root tips without exceeding available storage capacity. All images were saved on the controlling PC hard drive and backed up onto a 250GB Verbatim portable hard drive.

Image J was used to plot coordinates of the roots in the images which were later analyzed in MS Excel. Image J is an image processing program originally developed by the National Institutes of Health. Image J has the function to select points within an image and log the coordinates of the selected location. All coordinates plotted were automatically saved on to an excel spreadsheet which assisted analysis of the coordinates. This program was selected to plot the coordinates of the root tips because of its ease of use and its ability to open sequences of images and allow navigation through the sequence allowing root tips to be followed throughout an entire sequence.

2.1.8 Fertilizer

Miraclegro plant food was selected due to its composition of nitrogen, potassium and phosphorus along with trace elements full details are as follows:

- Nitrogen total 24%
- Ammoniacal nitrogen 3.5%
- Ureic nitrogen 20.5%
- Phosphourus pentoxide 8%
- Potassium Oxide 16%
- Boron 0.02%
- Copper 0.03%
- Iron 0.19%
- Manganese 0.05%
- Molybdenum 0.001%
- Zinc 0.03%

2.2 Method and Development

2.2.1 Compost preparation

Compost was packed into pots which were soaked for 24 hours to ensure that the compost was entirely saturated before they were moved to a tension table where 5k Pascal of suction was applied for 48 hours to allow the available water holding capacity for a growing plant to be calculated this was found to have a mean value of 279.92g with a standard error of 18.87. Five 50g samples were then dried at 105^{o}C for 24 hours and then re-weighed to calculate the gravimetric water content.

$$gravametric\ water\ content = \frac{Weight\ of\ wet\ sample - weight\ of\ dry\ sample}{weight\ of\ dry\ sample}$$

The mean value for this was 5.22 with a standard error of 0.16. Following this the compost was passed through a 10mm screen to remove any large fibrous lumps ensuring that an even spread of compost could be achieved across the plates. Prepared compost which was not in use was bagged off and stored at 5^{o}C.

2.2.2 Quantify sealant effect on growth

The first objective was to establish an effective way of sealing the plates during waterlogging that did not affect plant health. All four sealant methods were tested for effects on germination by filling four Petri dishes with prepared compost. Six tomato seeds were evenly sown across the dish and a sample of the sealant was applied around the edge of each dish. A control was also constructed which had no sealant present. All dishes were stored at 21.5^{o}C and the germination and growth of the seeds in each of the dishes were monitored each day at 10 am using a steel rule. All shoots were measured from the base of the seed to the furthest point on emerging shoots.

Within fourteen days sufficient data had been collected to be able to assess the effect each of the sealants had on seed germination and growth. If growth was to be monitored over a longer time a larger growth area would be required to allow sufficient room for each of the plants to grow.

2.2.3 Quantifying sealant integrity

Five different ways of using the sealants were tried during the test period:

1. A seal was cut out of a single sheet of foam and placed between the two plates and held in place using small clips.
2. A tape sealant was applied around the outside edge of the two plates. If this technique was successful it would be a quick and easy way to seal the plates before waterlogging.
3. The tape sealant was also applied in the similar way as the foam seal by cutting thin strips and sticking them along the inside edges before clipping the two plates in place with plastic clips.
4. Pond liner sealant which was in the tube applicator was applied around the inside edges of a set of plates before they were held together with clips.
5. Silicone sealant was applied along the inside edges of the plates before the two plates were held in place using small plastic clips.

All of the plates were then filled with 100mm of water before being placed into the growth cabinet at 21.5^{o}C and monitored every hour by measuring the water level from the bottom of the plates. As the foam seal failed quickly after being placed into to growth cabinet the plates were re-opened and the seal examined. It was found that under the warmth and the compression the foam was subjected to within the growth cabinet the foam structure had broken down. The foam was tried again but this time the seal was

made of two sheets of foam which meant that the seals integrity was not relying on a single layer of bubble structure which made up the foam.

2.2.4 Plate preparation for scanning Tomato seeds

Eight clear plates and eight black plates were cleaned and checked for scratches and damage to the structure of the plate. 290g of compost was weighed out for each clear plate and spread out evenly across the inside surface of the plate. It was found that 290g was the optimum amount of compost that allowed compost to be backed into the space between the two plates without distortion of the plates and allowed the compost profile between the two plates to remain distorted during the waterlogging process. A small hole was made in the compost 10 mm from the top of the plate directly below the centre opening along the top edge. One pre-soaked seed was inserted into this hole then covered over with compost. A black plate was then placed on top and held in place with clips. All plates were covered with foil and placed in the growth cabinet at an angle of 80^{o} with the clear face facing downwards. This would encourage the roots to grow towards the clear face and not disappear behind the compost.

2.2.5 Plate preparation for scanning Barley seeds

Eight clear plates and eight black plates were cleaned and checked for damage. 290g of compost was weighed out for each clear plate and spread out evenly across the inside surface of the plate. Three small holes were made in the compost 15 mm from the top of the plate directly below each of the openings along the top edge. The plates were then separated into two groups of four plates, one group was used to grow one line of barley and the other group was used to grow the comparative line of barley. One pre-soaked seed was inserted into each of the holes then covered over with compost. A black plate was then placed on top and held in place with clips. All plates were covered with foil and

placed in the growth cabinet at an angle of 80^{o} with the clear face facing downwards this would encourage that the roots to grow towards the clear face and not disappear behind the compost.

2.2.6 Watering and fertilizing

Plates were fertilised and watered using the same method. Plates were not sealed when they were first put into the growth cabinet at this would cause watering and fertilizing problems. Initially the Tomato plates had their water and fertiliser applied at 20ml each time by injecting the solution in the top of the plate and allowing the solution to run down across the face of the plate. This resulted in poor root elongation due to poor distribution of nutrients from the fertiliser during watering.

The second method required all of the plates to be removed from the growth cabinet and placed at an angle of 80^{o} with the clear face facing downwards in a large tray which could be filled to a level of 100 mm with a water or fertilizer figure 2.2. The plates remained in the tray for two hours before being returned to the growth cabinet. Watering and fertilizing occurred once a week until waterlogging commenced where they were fertilized two days before waterlogging was due to commence. The second method was found to be much more effective than injecting the fertiliser giving a more even distribution of nutrients across the plate.

Figure 2.2- Plates during watering and fertilizing process, 100mm of water or fertilizer added after plates were inserted, plates remained in this position for two hours.

2.3 Method Used for Data Collection

2.3.1 Experiment 1 (scanning Tomatoes)

Eight plates were prepared and allowed to grow in the growth cabinet until sufficient root elongation had occurred should have occurred within two weeks to allow scanning. The plates were fertilized by injecting the solution across the top of the plate every second day and it was allowed to run down through the profile within the plate. Unfortunately this resulted in poor root formation. As the root formation was so bad it was not possible to use these plants to test the technique as all roots were located in the top 80mm of the plate profile.

2.3.2 Experiment 2 (scanning Tomatoes)

Eight plates were prepared and allowed to grow in the growth cabinet until sufficient root elongation had occurred which should have occurred within two weeks to allow scanning. The plates were fertilized by removing them from the growth cabinet and then placed into a tray at an angle of 80^{o} with the clear face facing downwards. The tray was then filled to

a level of 100 mm with fertilizer once a week. Unfortunately on week two due to the circulation fan failing within the growth cabinet all eight plants had become severally heat stressed meaning that any data generated using these plants would be affected. As a future precaution two thermostats were installed into the growth cabinet which were clearly visible from the inspection window.

2.3.3 Experiment 3 (scanning Barley)

Eight plates were prepared and allowed to grow in the growth cabinet until sufficient root elongation had occurred which was within four days at which point all plates were fertilized. The plates were fertilized by removing them from the growth cabinet and then placed into a tray at an angle of 80^{o} with the clear face facing downwards the tray was then filled to a level of 100 mm with fertiliser following fertilizing the plates were sealed and returned to the growth cabinet. The three of each of the barley line plates were then placed into the scanners which were angled at 80^{o} and while the sealant was setting pre waterlogging scanning of the roots took place for 24 hours. After 24 hours all plates were waterlogged and scanning of the roots commenced for five days followed by two days without waterlogging.

2.3.3.1 Image Capture

All six Cannon Canoscan 5600F flat bed scanners were controlled by archiScan_multi which allowed scans across the six scanners to be taken at set times over a period of time. This program was set to take scans every 3 hours as this gave sufficient growth between scans for a change in coordinates to be plotted without exceeding the available memory required for the eight images of each plate generated ever 24 hours. The plates remained in the same location throughout each of the different phases meaning that a sequence of images could be generated over time showing the roots as they grew across the face of

the clear plate. Each plate was numbered to correspond to the scanner it was in and a small section of 5mm graph paper was applied to act as a reference scale in all images. The level of the waterlogging was also marked with a thin strip of coloured tape to assist in identifying the waterlogging level on the images. After each day the sequence of images could be analysed for each of the plates using Image J. Image J allowed a selection of root tip coordinates from above and below the waterlogging level to be tracked and plotted on an MS Excel spreadsheet.

The images generated could not only give quantitative data but also simple visual representation of the root elongation across the plate through any time scale figure 2.3.

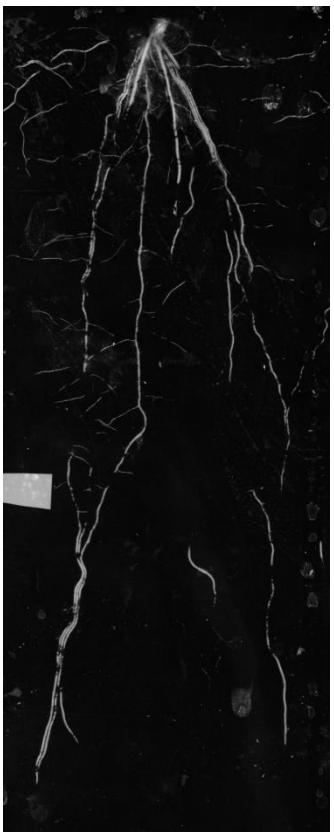


Figure 2.3- Example of Image generated by the scanners showing roots of one mutant line of Barley.

2.3.3.2 Displaying data

Using trigonometry it was possible to set up a spread sheet that would convert the coordinates into a trace of the root tip movement.

Distanced moved in pixels = $\sqrt{(X2 - X1)^2 + (Y2-Y1)^2}$

Where: X1 = x coordinate value 1

X2 = x coordinate value 2

Y1 = y coordinate value 1

Y2 = y coordinate value 2

In order to get a "true" measurement of the root elongation first the generated data had to be converted from pixels to mm. This was done by plotting the coordinates of key points on the 5mm graph paper on the plates to generate a conversion factor. This conversion factor which was 11 pixels to 1 mm could then be used to convert the measurements in pixels into measurements in mm.

mm distance moved = $\frac{Distance\ moved\ in\ pixels}{11}$

It was also possible to use the coordinate values to compare the movement of the root tops at different stages to look for variation in the way in which the two barley lines elongated their roots Figure 2.4 and 2.5.

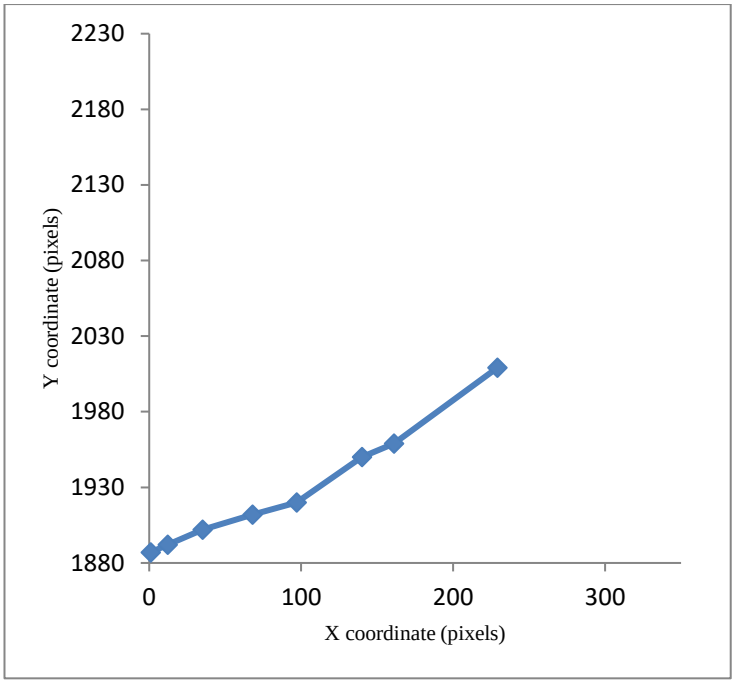


Figure 2.4- Mutant Barley seminal root tip movement trace over 24h above waterlogging level on Day 4 of waterlogging

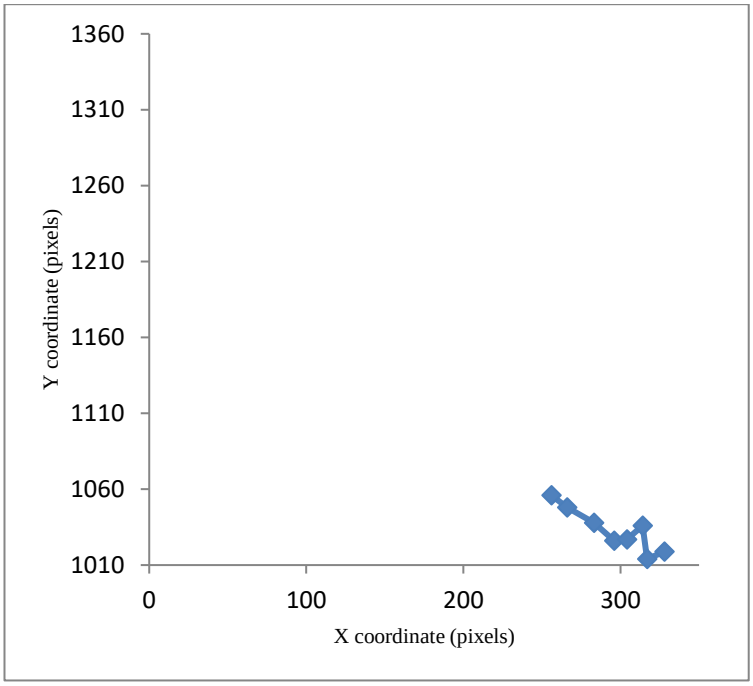


Figure 2.5- Wild Barley seminal root tip movement over 24h above waterlogging level on Day 4 of waterlogging

2.3.3.3 Visual assessment of plant leaf stress.

Nine leaves were selected and measured from the first node to the tip of each leaf for each of the six plates that were waterlogged and the two controls. Visual assessment of the health of each leaf during measurement was noted with the measurement paying particular attention to the area of leaf which had become wilted or were showing symptoms of chlorosis. Each plate was then photographed in both visible light and with a near infrared filter.

3 Results

3.1 Seal development

3.1.1 Effects on seed germination

Tests carried out on the previous sealant method showed that silicone had a marked effect on seed germination and growth when compared to seeds grown in the absence of any sealant figure 3.1.

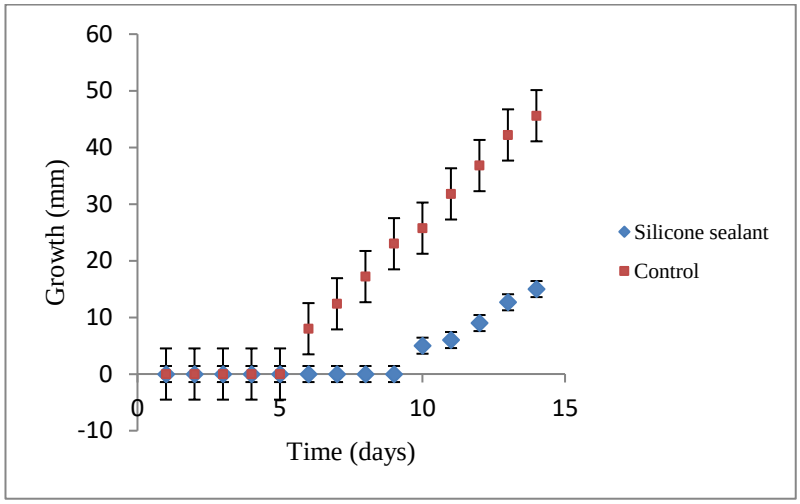


Figure 3.1- Comparing the growth of seedlings in the presence of silicone based sealant to a control showing standard error. All measurements were taken from base of the stem to the top of the stem.

This reinforced that this was a problem which had to be improved before continuing with this experiment. The three other sealant techniques had a marked improvement on the silicone based sealant as can be seen in figure 3.2.

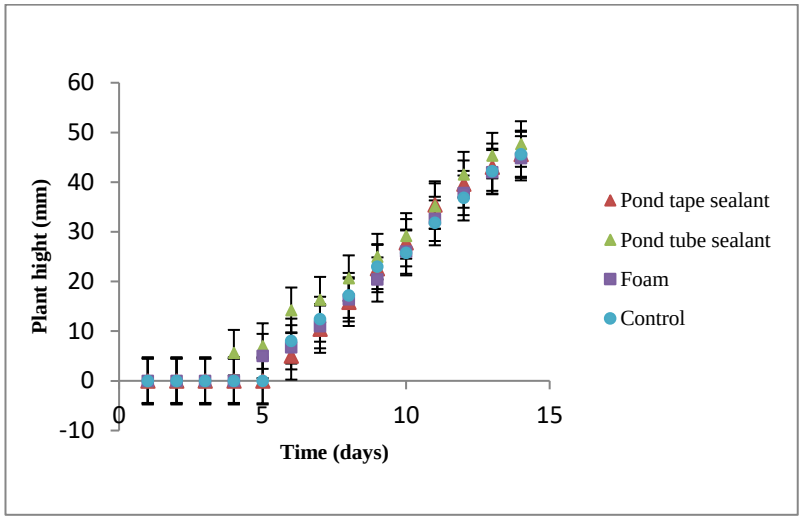


Figure 3.2 - Comparing the growth of seedlings in the presence of the new sealant methods showing standard error. All measurements were taken from base of the stem to the top of the stem.

3.1.2 Seal integrity

The next stage was to test how well the new sealant methods retained the water within the plates. In both cases the foam seal had failed within seven hours meaning that it was not strong enough to maintain a secure seal throughout any experiments done during this thesis. Using the MS Polymer based pond sealent tape applyed along the outside edges of the plate developed problems before being inserted into the growth cabinet. The tape could not adhear to the surface with enough force to withstand the pressure from the head of water and leaks developed as soon as water was pored between the plates. Both the pond sealant types when applyed to the inside surfaces preformed well Figure 3.3 until 31 hours where leaks in the pond tape seal started to appear the the corners where the individual strips of tape joined.

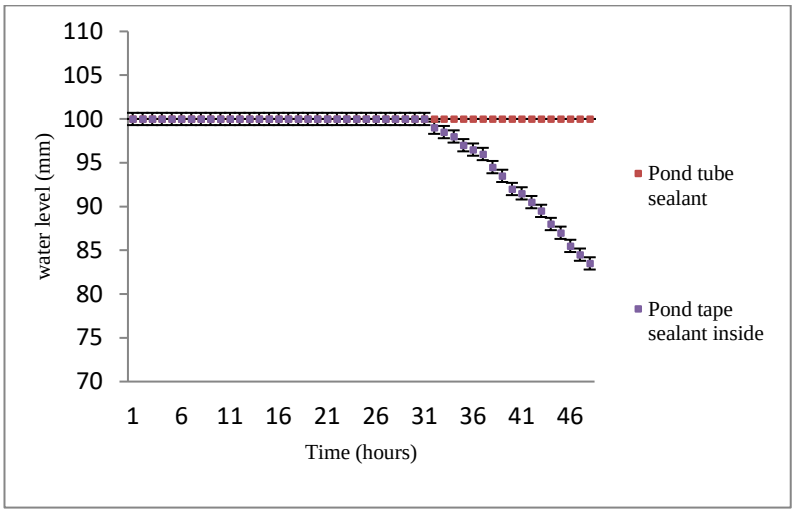


Figure 3.3 – Pond sealant test (Water holding capacity) showing the drop in water level against time.

The pond sealant in the tube applicator continued to retain water with no losses for two weeks at which point testing was ended. The decision that the pond sealant in the tube applicator was the most suited method of making a seal between the two plates during the waterlogging process. This decision was made on the basis that during tests it showed no significant effect on seed germination and growth and was able to contain water for in excess of the maximum period of seven days which was required for the experiments in this thesis.

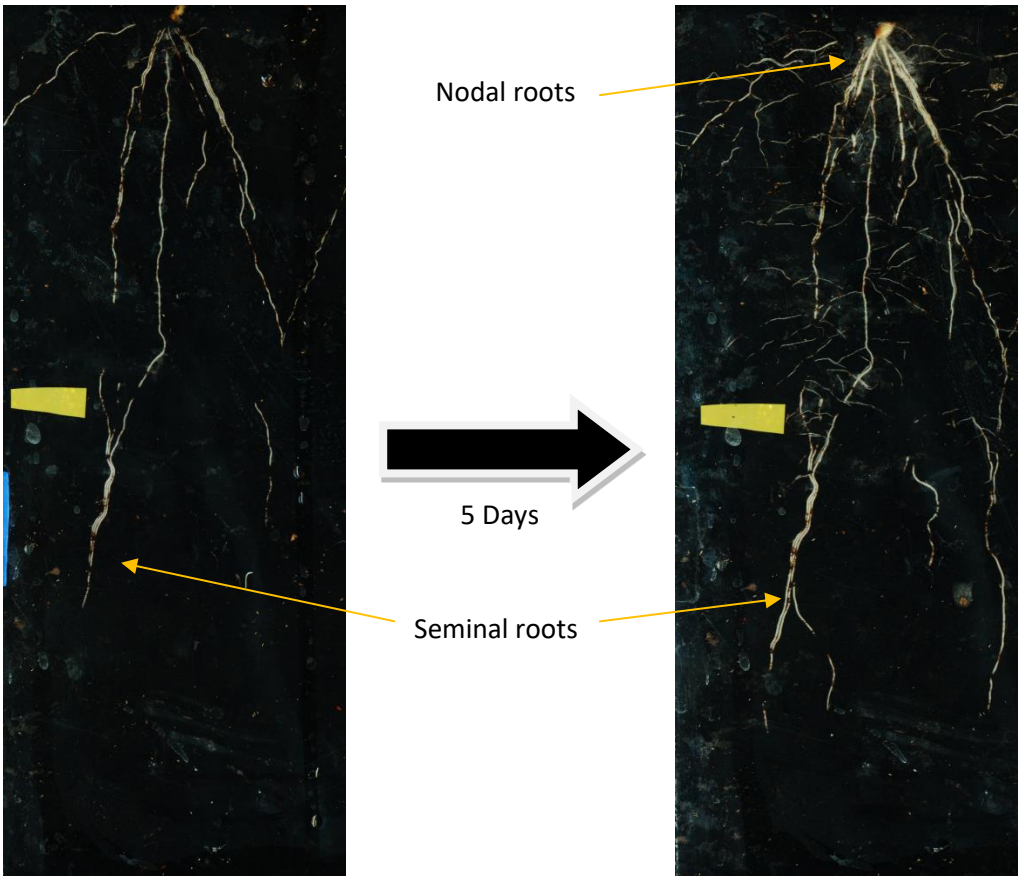
3.2 Raw data

Due to the problems with the growth cabinet no data was generated from tomatoes. We know that in the right conditions this technique will work with tomatoes as this technique has been carried out previously with the Silicone based sealant by Dresbøll (Dresbøll Personal communication 2010). In 2010 Dresbøll carried out experiments using the same technique as used in this thesis but plant health issues were reported during those experiments. I believe from my testing of the sealant used by Dresbøll (2010) this was

the cause of the health problems and the new sealant method would ensure healthy plants in a repeat of this experiment.

3.2.1 Scanned images

At the end of the experiment a sequence of images had been generated which showed the root elongation over the test period see figure 3.4.



Start of experiment End of experiment

Figure 3.4-Images generated from start to end of experiment. Initially only seminal roots develop, after 5 days of water logging nodal roots emerge similar to results of Palta (2007) who showed nodal roots developing after 3 days.

Once these images were imported into Image J one sequence of images at a time three root tips above and three root tips below the waterlogging level were plotted. This quickly

generated a large amount of data showing the coordinates of each of the roots as they elongated across the images.

3.2.2 Root tip elongation between barley lines MC 169 and MC182

The mutant line of barley *Hordeum Vulgare* L MC 169 is reported to have much straighter and faster growing roots when compared with its parental material *Hordeum Vulgare L MC182.* (A.E. Martinez et al 2003).

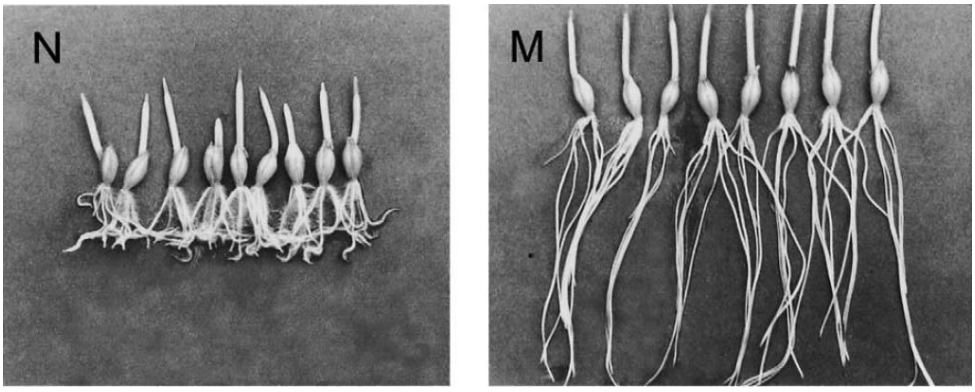


Figure 3.5-Image taken from paper generated by A.E. Martinez et al 2003 of parental line (N) compared to mutant lime (M)

Using the coordinates generated in Image J it is possible to plot the movement of the seminal root tips as they move through the profile contained within the plates. Figures 3.6 to 3.11 show the how the seminal root tips have moved in 24 hours on day two of the waterlogging above the waterlogging level. The standard error of plotting root tips in Image J is 0.02mm for each point.

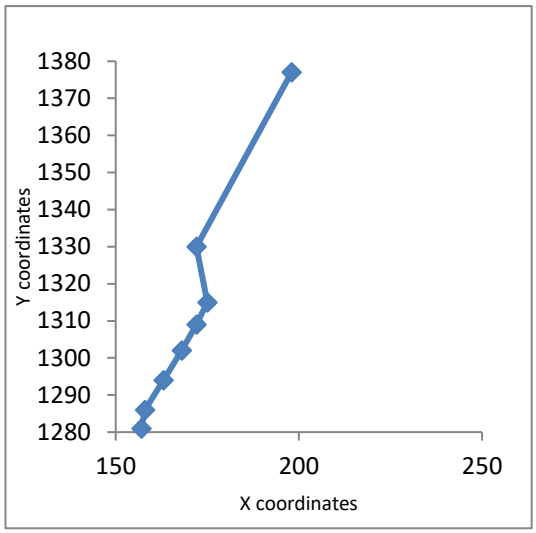


Figure 3.6-Wild type barley seminal root tip movement on day 2 above the waterlogging level.

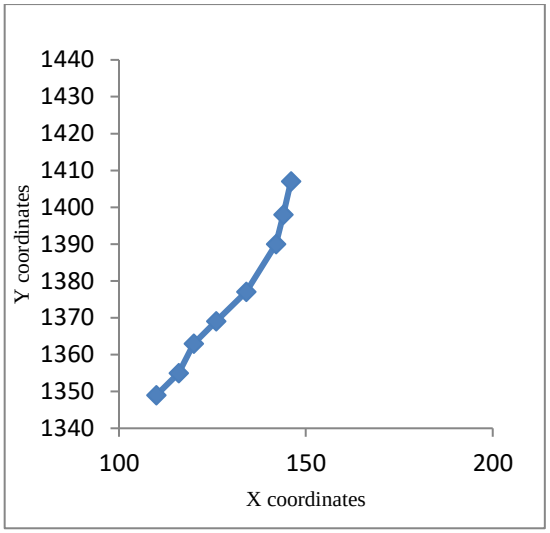


Figure 3.7- wild type barley seminal root tip movement on day 2 above waterlogging level

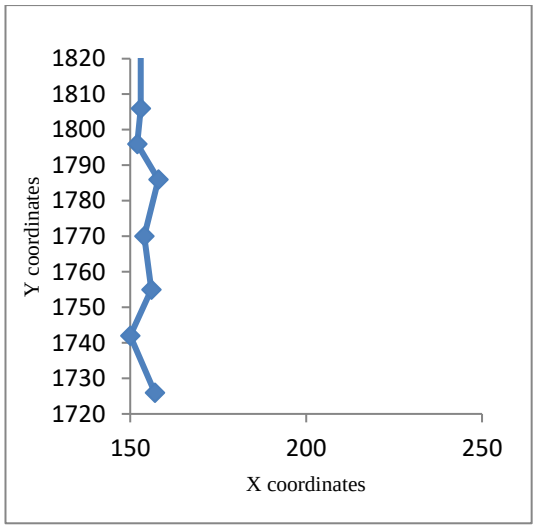


Figure 3.8- wild type barley seminal root tip movement on day 2 above waterlogging level

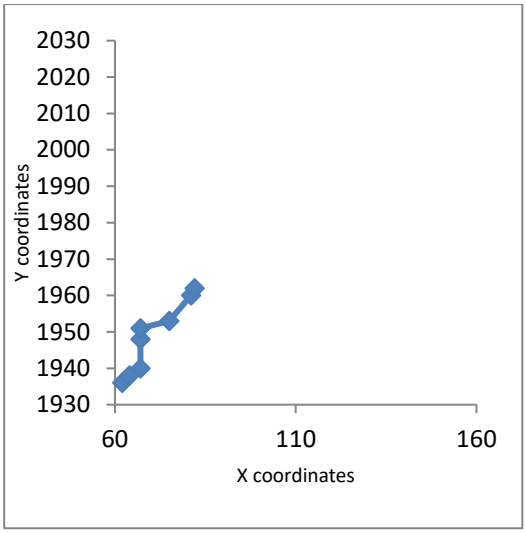


Figure 3.9-Mutant barley root tip movement on day 2 above the waterlogging level.

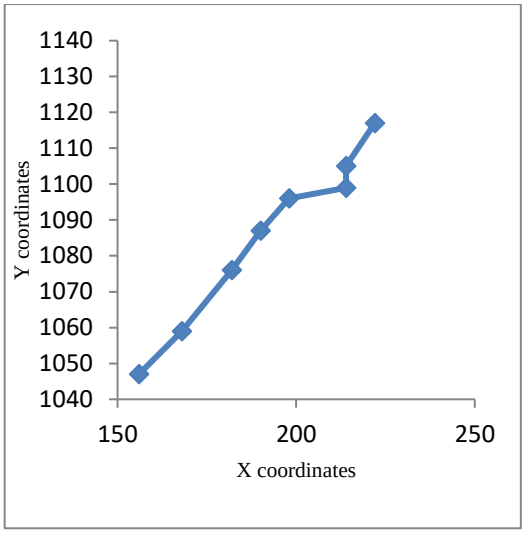


Figure 3.10-Mutant barley root tip movement on day 2 above the waterlogging level.

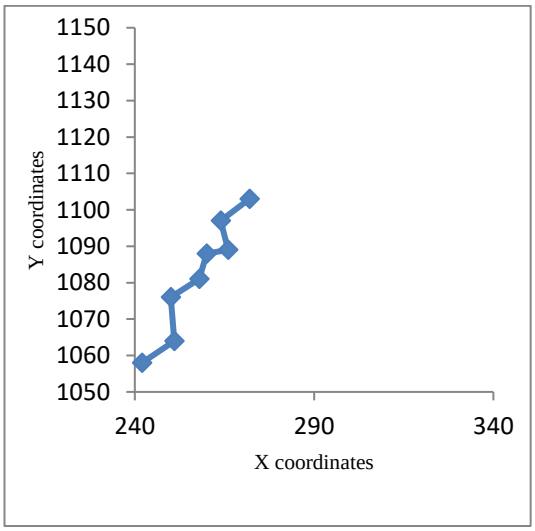


Figure 3.11-Mutant barley root tip movement on day 2 above the waterlogging level.

From figures 3.6 and 3.11 it would appear that the root tips of the wild type barley were growing at a higher rate than that of the mutant. The roots of both lines appear to be elongating without any significant difference in path.

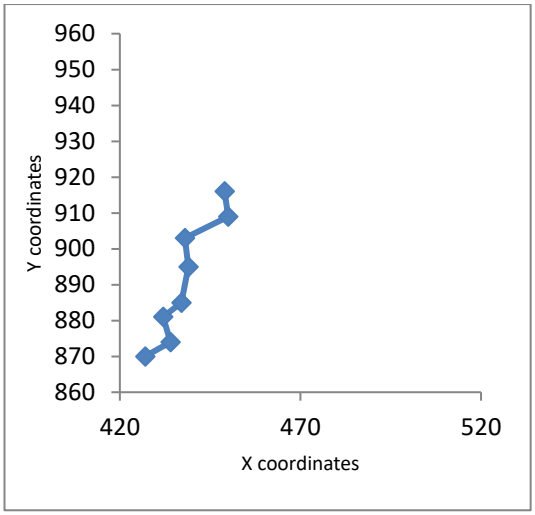


Figure 3.12-wild barley root tip movement

on day 2 below the waterlogging level.

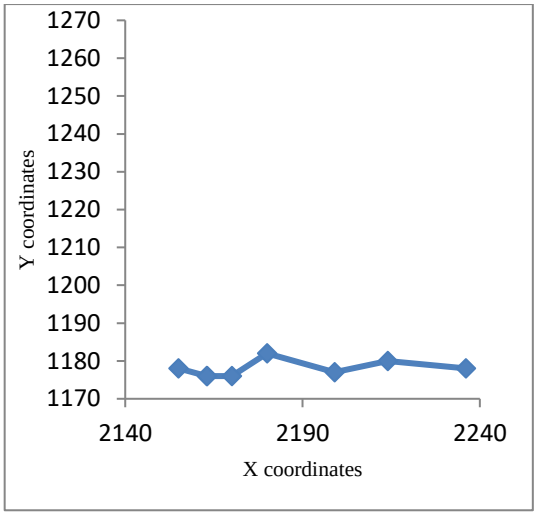


Figure 3.13-wild barley root tip movement

on day 2 below the waterlogging level.

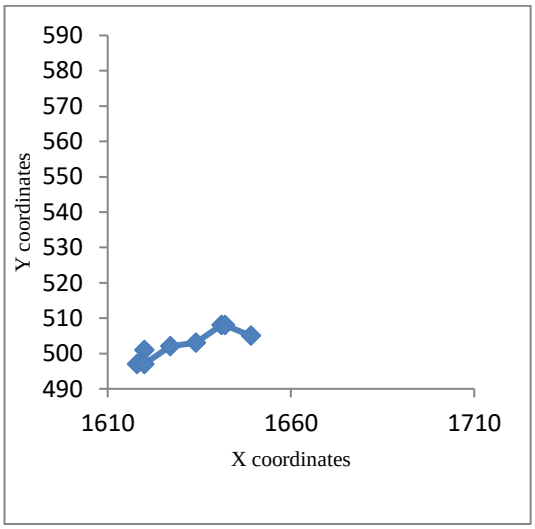


Figure 3.14-wild barley root tip movement

on day 2 below the waterlogging level.

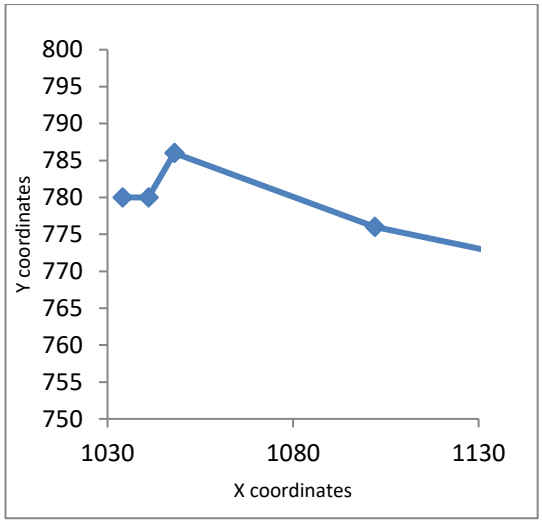


Figure 3.15-Mutant barley root tip movement on day 2 below the waterlogging level.

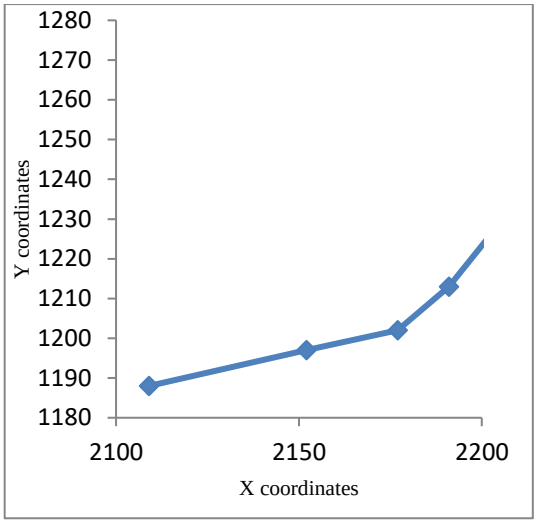


Figure 3.16-Mutant barley root tip movement on day 2 below the waterlogging level.

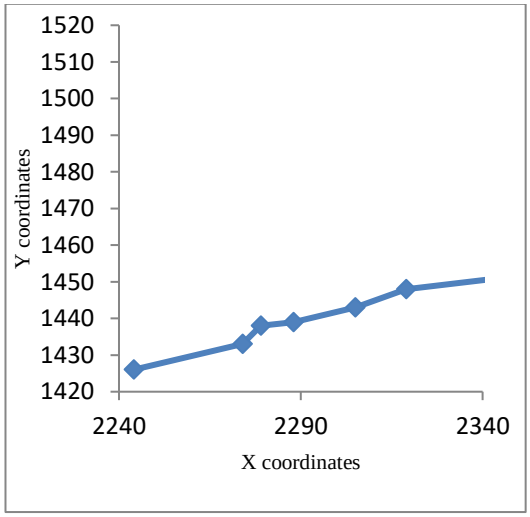


Figure 3.17-Mutant barley root tip movement on day 2 below the waterlogging level

Figures 3.12 to 3.17 show the root tip elongation of the two lines of barley under the waterlogging level at two days. The root tips of the mutant line are elongating in a much straighter line than that of the wild parental line along with there being a higher rate of

root elongation in the mutant line. This supports the results A.E. Martinez et al 2003 found when using hydroponics. This would suggest that the mutant line of barley consistently exhibits its reported characteristics in compost as well as in hydroponics.

3.2.3 Root elongation

It is recognised that during waterlogging seminal root elongation below the waterlogging level is reduced and roots above the waterlogging level are increased (Palta et al 2007). Using the coordinates generated in Image J the growth rate of roots in mm/h below the waterlogged level and above the waterlogged level could be calculated. Figures 3.18 and 3.19 show the difference between the roots above and below the waterlogging level in the mutant line of barley and its wild parental line.

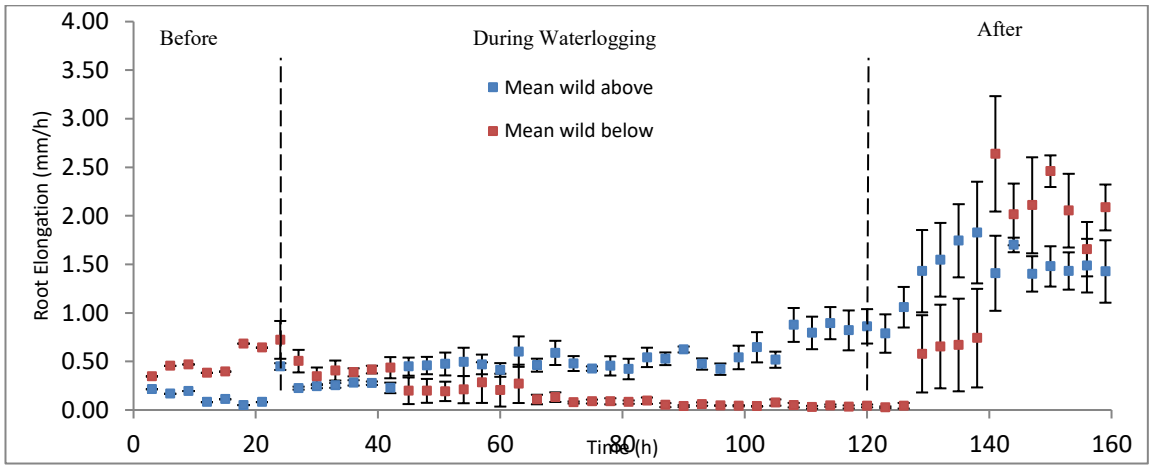


Figure 3.18-Wild barley root elongation rate with standard error. The dotted lines depict the three different phases of the experiment before waterlogging, during waterlogging and after waterlogging.

One can see that in figure 3.18 after 66 hours there appears to be a difference between the root elongation rate of the roots above the waterlogging level and the roots below the waterlogging level in the wild parental line. A T test of this area was done to test if this difference was significant. Table 3.1 shows the results from this T test.

	Variable 1	*Variable 2*
Mean	0.59844117	0.064543
Variance	0.02799408	0.000831
Observations	19	19
Hypothesized Mean Difference	0	
df	19	
t Stat	13.7073196	
P(T<=t) one-tail	1.3283E-11	
t Critical one-tail	1.72913279	
P(T<=t) two-tail	2.6566E-11	
t Critical two-tail	2.09302405	

Table 3.1- T test results assuming unequal variances and two tails from 66 hours to 120 hours comparing root elongation rate above and below the waterlogging level in the wild barley line.

Stating that the null hypothesis is that there is no difference between the two population means and that the t Critical values for the two tails is 2.09. It is possible to see from table 3.1 that the calculated t value exceeds the t Critical two tails value so we can say that the means of the results above and below the waterlogging level are significantly different at a 95% level of probability between 66 hours and 120 hours. A P value of 2.65^{-11} would suggest that there is a low chance of the t value occurring randomly.

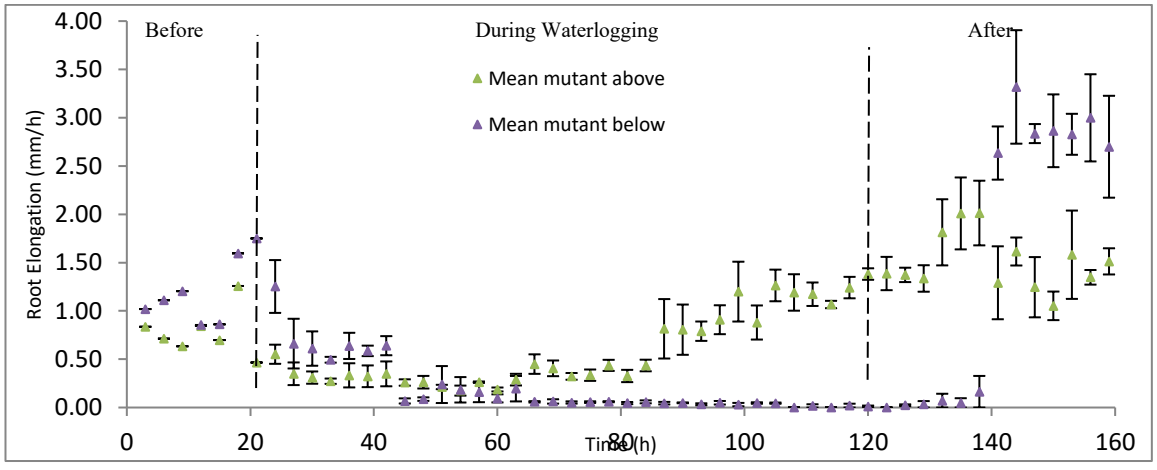


Figure 3.19-Mutant barley root elongation rate with standard error. The dotted lines depict the three different phases of the experiment before waterlogging, during waterlogging and after waterlogging.

Looking at figure 3.19 once again after 66 hours there appears to be a difference between root elongation above the waterlogging level and the roots below the waterlogging level in the mutant line. A t test of this area was done to test if this difference was significant. Table 3.2 shows the results from this t test.

	Variable 1	*Variable 2*
Mean	0.8118632	0.038883
Variance	0.1388656	0.000415
Observations	19	19
Hypothesized Mean Difference	0	
df	18	
t Stat	9.0281702	
P(T<=t) one-tail	2.102E-08	
t Critical one-tail	1.7340636	
P(T<=t) two-tail	4.204E-08	
t Critical two-tail	2.100922	

Table 3.2- T test results assuming unequal variances and two tails from 66 hours to 120 hours comparing root elongation rate above and below the waterlogging level in the wild barley line.

Using a null hypothesis that there is no difference between the two population means and that the t Critical values for the two tails is 2.10. It is possible to see from table 3.2 that the calculated t value exceeds the t Critical two tails value so we can say that the means of the results above and below the waterlogging level are significantly different at a 95% level of probability between 66 hours and 120 hours. A P value of 4.20^{-08} would suggest that there is a low chance of the t value occurring randomly.

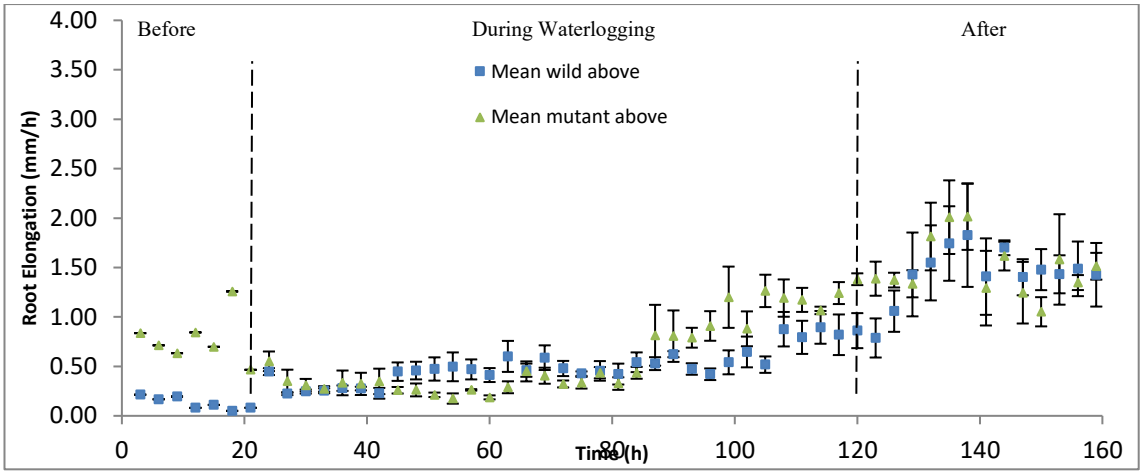


Figure 3.20-wild & Mutant barley root elongation rate above waterlogging level with standard error. The dotted lines depict the three different phases of the experiment before waterlogging, during waterlogging and after waterlogging.

There also appears to be a difference between the two lines. Initially it would appear that the wild parental line of barley has an increasing rate of root elongation above the waterlogging level which after 48 hours is higher than the mutant line figure 3.20. By 96 hours the mutant line has a significantly higher rate of root elongation compared to its parental line this is confirmed by a t test Table 3.3.

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	1.145016	0.707967
Variance	0.027182	0.032137
Observations	9	9
Hypothesized Mean Difference	0	
df	16	
t Stat	5.383365	
P(T<=t) one-tail	3.05E-05	
t Critical one-tail	1.745884	
P(T<=t) two-tail	6.09E-05	
t Critical two-tail	2.119905	

Table 3.3- T test results assuming unequal variances and two tails from 96 hours to 120 hours comparing root elongation rate above the waterlogging level in both lines of barley.

With the null hypothesis that there is no difference between the two population means and that the t Critical values for the two tails is 2.11. It is possible to see from table 3.3 that the calculated t value exceeds the t Critical two tails value so we can say that the means of the results above and below the waterlogging level are significantly different at a 95% level of probability between 96 hours and 120 hours. A P value of 6.09^{-05} would suggest that there is a low chance of the t value occurring randomly. Although the P value would suggest that there is a higher chance of the t value occurring in this situation than when comparing the root elongation rates of the two lines above and below.

From figure 3.20 the mutant line is shown to be higher root elongation rate in the top of the profile than its parental line before waterlogging. As the t value is higher than the t critical in a t test of the two mean values between 0 hours and 21 hours we can say that there is a significant difference between the two lines at a 95% probability before waterlogging table 3.4.

	Variable 1	*Variable 2*
Mean	0.778342	0.128525
Variance	0.0612	0.004024
Observations	7	7
Hypothesized Mean Difference	0	
df	7	
t Stat	6.731858	
P(T<=t) one-tail	0.000135	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.000269	
t Critical two-tail	2.364624	

Table 3.4- T test results assuming unequal variances and two tails from 0 hours to 21 hours comparing root elongation rate at the top of the profile in both lines of barley.

The root elongation rate between the two lines below the waterlogging level after 66 hours has almost stopped in both lines this could be due to ethylene levels become high and causing root elongation to stop this cannot be confirmed as ethylene levels were not measured (figure 3.21). Seminal roots elongation can be permanently stopped or there elongation rate can be reduced greatly by high ethylene levels caused by waterlogging (Huang & Johnson, 1995).

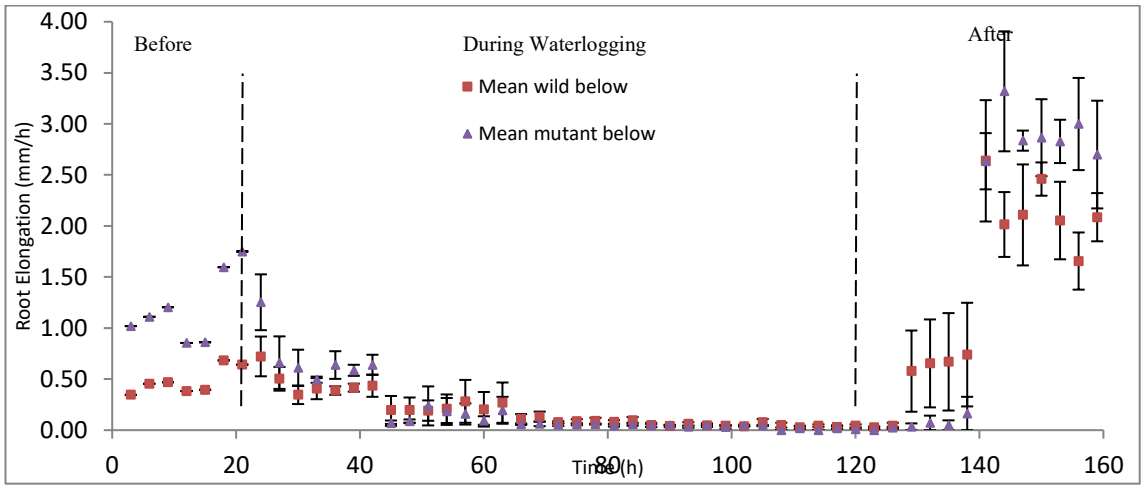


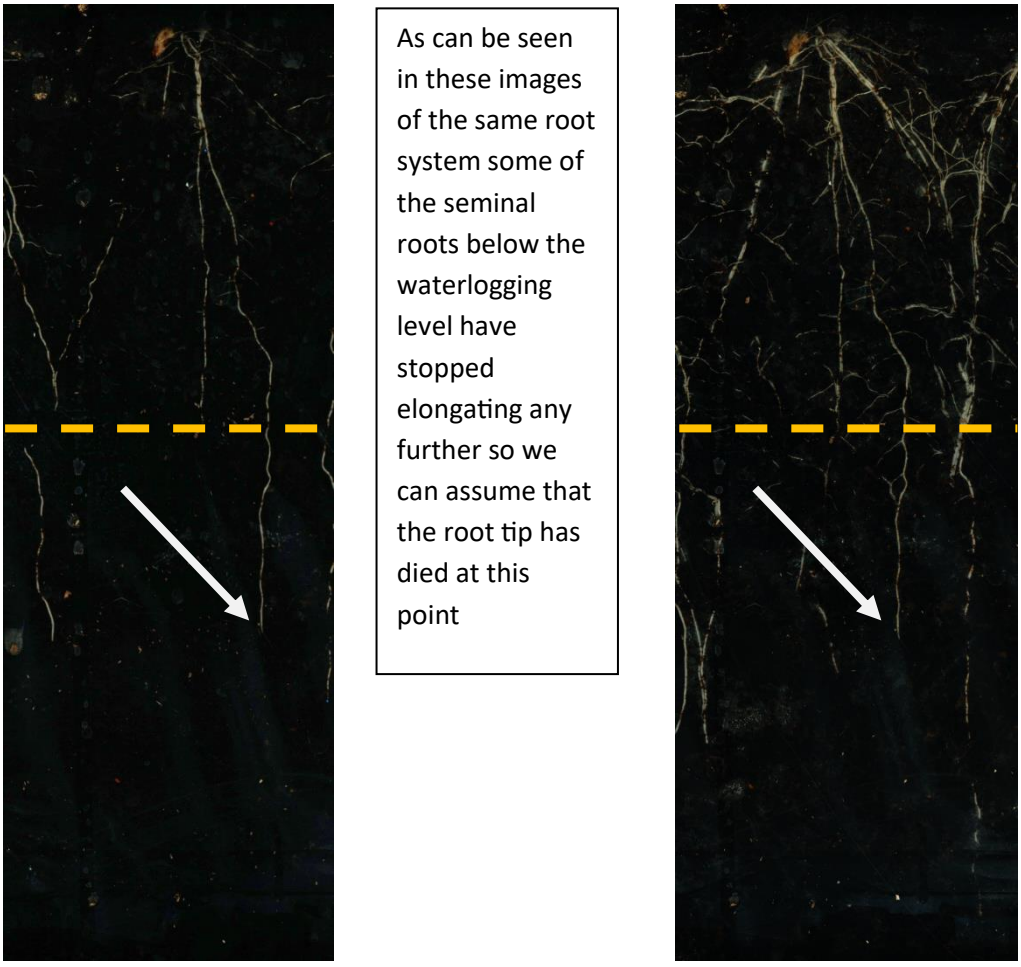
Figure 3.21-wild & Mutant barley root elongation rate below waterlogging level with standard error. The dotted lines depict the three different phases of the experiment before waterlogging, during waterlogging and after waterlogging.

The root elongation rate of the mutant line is shown to be higher in the bottom of the profile than its parental line before waterlogging in a similar way to the roots at the top of the profile. As the t value is higher than the t critical in a t test of the two mean values between 0 hours and 21 hours we can say that there is a significant difference between the two lines at a 95% probability before waterlogging. A P value of 0.001 would suggest that there is a low chance of the t value occurring randomly table 3.5.

	Variable 1	*Variable 2*
Mean	1.199367	0.482117
Variance	0.122458	0.017161
Observations	7	7
Hypothesized Mean Difference	0	
df	8	
t Stat	5.078637	
P(T<=t) one-tail	0.000477	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.000955	
t Critical two-tail	2.306004	

Table 3.5- T test results assuming unequal variances and two tails from 0 hours to 21 hours comparing root elongation rate at the bottom of the profile in both lines of barley.

By looking closely at images of the seminal roots after this point it can be said that this is likely to be the case that after 66 hours (42 hours into waterlogging) that the seminal root tips have reached their critical ethylene levels resulting in the root elongation of these roots to stop permanently (Huang & Johnson, 1995) figure 3.22.



Roots at 66 hours Roots at 160 hours

Figure 3.22-Visual comparison of root elongation at 66 hours to 160 hours. The dotted lines depict the waterlogging level

When the images from the two different lines are observed there are marked differences in the axes in which the roots have grown figure 3.23 and 3.24. The roots of the mutant line have far more lateral roots above the waterlogging level and the roots below the waterlogging level are much straighter than that of the wild parental line. H Schneider et al (2007) found that xylem pressures varied significantly when plant roots were under stress. As xylem pressure is linked to changes in turgor pressure further examination of the roots of these two different lines may show that the reasons for the variation in root elongation rate and the way in which the roots elongate may be due to changes in root turgor pressure.

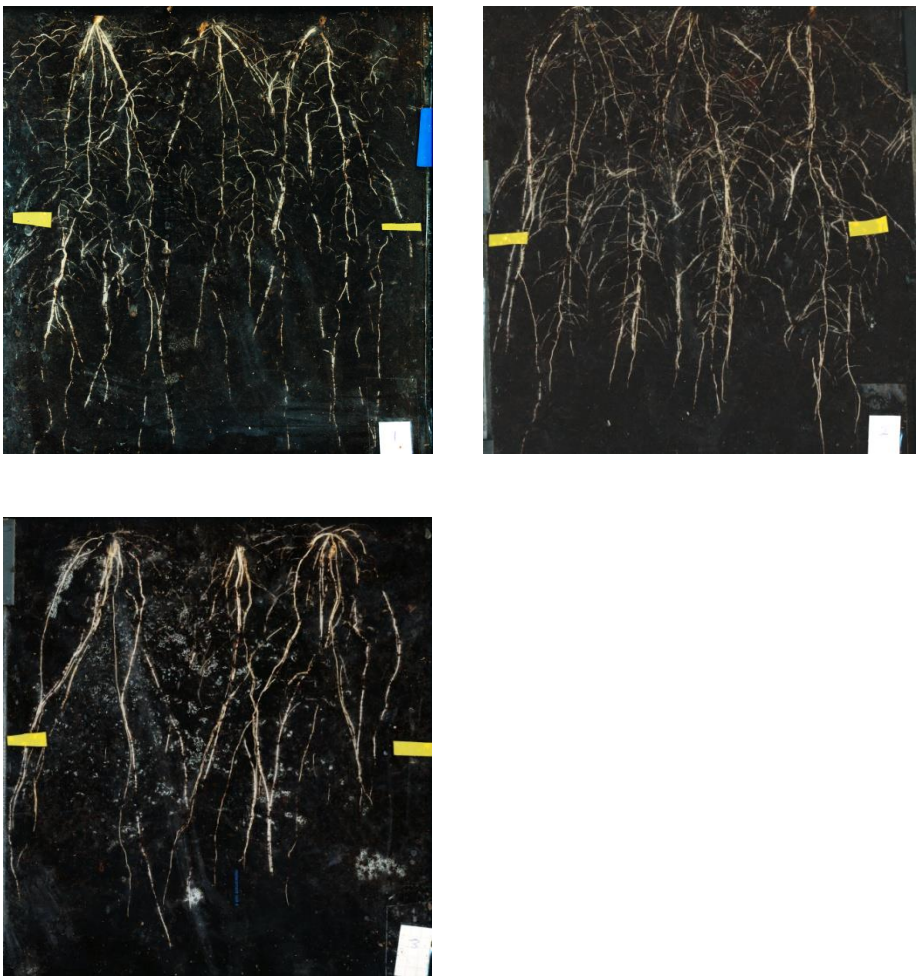


Figure 3.23- root elongation after experiment in the wild parental line. Yellow tape denotes the waterlogging level.

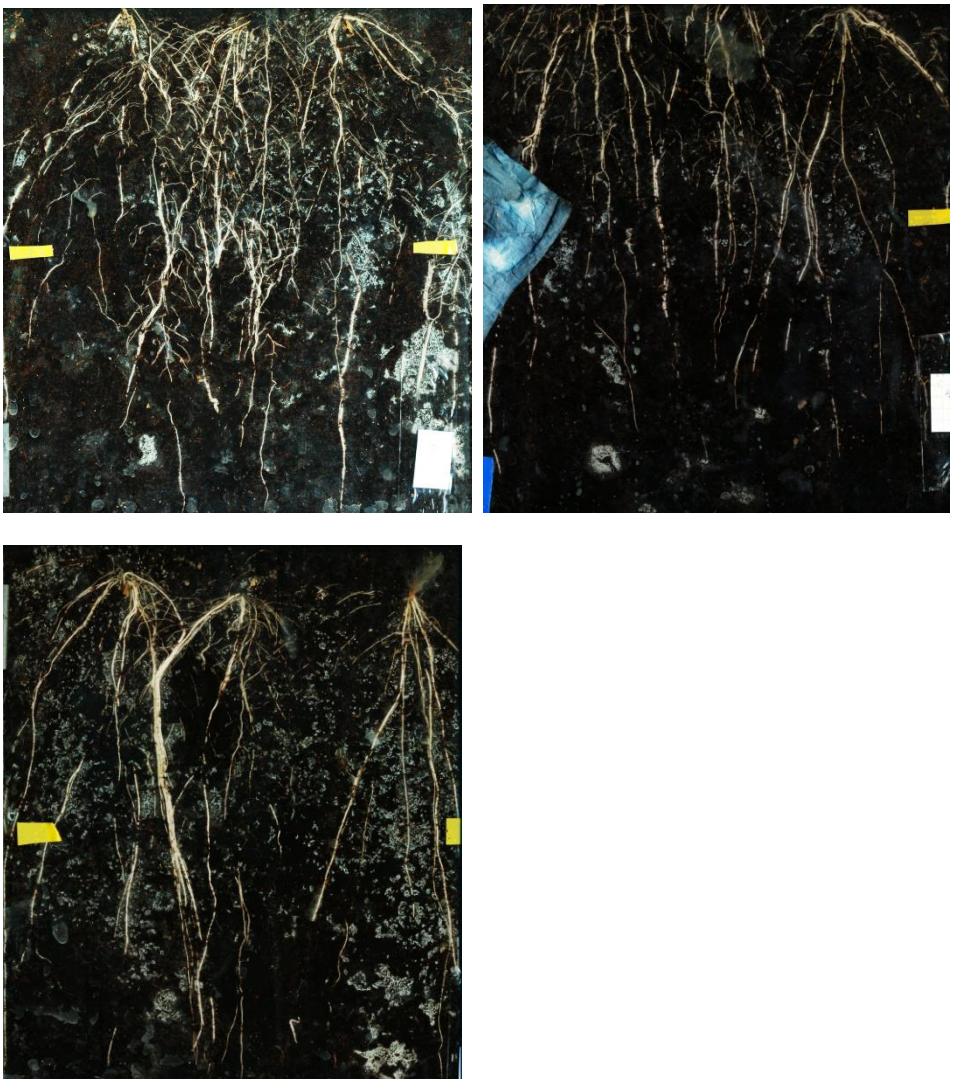


Figure 3.24-root elongation after experiment in the mutant. Yellow tape denotes the waterlogging level.

3.3 Plant stress

After the waterlogging process the physical health of the plants was assessed. The plant leaf length was measured to give an indication of leaf area produced by the two lines. All leaves were measured from the first node to the tip of each leaf. Figure 3.25 and 3.26 show the results of both the mutant and its parental line. All of the plates that had been waterlogged the leaf area index was effected. The wild parental line appeared to have suffered less stress and was able to maintain growth of its leaf area during waterlogging compared visually to the mutant line. Although when you look at the control taking the error into account the mutant line developed a larger leaf area index compared to its parental line. This would indicate that during waterlogging the leaf area of the mutant line was affected to a greater extent than that of its wild parental material. This indicates that the mutant line was under a higher level of stress than its parental line (Grant R. Cramer 1992). Leaf development in early stages of plant development affects plant health in later growth stages which will in turn affect yield (Yuncai Hu et al (2000).

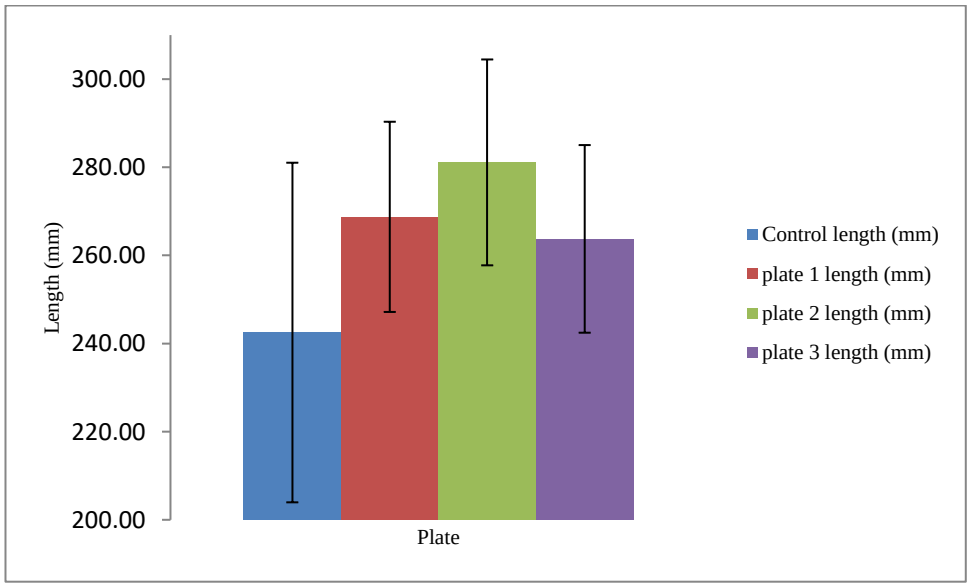


Figure 3.25-Mean wild leaf length from first node with standard error, n=9

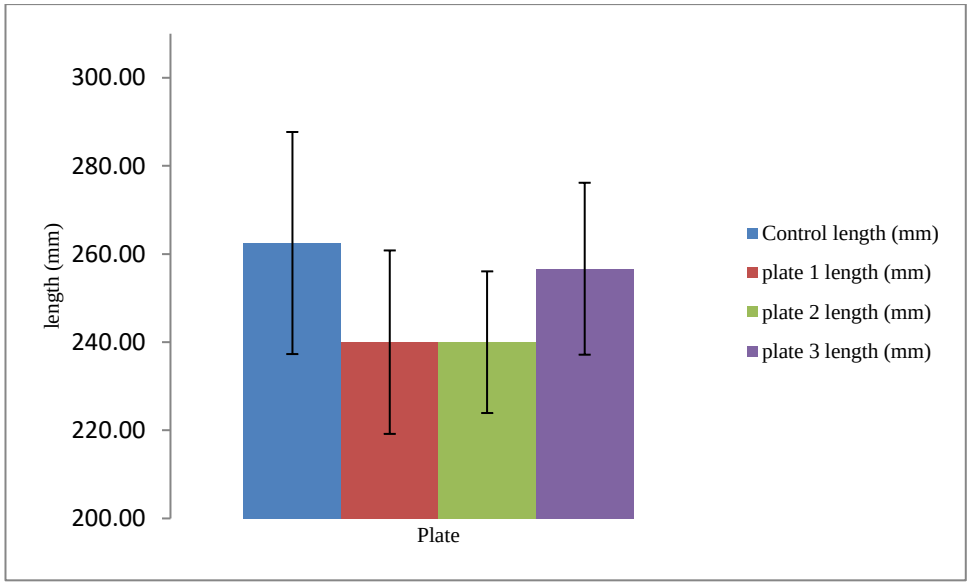


Figure 3.26-Mean mutant leaf length from first node with standard error, n=9

Leaf health was also visually assessed after waterlogging both lines showed signs of chlorosis (figure 3.27 and 3.28).

Figure 3.27-Leaf tip from wild parental line of barley after waterlogging showing chlorosis.

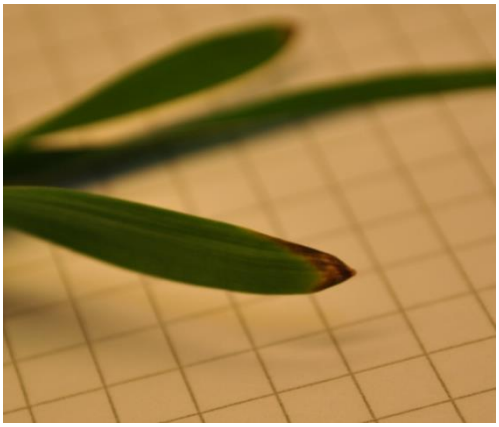


Figure 3.28-Leaf tip from mutant line of barley after waterlogging showing chlorosis.

From figure 3.29 and 3.30 it can be seen that the mutant line appeared to have a higher percentage of its leaf area affected by chlorosis when compared to its parental line.

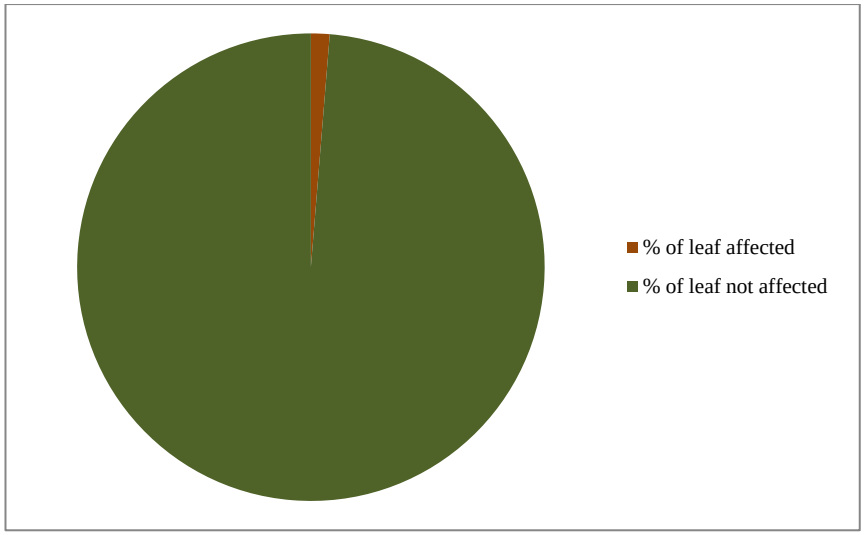


Figure 3.29- % of leaf affected by chlorosis in the wild parental line after waterlogging, n=9

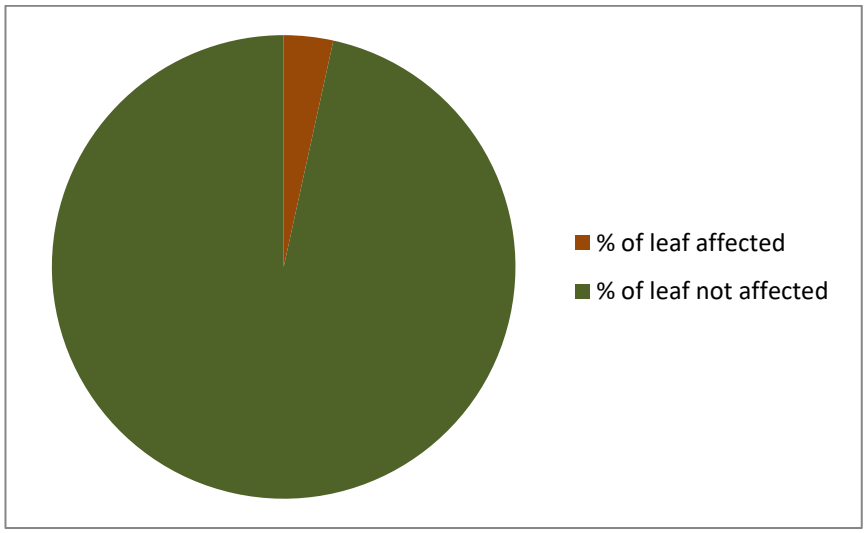
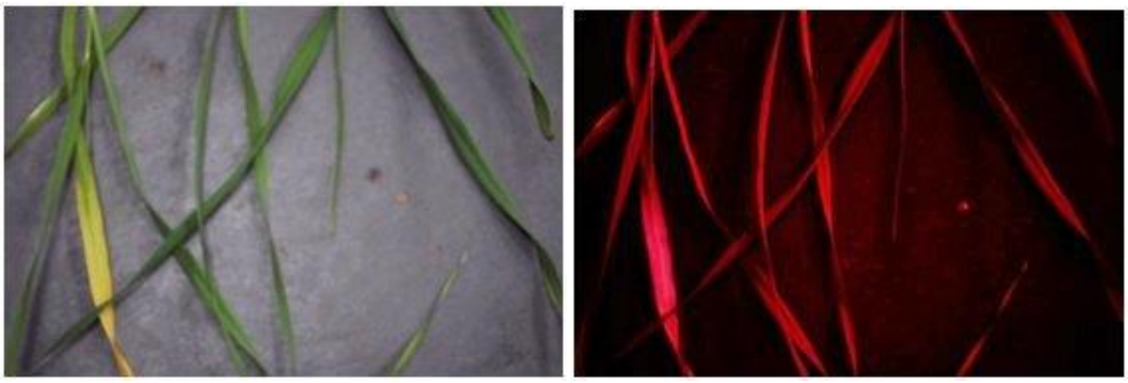


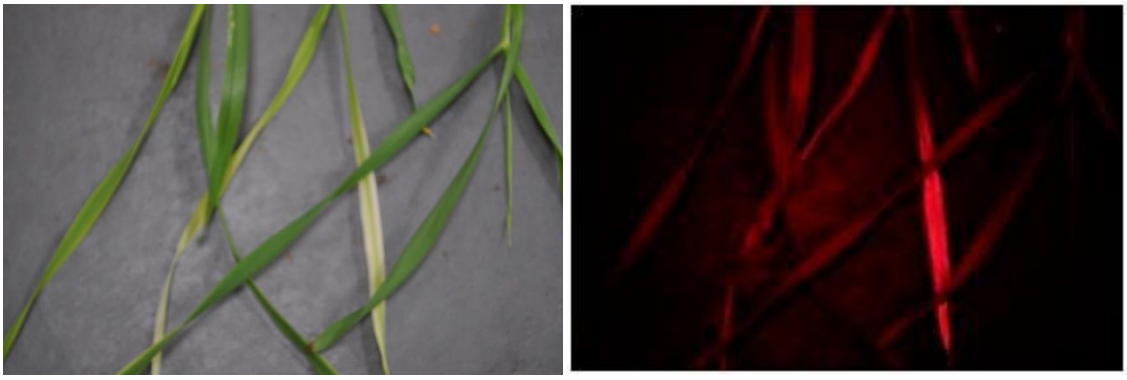
Figure 3.30- % of leaf affected by chlorosis in the mutant line after waterlogging, n=9

By using a near infrared filter it is possible to see the effects on the leaf structure. Photosynthetic efficiency can be assessed by a photo taken with a near infrared filter. a leaf with high levels of chlorophyll will fluorescence less than a leaf with low levels of chlorophyll therefore an increase in fluorescence implies a decrease in photosynthesis (Laury Chaerle et al 2004**)** Figures 3.31 and 3.32 show this effect on the mutant line and its wild parental line.



Wild visible light Wild near infrared light

Figure 3.31-Wild barley assessment of decrease in photosynthesis



Mutant visible light Mutant near infrared light

Figure 3.32-Mutant barley assessment of decrease in photosynthesis

Although this technique does not allow more accurate analysis of leaf chlorosis than simple visually judging the percentage of leaves affected by chlorosis it does give a visual representation of the damage to a plant's ability to photosynthesise. In order to quantify data generated using near infrared light this would require a photo sensitive cell or software that can measure the reflectance. This is the fundamental concept that analytical equipment such as N tech industries, Greenseeker and Yara's Nitrogen sensor operate.

4 Discussion

4.1 Seal development

It was important to initially develop a method of sealing the two plates together which did not affect the growth and development of test plants. The foam that was used to form a seal was simple and easy to use but breaking down of the foam structure when under pressure resulted in the seal failing to meet necessary requirements.

The MS polymer based sealant proved to a suitable alternative to silicone based sealant. The MS polymer sealant was available in two forms, tape and in a tube applicator. Different ways of applying is sealant was tried but application between the two plates remained the most efficient. This sealant was designed to be used between two layers of EPDM or PVC pond liner and therefore did not have a high level of adhesion strength which is required for external application. It was the MS polymer based sealant in the tube applicator that was finally used due to its structural integrity, ease of use and low effect on plant health. This method of sealing equipment which is to be used in close proximity to plants could be applied to future applications where other sealants could affect plant health.

Future development could be made in the foam seal by trying different types of foam which have a stronger structure. By developing the foam seal a simple reusable seal could be possible.

4.2 Root scanning

Once the initial problems with the growth cabinet were repaired successful scanning of barley roots was achieved. By plotting of root tip elongation rate above and below the waterlogging level confirmed that during waterlogging root tips below the waterlogging

level start to slow their elongation rate while roots above the waterlogging level increase their elongation rate (Palta et al 2007). Results also confirmed that approximately after 48 hours of waterlogging seminal root elongation stops (Ari Rajala, 2002). Without further analysis of the roots it is not possible to confirm that this is due to ethylene levels resulting in aerenchyma formation (Huang & Johnson, 1995). In future replications of this experiment ethylene levels could be monitored in order to confirm the cause of the seminal root tips reduction and final stop in elongation rate. Analysis of the results show a significant difference in the elongation rate of the roots formed by the mutant line (*Hordeum Vulgare* L MC 169) when compared to its wild parental line (*Hordeum Vulgare L MC182)*. This supports the hypothesis that there are varietal differences with regards to waterlogging tolerance within species. This knowledge has practical applications in breeding programs. The importance of selecting for waterlogging tolerance may become an important consideration with increased precipitation predicted in future years (Maraun et al 2008).

The mutant line of barley *Hordeum Vulgare* L MC 169 is reported to have much straighter and faster growing roots when compared with its parental material *Hordeum Vulgare L MC182* when grown in hydroponics (A.E. Martinez et al 2003). Results generated during this thesis would suggest that this is also the case when the two lines are grown in compost. This further secures the possibilities of the mutant line being able to be used as experimental material for studies of root genetics and function when being influenced by environmental influences. By having a selection of model lines which have proven significance to certain environmental influences will allow further developments to be made in specific areas of research.

From visual observations of the images after the experiment, differences in the angular spread of the roots were visible. The mutant line appeared to have consistently higher root

angle axes when compared to its wild parental line. Not only does this mean that the mutant lines roots would have a greater ability to exploit zones of high phosphate concentration in the topsoil which has the potential to increases yield (Liao et al, 2001) but would also have a higher tolerance to lodging. Lodging can be caused by waterlogging which results in yield reductions (Macmillan F. 2010. personal communication). For this reason lodging tolerance that should be considered when assessing plant tolerance to waterlogging.

4.3 Visual plant stress

Measurements of the leaf lengths of the two lines of barley indicate that leaf growth during waterlogging is affected to a greater extent in the mutant line of barley when compared to its wild parental line. Poor leaf development in early stages of plant development has a detrimental effect on yield and final crop quality (Yuncai Hu et al, 2000). Further analysis of the two lines at growth stage 87 after waterlogging treatment would allow this effect to be quantified (Watson et al, 1976). The reduction in leaf length may explain why root elongation below the waterlogging level dropped lower faster in the mutant line than in its parental material. This may have been caused by a reduced supply of oxygen to the aerenchyma tissue as a result of the shorter leaves (Samad et al, 2000).

Leaf chlorosis can be used to measure waterlogging tolerance (Boru et al. 2001). From the results generated in this thesis the mutant line of barley appeared to have a higher % area of leaf area affected by chlorosis even when mean root elongation rate was higher. This might support suggestions that the mutant line has lost the ability to sense water gradients (Martinez et al, 2003). The reduction in leaf health may be due to the continued carbon demand for root elongation which perhaps became higher than could be harnessed

by the plant (Drouet et al, 2007). Further research into how the mutant line recovers after waterlogging may prove useful as the larger rooting system of the mutant line may allow an improved recovery then compared to its wild parental line.

The spectral characteristics of radiation reflected or absorbed by leaves can be used to develop a thorough understanding of plant physiology (Gregory et al, 2001). Leaf reflectance at wavelengths between 400 – 720nm is affected by stress more consistently than visible light (Carter. 1993). It is possible to estimate leaf chlorophyll concentration from indices based on leaf reflectance in far red light (Gitelson and Merzlyak. 1997). From the images generated using the 720nm infrared filter it was possible to visually see the reduction in chlorophyll within leaves. It was not possible on this occasion to quantify the levels of chlorophyll from these images. It does show that it is possible to visually observe changes in chlorophyll levels. Future analysis of these two barley lines may use this technique to quantify plant stress during the waterlogging process. By using this technique plant mass above the soil may also be assessed in response to waterlogging over a period of time.

Lemna tec use near infrared imaging to asses spatial root performances of plants in their high throughput testing. Lemna tec use near infrared imaging to quantify water distribution in soil during drought testing. This same approach could be applied to waterlogging tests to generate quantitive data of the water distribution within the plates which allow more accurate models to be constructed to simulate root response to waterlogging.

5 Conclusion

This Thesis set out to look at whether it was possible to quantify root response to waterlogging above and below the waterlogging. This was successfully achieved by extensive research into other phenotyping techniques and the development of an existing technique.

The results gained from experiments carried out in this thesis have confirmed that it is possible to quantify root response to waterlogging above and below the waterlogging level. From the experiments carried out in this thesis it was possible to compare two barley lines. Mutant line MC 169 and its wild parental line MC182 were selected to be used. This was because there is documented evidence to suggest that the mutant line MC 169 had a significantly higher rate of root elongation and straighter growing root tips when compared to its wild parental line MC182 (A.E. Martinez et al 2003). The mutant line was confirmed of having significantly higher root elongation rate compared to its wild parental line both above and below the waterlogging level. Coordinate plots of the root tips confirmed that the mutant line has got straighter growing roots. This may support suggestions made by A.E. Martinez et al 2003 that the mutant line has lost the ability to detect water gradients.

Although the mutant line had better root tolerance to waterlogging its leaf area was affected to a greater extent. Wild parental line MC 182 was affected the least which may give the line an advantage when recovering from waterlogging where early leaf development is important to plant health in later growth stages (Yuncai Hu et al. 2000). Further research into this with these two lines would be advantageous to see how plant health and cropping yield is affected at growth stage 87. The results of this thesis suggest

that there are varietal differences between lines towards waterlogging tolerance. Further analysis of different lines in this manner may assist in finding a common gene that controls the desirable traits within species that improve waterlogging tolerance (Cao et all. 1995).

This technique may prove to be a useful tool to quantify root response to waterlogging in different lines within species to assist in future breeding programs where waterlogging tolerance is important. This technique may also prove to be useful in further aspects of root research where roots are required to be monitored at scheduled intervals in a non invasive manner. This technique has the potential to reduce the development time in which it takes to breed new lines by allowing more concise selection of parental material and following generations.

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Appendices

Root elongation results

wild above

time (h)	all trays average Mean wild above (mm)	SD	SE
3	0.21		0.00
6	0.17		0.00
9	0.20		0.00
12	0.08		0.00
15	0.11		0.00
18	0.05		0.00
21	0.08		0.00
24	0.45	0.06	0.03
27	0.23	0.03	0.02
30	0.25	0.04	0.02
33	0.25	0.04	0.02
36	0.28	0.05	0.03
39	0.28	0.03	0.02
42	0.23	0.09	0.05
45	0.45	0.16	0.09
48	0.46	0.15	0.09
51	0.47	0.20	0.12
54	0.50	0.25	0.15
57	0.47	0.18	0.10
60	0.41	0.12	0.07
63	0.60	0.27	0.16
66	0.46	0.12	0.07
69	0.59	0.22	0.12
72	0.48	0.13	0.08
75	0.43	0.04	0.02
78	0.45	0.17	0.10
81	0.42	0.18	0.11
84	0.54	0.17	0.10
87	0.53	0.11	0.07
90	0.62	0.05	0.03
93	0.47	0.10	0.06
96	0.42	0.10	0.06
99	0.54	0.21	0.12
102	0.65	0.27	0.16
105	0.52	0.14	0.08
108	0.88	0.30	0.17
111	0.79	0.29	0.17

wild below

time (h)	all trays average Mean wild below (mm)	SD	SE
3	0.35		0.00
6	0.45		0.00
9	0.47		0.00
12	0.38		0.00
15	0.40		0.00
18	0.68		0.00
21	0.64		0.00
24	0.72	0.34	0.19
27	0.50	0.20	0.12
30	0.35	0.16	0.09
33	0.41	0.18	0.10
36	0.39	0.07	0.04
39	0.42	0.07	0.04
42	0.44	0.19	0.11
45	0.20	0.24	0.14
48	0.20	0.21	0.12
51	0.19	0.17	0.10
54	0.21	0.24	0.14
57	0.28	0.36	0.21
60	0.20	0.29	0.17
63	0.27	0.34	0.20
66	0.11	0.09	0.05
69	0.13	0.09	0.05
72	0.08	0.02	0.01
75	0.09	0.03	0.02
78	0.09	0.05	0.03
81	0.08	0.03	0.02
84	0.10	0.06	0.03
87	0.05	0.04	0.02
90	0.04	0.02	0.01
93	0.06	0.03	0.02
96	0.05	0.01	0.00
99	0.04	0.01	0.01
102	0.04	0.01	0.00
105	0.08	0.06	0.03
108	0.05	0.04	0.02
111	0.03	0.02	0.01

Root elongation results

wild above

time (h)	all trays average Mean wild above (mm)	SD	SE
114	0.89	0.29	0.17
117	0.82	0.36	0.21
120	0.86	0.31	0.18
123	0.79	0.34	0.20
126	1.06	0.36	0.21
129	1.43	0.73	0.42
132	1.55	0.66	0.38
135	1.74	0.65	0.38
138	1.83	0.91	0.52
141	1.41	0.67	0.39
144	1.70	0.13	0.07
147	1.40	0.32	0.18
150	1.48	0.36	0.21
153	1.43	0.33	0.19
156	1.49	0.48	0.28
159	1.43	0.56	0.32

wild below

time (h)	all trays average Mean wild below (mm)	SD	SE
114	0.04	0.02	0.01
117	0.03	0.03	0.02
120	0.04	0.02	0.01
123	0.03	0.02	0.01
126	0.04	0.05	0.03
129	0.58	0.69	0.40
132	0.65	0.75	0.43
135	0.67	0.83	0.48
138	0.74	0.88	0.51
141	2.64	1.03	0.59
144	2.01	0.55	0.32
147	2.11	0.86	0.49
150	2.46	0.28	0.16
153	2.05	0.66	0.38
156	1.66	0.48	0.28
159	2.09	0.41	0.24

Root elongation results

Mutant above

time (h)	all trays average Mean mutant above (mm)	SD	SE
3	0.84		0.00
6	0.71		0.00
9	0.63		0.00
12	0.84		0.00
15	0.70		0.00
18	1.26		0.00
21	0.47		0.00
24	0.55	0.17	0.10
27	0.35	0.20	0.12
30	0.31	0.11	0.06
33	0.27	0.05	0.03
36	0.33	0.22	0.13
39	0.32	0.19	0.11
42	0.35	0.22	0.13
45	0.26	0.06	0.03
48	0.26	0.11	0.07
51	0.21	0.04	0.02
54	0.17	0.09	0.05
57	0.26	0.01	0.01
60	0.19	0.03	0.02
63	0.29	0.10	0.06
66	0.45	0.18	0.10
69	0.41	0.14	0.08
72	0.32	0.06	0.03
75	0.33	0.10	0.06
78	0.44	0.10	0.06
81	0.33	0.11	0.06
84	0.43	0.10	0.06
87	0.81	0.53	0.31
90	0.81	0.45	0.26
93	0.79	0.17	0.10
96	0.91	0.26	0.15
99	1.20	0.54	0.31
102	0.88	0.31	0.18
105	1.26	0.28	0.16
108	1.19	0.33	0.19
111	1.17	0.21	0.12

Mutant below

time (h)	all trays average Mean mutant below (mm)	SD	SE
3	1.02		0.00
6	1.11		0.00
9	1.20		0.00
12	0.85		0.00
15	0.86		0.00
18	1.60		0.00
21	1.75		0.00
24	1.25	0.47	0.27
27	0.66	0.45	0.26
30	0.61	0.31	0.18
33	0.49	0.05	0.03
36	0.64	0.23	0.14
39	0.59	0.09	0.05
42	0.64	0.17	0.10
45	0.07	0.05	0.03
48	0.09	0.03	0.02
51	0.24	0.33	0.19
54	0.18	0.23	0.13
57	0.16	0.18	0.10
60	0.09	0.07	0.04
63	0.19	0.23	0.13
66	0.06	0.01	0.01
69	0.06	0.04	0.02
72	0.05	0.02	0.01
75	0.06	0.01	0.01
78	0.06	0.01	0.01
81	0.05	0.02	0.01
84	0.06	0.03	0.02
87	0.05	0.02	0.01
90	0.05	0.01	0.00
93	0.03	0.02	0.01
96	0.05	0.03	0.02
99	0.03	0.03	0.02
102	0.05	0.01	0.01
105	0.05	0.01	0.01
108	0.00	0.00	0.00
111	0.02	0.03	0.02

Root elongation results

Mutant above

time (h)	all trays average Mean mutant above (mm)	SD	SE
114	1.07	0.07	0.04
117	1.24	0.19	0.11
120	1.38	0.10	0.06
123	1.39	0.30	0.17
126	1.37	0.13	0.07
129	1.34	0.24	0.14
132	1.81	0.59	0.34
135	2.01	0.64	0.37
138	2.01	0.58	0.33
141	1.29	0.65	0.38
144	1.62	0.25	0.14
147	1.25	0.54	0.31
150	1.05	0.26	0.15
153	1.58	0.79	0.46
156	1.35	0.13	0.08
159	1.51	0.23	0.14

Mutant below

time (h)	all trays average Mean mutant below (mm)	SD	SE
114	0.00	0.00	0.00
117	0.02	0.03	0.02
120	0.01	0.02	0.01
123	0.00	0.00	0.00
126	0.02	0.02	0.01
129	0.03	0.06	0.03
132	0.07	0.12	0.07
135	0.05	0.08	0.05
138	0.16	0.28	0.16
141	2.63	0.48	0.28
144	3.32	1.02	0.59
147	2.84	0.17	0.10
150	2.86	0.65	0.38
153	2.83	0.37	0.21
156	3.00	0.78	0.45
159	2.70	0.91	0.53

plant height after waterlogging

wild type

	control	scanner 1	scanner 2	scanner 3	mean of scanners
	360	330	390	370	363.33
	300	240	300	260	266.67
	390	300	220	200	240.00
	310	170	340	310	273.33
	170	240	290	280	270.00
	130	280	210	240	243.33
	120	360	280	270	303.33
	160	230	330	180	246.67
	wild type control				wild type after waterlogging
mean	242.5				275.83

mutant

	control	scanner 1	scanner 2	scanner 3	mean of scanners
	300	320	230	340	296.67
	360	180	190	210	193.33
	220	230	230	250	236.67
	290	180	180	300	220.00
	310	260	310	200	256.67
	160	170	290	280	246.67
	170	240	220	330	263.33
	290	350	210	220	260.00
	mutant control				mutant after waterlogging
mean	262.5				246.67

	wild type	mutant
control	242.5	262.5
after waterlogging	275.83	246.67