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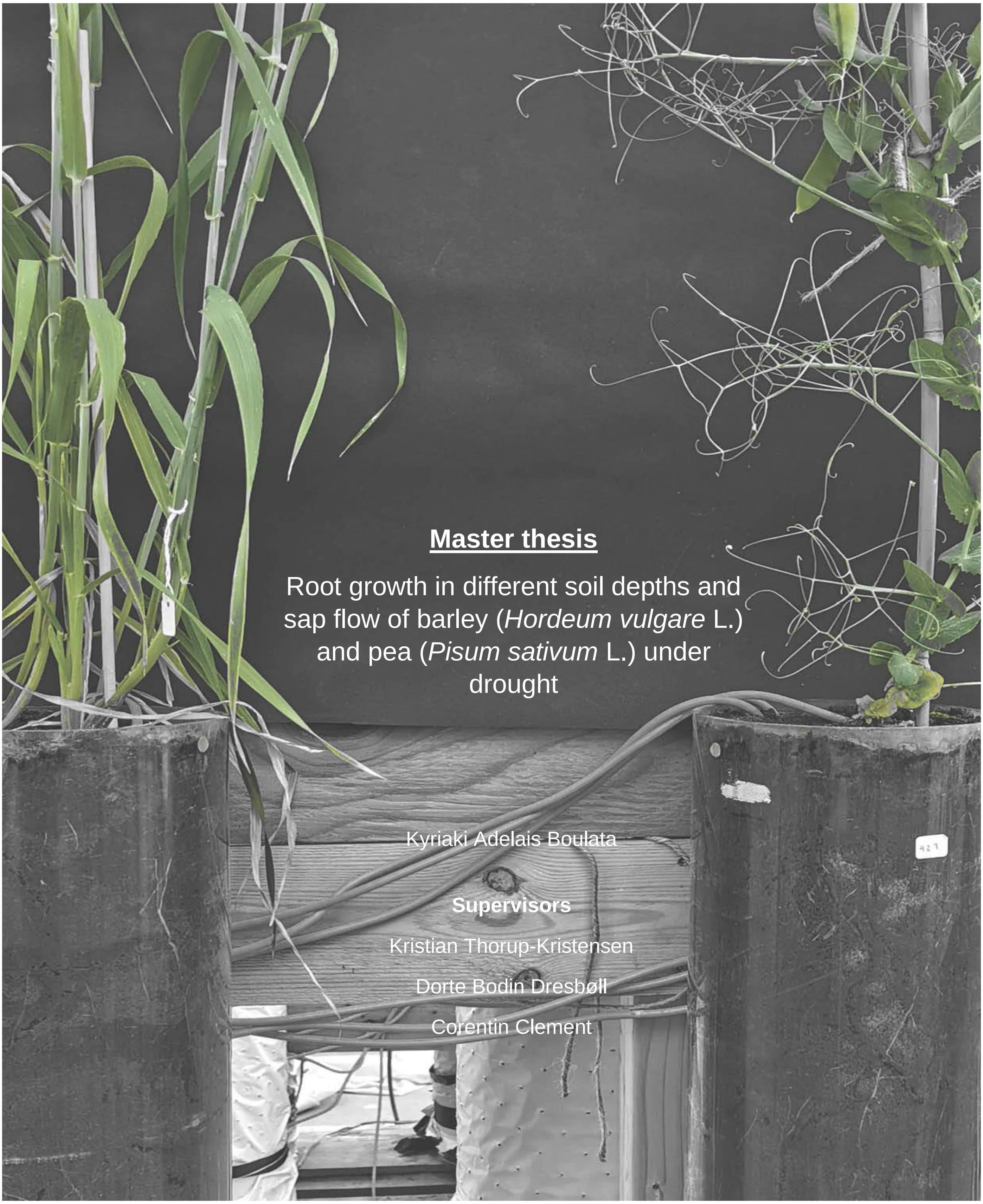

Master thesis

Root growth in different soil depths and sap flow of barley (*Hordeum vulgare* L.) and pea (*Pisum sativum* L.) under drought

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Abstract

Root systems that proliferate in deep soil domains, when the upper most layers are progressively drying, have been proposed to confer crop drought resistance through the utilisation of deep water. A greenhouse study was conducted to identify root growth dynamics in three soil layers (0-26, 36-62 and 71-97 cm) and to relate them to sap flow rates and soil water content under mild drought. To that end, two crops with different root systems, peas (dicot) and barley (monocot), were grown in a loamy sand soil in 14x100 cm transparent pots and subjected to intermittent and terminal drought. In peas, no increased root proliferation (measured as root length) was observed in the lowest soil layers when the topsoil was dry and sap flow rates, during terminal drought, were similar in the control and the water stressed treatment. However, barley showed an enhanced root growth in the 36-62 cm layer due to the intermittent drought and in the 71-97 cm layer due to terminal drought. Moreover, sap flow rates, as measured during terminal drought, demonstrated increased water use in barley during the early stages of the drought. Our findings show the importance of the drought characteristics (timing, duration and severity) in plant response to drought and highlight the need for development of genotypes with increased root length in deeper soil domains under drought conditions.

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Introduction

Water scarcity in crop production

Water is an essential resource for terrestrial plants and is required in vast amounts. About 97% of the water taken up by the plants is transported, through the transpiration stream, to the leaves where it is diffused as water vapour through stomatal pores as an inevitable consequence of carbon assimilation (Taiz and Zeiger, 2010). A far smaller amount is needed for plant growth. Under some conditions, plant available water in the soil fails to meet transpiration demand and crops eventually experience water stress, one of the greatest yield limitations worldwide.

Water scarcity is a serious threat to crop production and food security in irrigated and rainfed areas, worldwide. Freshwater resources are continuously dwindling: the ever-growing world population and the growth of the industrial and agricultural sector account for a significant increase of universal water use with irrigated agriculture being responsible for the major part of it (Babkin and Klige, 2003). Therefore, irrigated agroecosystems are facing the challenge of maintaining a sustainable and relatively high-yield production under such conditions. On the other hand, rainfed agriculture is also affected by water limitations. Climate change alters precipitation patterns (frequency, intensity, and timing of rainfalls) (Bakhsh and Kamran, 2019) and increases average air temperature. As rainfed agriculture is completely based on rain incidents and as rising temperatures increase crop evapotranspiration, these agroecosystems are particularly vulnerable to drought.

Drought stress characteristics

Drought affects crop performance through the timing, duration and intensity of the water shortage (Farooq *et al.*, 2017). During the growing season, crops can experience water limitations at all developmental stages: during the vegetative (intermittent drought) or the reproductive phase (terminal drought). Every stage has a different degree of sensitivity to water shortages (Cattivelli *et al.*, 2008). Intermittent drought can occur in one or more episodes while the terminal drought usually affects crops sometime during the reproductive phase and continues until harvest. Drought severity can be mild, moderate or severe and greatly depends on the climate parameters that influence the evaporative demand: high vapour pressure deficit temperature, solar irradiance and wind speed increase water loss from plant and soil surfaces. This means that i.e. a terminal drought of eight days can have different results on crops grown in different locations due to the differences in the above-mentioned parameters. Furthermore, drought severity is also determined by the speed at which the soil moisture recedes. During a mild drought, soil water would recede slowly and a fraction of it would still be available for plant use. The combined effects of timing, duration and intensity of drought influence the extent of yield loss.

Intermittent drought is hardly ever lethal to the plant but can diminish plant growth and thus yield capacity. Terminal drought can result in big yield reductions in rainfed systems (Richard *et al*., 2015). As several yield components are sensitive to drought, water shortages during their formation adversely affects yield. Pollen infertility (Yu *et al*., 2019), seed weight (Muñoz-Perea *et al*., 2006), seed number (Jamieson *et al*., 1995), number of pods (Gorim and Vandenberg, 2017), tiller density (Cai *et al*., 2018) call all be adversely influenced by water shortage.

Role of roots under water stress conditions

Improvement of drought tolerance has primarily come from efforts focusing on the above ground plant characteristics. Roots are concealed within the soil, which cause technical and experimental difficulties in studying them. However, during the last decades, there has been an increased interest in root studies and there is growing evidence that root systems are important for drought tolerance.

In the absence of rain events, the soil dries gradually from the top layers due to drainage and evapotranspiration. This results in deeper soil strata maintaining higher moisture contents at the end of the growing season and rainfed crops, under these circumstances, could exploit the stored soil water to cover their needs during the reproductive phase. By capturing this untapped water during drought events plants could alleviated the consequent stress or delay the onset of a more severe one (Hurd, 1968), and indeed there are numerous studies which demonstrated the role of deep and vigorous roots during droughts.

Deep rootedness and root length

Root system architecture plays an essential role in plant productivity under limited soil water conditions (Lynch, 1995). Its role in plant adaptation to drought environments has been outlined in Manschadi *et al.* (2006) who showed that a relatively dense and deep root system in wheat increased water access to deeper soil strata during the grain-filling period. Moreover, root length (RL) was greater in the deeper soil layers. They also suggested that the extra water extracted during the grain-filling period can positively influence yield. This is supported by

Kirkegaard et al (2007) who found that even relatively small amounts of deep water contribute significantly to grain yield in wheat under moderate terminal drought.

The angle of early roots seems to influence rooting depth (Wasson *et al.*, 2012). Steeper root angles result in deeper root systems and, in rice, this trait has been demonstrated to allow drought avoidance (Uga *et al.*, 2013). On the other hand, shallower roots resulting from narrower root angles can be of importance for e.g. phosphorus foraging from the upper soil layers (Ho et al., 2005; Rubio et al., 2003).

Besides cereals, deep rootedness has also been found to play a role in vegetable and legume performance under drought. In an experiment with lettuce, Johnson *et al.* (2000) found that QTL's for taproot length, found in a wild relative, enhanced water uptake from deep soil layers. Deep roots alleviated stress in groundnut (Reddy *et al.*, 2003), soybean (Prince *et al.*, 2015) and common bean (Polania *et al.*, 2017).

Root length is important for water extraction

Soil water depletion from the soil profile, over a growing season, is highly correlated with maximum rooting depth and root length density (RLD), with the former having a stronger correlation (Hamblin and Tennant, 1987).

RLD is an important trait for water and nutrient uptake. The greater its value is in a given volume of soil, the greater the rates of water uptake will be (Passioura, 1983). However, there is an upper limit of RLD that is valuable for water extraction. Ludlow and Muchow (1990) argue that a RLD of 0.5 cm cm^{-3} and more is sufficient to extract all plant available water and variation in RLD between 0.3 to 6 does not induce variation in the extracted water. In chickpea, RLDs of more than 0.4 cm cm^{-3} did not contribute in extra water extraction (Gregory, 1988). These findings suggest that there is an additional metabolic cost for roots that, in the long term, do not contribute significantly to water uptake. However, within a shorter time scale of consideration, an increase in RLD can be beneficial for water uptake, especially under soil water deficits. According to Zhan et al (2015) this cost is avoided in plants with reduced lateral root branching density which results in deeper root systems that can utilise deep water resources and reduce the yield penalty. Therefore, alterations to root architecture towards deeper roots can contribute to enhanced water acquisition from deeper soil domains.

Roots tend to proliferate to a greater extend in soil domains with higher nutrient and water content (Johnson et al., 2000). Under drying topsoil conditions (i.e. terminal drought), maize root proliferation was observed to be restricted in the upper soil layers but increased in the lower layers where roots extracted water at greater rates per unit length when compared to the well-watered plants (Sharp and Davies, 1985). Similarly, soybean, increased its RL deeper in the soil in response to drier upper layers (Hoogenboom *et al*., 1987). Thus, the development of greater root densities deeper in the soil under terminal drought events can increase water foraging.

Monocotyledonous vs dicotyledonous root systems

Monocotyledonous species, such as cereals, develop fibrous root systems. The embryo in the seed gives rise to one or more "primary" roots and the mesocotyl to "basal" roots. Both root types branch into successive orders of "laterals". On the other hand, dicotyledonous species, such as legumes, have root systems comprising of a single primary root, the "taproot" which originates from the embryo and can also give rise to different orders of "lateral" roots. "Shoot-borne" roots can be found in both plant types and are created from aerial parts of the plant (Rich and Watt, 2013; Zobel and Waisel, 2010).

Cereal crops typically develop greater total RL per area than grain legumes and in some cases this difference can be 5 or even 10-fold (Hamblin and Tennant, 1987). On the other hand, legumes extract more water per unit RL than cereals (Katayama *et al*., 2000). However, legumes continue to expand their root densities after the pod-filling phase (at a slower pace than before) while most cereals stop even earlier (anthesis phase) (Gregory, 1988).

Differences in root system architecture between legumes and cereals may be attributed to the different types of vegetative growth, which is indeterminate and determinate, respectively (Gregory, 1988) however the majority of modern grain legume crops have a determinate growth pattern.

Transpiration and sap flow under water stress

Transpiration reflects plant water use and is directly affected by drought. To adapt under water stress conditions, plants, through a series of regulatory events, induce stomatal closure (Osakabe *et al*., 2014). This in turn affects the amount of water vapour diffusing out of the leaves hence transpiration rates are inhibited.

A direct measure of transpiration is obtained by monitoring sap flow rates on intact plant stems: the mass of sap ascending through a stem section per unit time is determined (usually g h^{-1}). This method is based on the heat-balance theory that was developed by Sakuratani (1981) and Baker and van Bavel (1987). A brief description follows below:

According to the heat-balance, a constant and steady state heat flows from a heater strip to a stem segment as seen in Figure 1. This heat is partitioned to the sap, the stem and the outer environment (if no heat storage is assumed):

$$P_{in} = Q_f + Q_u + Q_d + Q_r \qquad \text{(Equation 1)}$$

where:

P_{in}= power input from the heater

Q_f= energy convection carried by the sap flow

Q_u, Q_d= the two components of the axial heat conduction; energies transferred upwards and downwards along the stem

Q_r= radial energy loss by conduction to the ambient

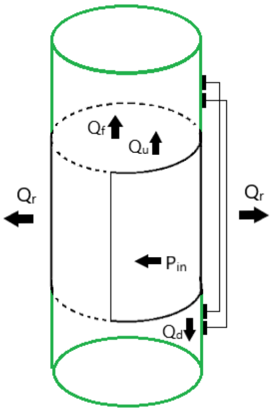


Figure 1 An annular heater on a stem section and heat fluxes of the heat balance theory. A constant and steady state energy input is applied (P_{in}) to the stem section which results in heat fluxes radially to the ambient (Q_r), vertically in the stem (Q_u, Q_d) and the one carried by the sap flow (Q_f).

The rate of sap flow is then calculated using the following relation:

$$F = Qf / C_p * dT = (P_{in} - Q_u - Q_d - Q_r) / C_p * dT \qquad \text{(Equation 2)}$$

where:

Cp= specific heat of water (4.186 J/g*C)

dT= temperature increase in sap

The axial (Q_u + Q_d) and radial (Q_r) heat losses are measured through thermocouples and thermopiles and F is being calculated from equation (2).

Sap flow sensors have successfully been used to monitor sap flow rates in many plant species. Most of the research has been conducted on trees, however this method has also been used in many agricultural crops (Table 1). Sap flow sensors can also be applied in thin stems (microsensors) with stem diameters ranging from 2.1 to 7 mm (Dynamax, Inc.) but literature on the lowest end of this range is very rare.

Table 1. Examples of herbaceous crops in which sap flow measurements based on the heat balance theory were performed.

Crop	References
Cereals	
Winter wheat (*Triticum aestivum* L.)	Cai et al, 2018; Rafi et al, 2019
Corn (*Zea mays* L.)	Han et al, 2018
Legumes	
Soybean (*Glycine max* (L.) Merr.)	Cohen et al, 1993; Sauer et at, 2007
Vegetable crops	
Tomato	De Swaef et al, 2012
Potato (*Solanum tuberosum* L.)	Gordon et al, 1997; Byrd et al, 2015
Industrial crops	
Cotton (*Gossypium hirsutum* L.)	Dugas et al, 1990; Isoda and Wang, 2002
Sunflower (Heliantus annus L.)	Zhang and Kirkham, 1995
Ornamental crops	
Rose (*Rosa hybrida* L.)	Rose and Rose, 1994

In Zhang and Kirkham (1995) the sap flow of sorghum (monocotyledon) is compared to that of sunflower (dicotyledon) under well-watered and water stressed conditions. In the well-watered treatment sunflower showed much higher rates of sap flow than sorghum. During water stress both crops exhibited lower sap flow rates but in sunflower the difference between the water treatments was more pronounced. They also noted that in sorghum, radial heat conduction dominated the energy balance under all water treatments while this was only true for water stressed sunflower (except midday). Finally, they concluded that the gauges were reliable only when sap flow rates exceed that of 20 g h^{-1}. In another study, an effect of soil texture on sap flow rates was found under drought as no differences were detected among control and watered stressed plants in a silty soil but noted in the stony (Cai *et al*, 2018).

Some authors underline the problems arising when sap flow sensors are used under specific circustances. Langensiepen *et al.* (2014) experimented in wheat under field conditions and found that the heat fluxes of the heat-balance theory did not allow enough energy to be carried by the sap resulting in noisy sap flow measurements. This is also noticed in Gordon *et al.* (1997) where at very low sap flow rates in potato stems most of the energy was lost radially. They concluded that under these circumstances, a considerable amount of heat is stored in the stem. This agrees well with Grime *et al.* (1995) who suggest that a heat storage term (Q_s) should be included in the heat balance equation, especially under low sap flow rates (Groot and King, 1992).

Aim of research

The aim of this research was to study the root growth of a monocotyledonous (barley) and a dicotyledonous (pea) species, in different soil depths in relation to soil water depletion and transpiration under intermittent and terminal drought. It is hypothesised that under drought conditions, root length increases in the lowest soil layers in response to drying in the topsoil layer. Furthermore, it is hypothesized that this increase in root length will be accompanied by maintenance in transpiration rates.

Materials and methods

Study site and experimental setup

The experiment was conducted in a greenhouse at the University of Copenhagen, (55°40′08.5”N 12°18′19.4″E), Denmark from May to August 2020.

Spring barley (*Hordeum vulgare* L. cv. RGT Planet) was sown in 12 root-observation pots on May 4. Field pea (*Pisum sativum* L. cv. Javlo) was sown into a nursery on May 13 and later transplanted in 12 root-observation pots, on May 20. All 24 pots were organized in a complete randomized design (Figure 2). To simulate commonly practiced plant densities in the field, each pot contained either one pea (55 plants m^{-2}) or two barley plants (150-180 plants m^{-2}).

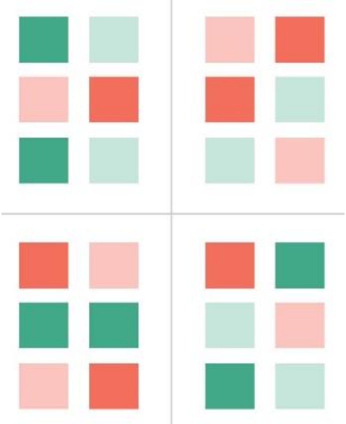


Figure 2 Experimental design: a completely randomized design of peas (orange) and barley (green) under control (darker shades) and WS (lighter shades) treatments.

The pots, 14 cm wide and 100 cm deep, were transparent and closed with a lid at the bottom. A filter paper with an extension to the outer environment (through a hole on the side of the pot) was placed on the lid to allow excess water to drain from the soil. All pots were filled with sieved soil (Table 2) to a soil bulk density of 1.49 g/cm^3. To achieve this density, the soil mass to be added to each pot was calculated:

$$m = p * V = p * (\pi * r^2 * h) = 1.49\ g \div cm^3 * (3.14 * 7^2\ cm^2 * 100\ cm) = 22.9\ kg$$

where m= soil mass in each pot, p= soil bulk density, V= pot volume, π= 3.14, r= pot radius, h= pot height.

The soil was added in each pot in portions and in between two portions pressure was manually applied to limit soil receding effects during the experimental period.

A white non-transparent plastic film covered each pot to prevent light exposure to the soil. Finally, pots were tied to wooden frames for support (Figure 3).

Table 2 Chemical composition and physical characteristics of the soil (*Source: USDA)

Chemical composition & pH				
Mg (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Organic matter (%)	pH
3.5	3.2	9.5	1.9	6.9
Physical characteristics				
Soil type	Clay <0.002 mm	Silt 0.002 – 0.02 mm	Fine sand 0.02 – 0.2 mm	Coarse sand 0.2 – 2.0 mm
Loamy sand*	7.7%	6.1%	42.2%	42.2%

Figure 3 Root observation-pots (24) attached to wooden pallets for support. The white plastic film is wrapped around the pots to prevent light exposure to the soil.

Water treatments & fertilization

Two water treatments were applied in both plant species: 1) a control treatment, where pots were continuously provided with water (0.2 L/24 h) through an irrigation system of a clay cone (Blumat, Easy, Telfs, Austria) inserted into a 0.5 L plastic bottle containing water, and 2) a water stressed (WS) treatment, where the irrigation system was withdrawn from the pots at selected drought stress periods.

Drought was imposed in two cycles. Six randomly selected pots per species were given the WS treatment during both cycles (Figure 2). In both crops, the first drought event occurred during the vegetative phase (21 DAS) and lasted for 22 days. In peas, however, the end of the cycle concurred with the first pod formation. The second drought cycle took place during the reproductive phase for both crops. It started at 51 and 60 DAS in peas and barley, respectively, and lasted until harvest. A recovery phase took place between the drought cycles where the WS pots were receiving the same amount of water as the control pots. An additional 400 ml was added daily in each pot, 3 days before the onset of the second drought event, to allow initiation of the second drought cycle with relatively high soil water content. The treatments and their corresponding dates are shown in Figure 4.

Due to progressive drying of the topsoil layer of some pots of the control treatment that was observed at around 36 and 45 DAS (peas and barley, respectively), 100 mL of additional tap water was added daily on the soil of the control pots (and the WS pots during recovery) from the above mentioned dates until the end of the experimental period.
Shortly after sowing, 70 ml of liquid fertilizer (5-1-4) (Min have næring) was mixed with 1.4 L of tap water to create a solution of which 60 ml was added to each irrigation bottle.

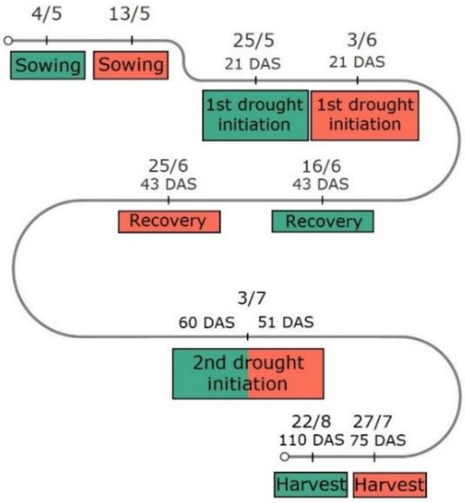


Figure 4 Water treatments flow. The second drought cycle continued until harvest. Green shade represents barley and orange represents peas.

Soil water content

Soil volumetric water content (VWC%) was monitored using time-domain reflectometry sensors (TDR-310S, Acclima, Inc., Meridian, ID, USA). Three sensors were vertically inserted into each pot (n=24) at three different depths: the top sensor was placed at 10-20 cm, the middle at 50-60 cm and the bottom at 80-90 cm from the top of the pot. All sensors were connected to a CR6 datalogger (Campbell Scientific, Inc., Logan, USA) and the data were logged every 24 h from sowing until harvest.

Canopy cover and plant height

Plant growth was measured in terms of canopy cover and plant height. Canopy cover was estimated as a percentage of green areas on a picture, using the Canopeo App (1.1.7, Oklahoma State University, USA). Images of every pot (n=24) were taken on 7-day intervals using a smartphone camera, from two fixed locations: the top view pictures, taken from a 120 cm distance from pot level and the side view pictures, 150 cm from ground level and 60 cm from the pot level. The pictures were taken from these two angles to catch the canopy growth in both the horizontal and vertical direction. Black backgrounds were used to avoid green areas not belonging to the plant(s) of interest. All images were later edited with Paint (Windows 10), to cover most of the background in black, and processed by the app using the 50% adjustment function (Figure 5).

Plant height was measured in all plants (n=24) from the soil level using a ruler. One main tiller was selected out of every barley pot whereas in peas height was measured on the main stem.

a) b) c)

Figure 5 Canopy images used for canopy cover estimation in Canopeo a) original image b) processed image in Paint and c) Canopeo output image.

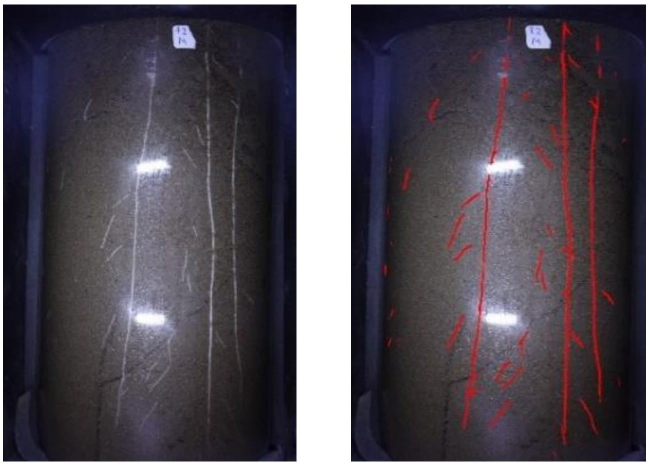
Root growth

Root growth was estimated non-invasively by taking weekly images of the pot sides and later extracting RL from the pictures using an image analysis software (Smith *et al*., 2020). A ‘’PhotoBox’’ consisting of a camera lens (Sony, DSC-QX10) and LED lights (Figure 6) was used to take images of roots growing at the pot-soil interface. Every pot was divided into three horizontal layers (measured from the top of the pot): 1) top layer 0-26 cm, 2) middle layer 36-62 cm and 3) bottom layer 71-97 cm. From each pot (n=24), one image was taken from each layer on 7-day intervals. All images from one pot were aligned on a randomly selected vertical axis around the pot.

a) b)

Figure 6 The PhotoBox used for obtaining root images from the pot-soil interface as seen from a) the side view and the b) front view where the lens and the LED lights are shown.

RootPainter was used to extract RL from all images obtained during the experimental period (n= 606). The dataset used to train the model consisted of all the above-mentioned images. Maximum tiles per image was set to "1" and target pixels to "1200". A new model was created ("random weights") and trained according to the "Corrective Training Protocol" in Smith *et al.*, 2020. An example of root predictions based on the model used for this dataset is seen in Figure 7. RL was extracted from all images in pixels and was later converted to cm using the following function: $RL\ (cm) = RL\ (pixels) * pixel\ size\ (cm)$



a) b)

Figure 7 a) Roots in soil and b) model predictions of the roots

Stomatal conductance

Measurements of stomatal conductance (g_s) started at 28 and 29 DAS for peas and barley, respectively, with a leaf porometer (SC-1, Decagon, Washington, USA). Three measurement cycles were performed in a day (morning: 9-11 am, midday:12-14 pm and afternoon:15-17 pm) on a weekly basis. In every measurement, a young fully expanded leaf was randomly chosen from each pot (n=24) and g_s was determined at both the adaxial and abaxial surfaces. The sum of both leaf sides was used to make the total gs. The average gs of a day was calculated (morning, midday and afternoon measurements) within each treatment combination. The porometer was calibrated before every measurement cycle.

The first dataset of gs in barley (29 DAS) was obtained with a different porometer than the one used for all following measurements.

Sap flow

To determine sap flow rates (g h^{-1}), a Flow32A-1K sap flow system (Dynamax Inc., Houston, TX, USA) with SGA2 gauges, suitable for 2.1-3.5 mm stem diameters, and a CR1000 data logger (Campbell Scientific, Inc., Logan, UT, USA) was used. Eight gauges were installed in two random plants/tillers per treatment at the beginning of the second drought cycle. A 2.3 V input voltage was applied to all gauge heaters. Stem thermal conductivity (Kst) was set to 0.54 W/m*K for peas and 0.28 W/m*K for barley which are typical for herbaceous and hollow stems, respectively. Initial Ksh was set at 0.2 and for later calculations an auto zero algorithm was running every other day between 3-5 am.

Sap flow calculations were performed every 15 minutes using the average of sensor measurements made every 60 seconds. When the temperature difference above and below the heater was less than 0.5 all data were ignored.

Data analysis

The statistical analysis was performed with IBM SPSS Statistics 26. To detect differences between the control and the WS treatment (traits: canopy cover, height, gs, RL) within consecutive measurements a two-way ANOVA was performed. An independent samples t-test was used to determine differences between the control and the WS treatment, within single measurements (DAS). For all tests, significance level (a) was set to 0.05. To summarize data, averages and standard errors were used in figures and tables.

Results

Soil water content

Drought influenced the soil volumetric water content (VWC) in all soil layers, as by the end of each drought cycle a difference between the water treatments is noted (Figure 8 & 9).

The VWC of both crops followed the same trend within all soil layers: during drought events, it remained constant (control) or declined (control and WS) down to a minimum value.

Peas

The effect of the first drought cycle on the VWC was noticed: a) in the topsoil layer (Figure 8a) between 22-44 DAS where the VWC fell from 14 to 5% (control remained stable at 15%), b) in the middle soil layer (Figure 8b) between 28-48 DAS where the VWC decreased from 16 to 5% (control from 17 to 8%) and c) in the bottom soil layer (Figure 8c) between 31-49 DAS as the VWC dropped from 24 to 5% (control from 26 to 10%). Thus, the first drought event caused the VWC in the WS treatment to reach a minimum of 5% in all soil layers, although at different dates. It was observed that from the onset of the drought to the appearance of its effect in the top, middle and bottom soil layer, a period of 1, 7 and 10 days elapsed, respectively.

The recovery period between the two drought cycles restored VWC in both treatments at about 27% in the top, 18% in the middle and 20% in the bottom soil layer. The top, middle and bottom soil layer got rewetted 2, 6 and 7 days, respectively, after the initiation of the recovery.

The second drought cycle decreased the VWC of the WS treatment until 64 DAS to a) 7% in the topsoil layer (19% in the control) , b) 8% in the middle soil layer (14% in the control) and c) 9% in the bottom soil layer (17% in the control).

Barley

The first drought cycle affected the VWC of the WS plants: a) in the topsoil layer (Figure 9a) between 22-44 DAS as the VWC dropped from 17 to 5% (control from 17 to 10%), b) in the middle soil layer (Figure 9b) between 28-56 DAS where the VWC fell from 16 to 5% (control from 17 to 10%) and c) in the bottom soil layer (Figure 9c) between 23-57 DAS where VWC decreased from 25 to 5% (control from 28 to 13%). As in peas, the first drought event resulted in 5% VWC of the WS treatment in all soil layers at different dates. The time lag between drought initiation and the appearance of its effect on the top, middle and bottom soil layer was 1, 7 and 7 days respectively.

The recovery period between the two drought events raised VWC up to 27% in the top, 19% in the middle and 20-25% in the bottom soil layer.

During the second drought cycle, the VWC of the WS treatment, decreased until 73 DAS to a) 8% in the topsoil (control reached 19%), b) 13% in the middle soil layer (control to 17%) and c)15% in the bottom soil layer (control at 25%).

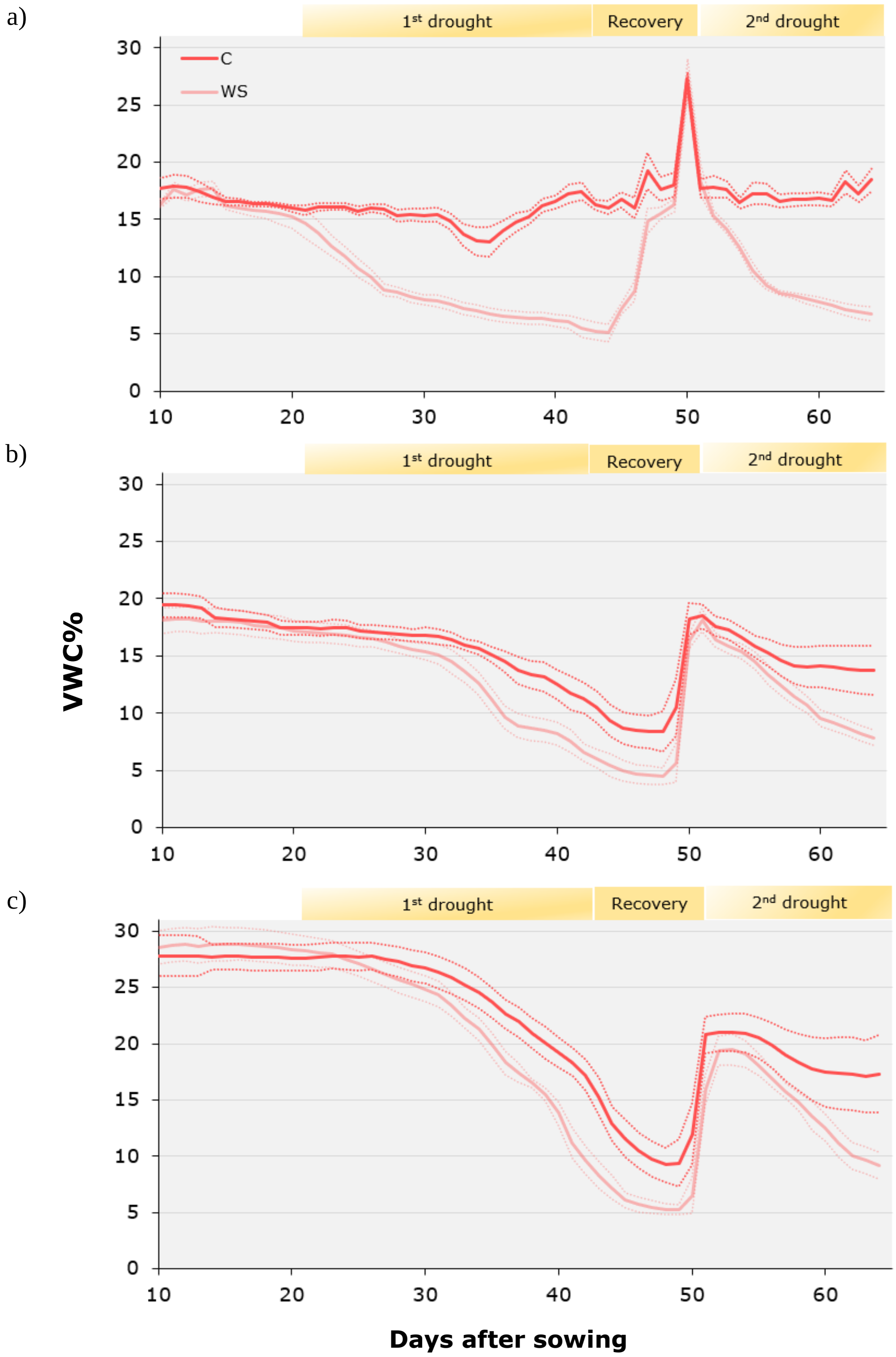


Figure 8 Soil volumetric water content (VWC) (%) of control (C) and water stressed (WS) peas in the a) topsoil, b) middle soil and c) bottom soil. Solid lines represent averages and dotted lines standard errors (n= 6).

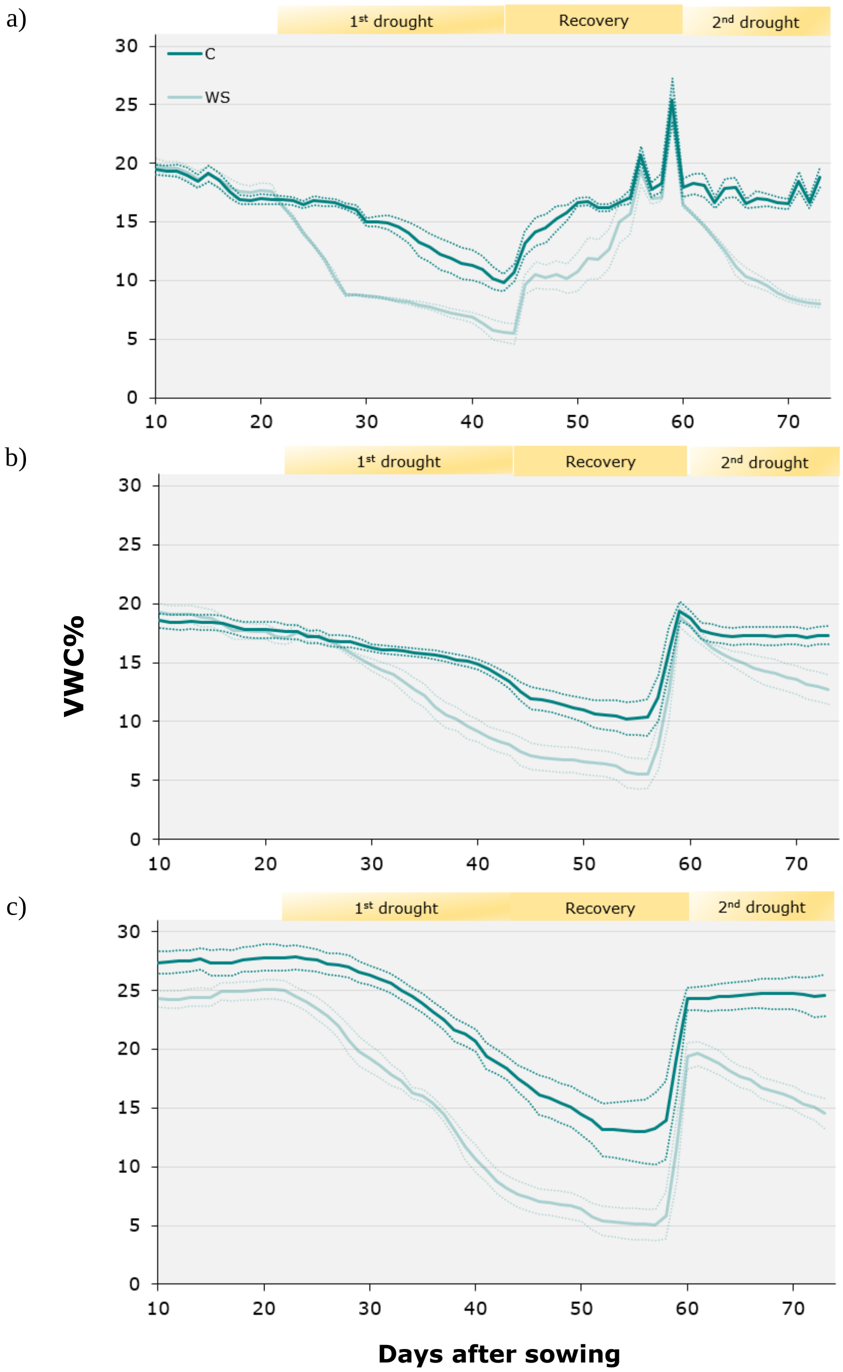


Figure 9 Soil volumetric water content (VWC) (%) of control (C) and water stressed (WS) barley in the a) topsoil, b) middle soil and c) bottom soil. Solid lines represent averages and dotted lines standard errors (n= 6).

Canopy cover and plant height

Considering the whole experimental period, drought did not have an impact on the canopy cover of peas but did reduce the canopy cover of barley (P= 0.001) (Figure 3). When control plants were compared to the WS at the same DAS, no differences were found in either plant species. Plant height was also not affected by drought in peas or barley.

Peas

The canopy cover of both treatments increased until 49 DAS and then 56 DAS (onset of 2nd drought) it started to decrease (Figure 10a).

Canopy cover was not affected by either drought events. A small, however non-significant, difference between the treatments was observed between 56 and 63 DAS.

Barley

The canopy cover of the control treatment increased until 44 DAS and showed a slight decrease 65 DAS (Figure 10b). In the WS treatment, canopy cover peaked a little later, at 51 DAS, and started to markedly decrease until 72 DAS.

The first drought event had an impact on canopy cover which was observed during the early days of recovery. At 44 DAS, control plants showed a 56 % increase in canopy cover, compared to the previous measurement, as opposed to the 3% of the WS plants (Figure 10b). The second drought cycle had a stronger influence on canopy cover than the first (P=0.04). At 65 (P= 0.184) and 72 DAS (P= 0.099) the difference between the control and the WS plants was noticeable.

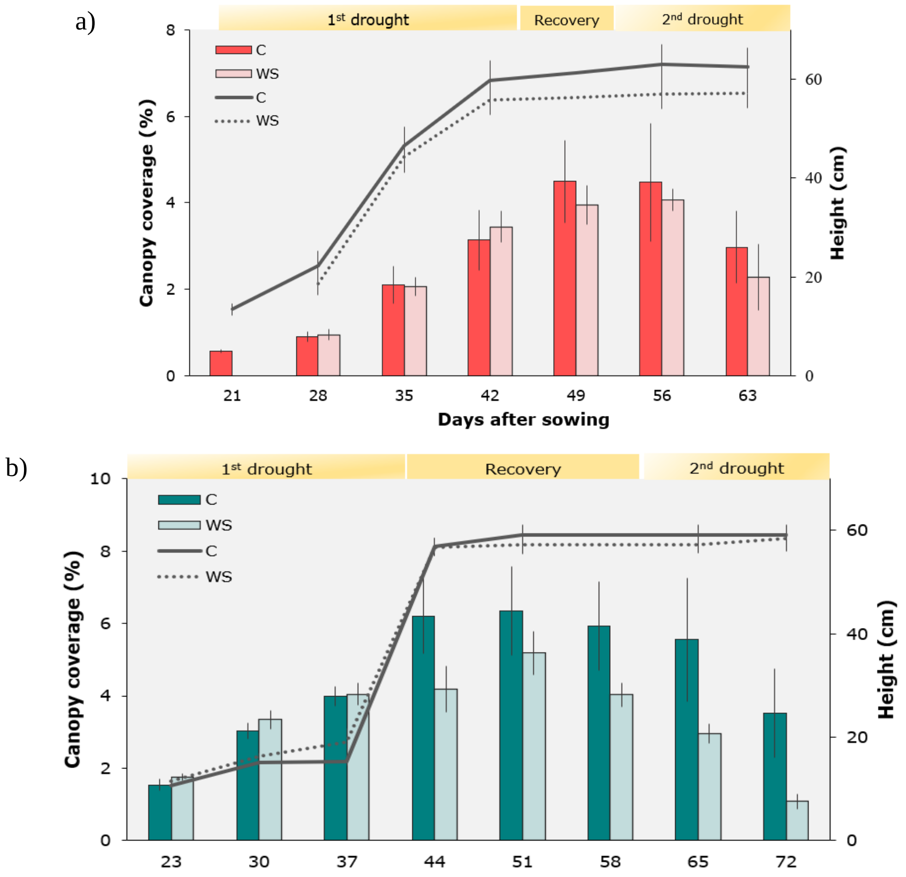


Figure 10 Canopy cover (%) and plant height (cm) of control (C) and water stressed (WS) a) peas and b) barley. Bars represent canopy cover (n=3-6 ±SEM) and lines plant height (n= 5-6 ±SEM).

Root length

Pea had a more extensive root system than barley. This is mostly noticed at the top and middle soil layers. At the bottom soil layer, there were no significant differences in RL between the plant species (Table 3).

Considering the whole experimental period, within each soil layer, RL did not differ significantly between the treatments in both crops (Table 3). When RL was compared between the treatments during drought cycles (as defined by VWC, see *Soil water content* section) significant differences were found in barley in all three soil layers whereas in peas, no such differences were detected.

Peas

Root growth was more prominent in the topsoil layer where the RL of both treatments averaged 82 cm (Table 3). Fewest roots were observed in the middle (55 cm) and even fewer in the bottom soil layer (29 cm).

In the topsoil layer (Figure 11a), RL increased until 26 DAS from where it remained constant until the last measurement. The same trend is noticed in the middle and bottom soil layer (Figure 11a, 11b), only here the increase seemed to stop at 33 and 40 DAS, respectively.

Within each soil layer, neither of the drought events seemed to significantly influence root proliferation and there were no significant differences in RL within single dates.

In the topsoil layer, a small rise, however non-significant, in the RL of the WS treatment was observed after the onset of both drought cycles.

In the middle soil layer, a small but not significant difference between the treatments is observed between 40-47 DAS, as control plants seem to have higher RL than the stressed plants. This difference is maintained throughout the second drought cycle.

In the bottom soil layer, the RL of both treatments seemed similar until 33 DAS. At 40 DAS, a rise in the RL of the WS treatment is observed. At 47, 54 and 61 DAS WS peas had higher, but non-significant, RL than the control.

Barley

Roots proliferated more in the topsoil (55 cm on average) than in the middle and the bottom (35 and 31 cm, respectively). Moreover, there were no significant differences in the RL between the middle and the bottom soil layer (Table 3).

In the topsoil layer, RL gradually increased and peaked at 42 DAS in the control and 49 DAS in the WS treatment. A decrease followed in both treatments and at 63 and 70 DAS RL remained constant and similar.

In the middle soil layer, the RL of the control treatment reached a maximum value at 21-35 DAS, then decreased between 35 and 42 DAS and decreased again between 56-63 DAS. On the other hand, the RL of the WS treatment peaked at 35 DAS and was gradually decreasing until the last measurement, at 70 DAS.

In the bottom soil layer, the RL of the control treatment increased until 35 DAS, then it decreased between 42-56 DAS and was stable at 56-70 DAS. The RL of the WS treatment exhibited two peaks: one, 35 DAS and another at 63-70 DAS.

Significant differences in RL were found between the treatments during both drought events and recovery. Shortly after the end of the first drought, at 49 DAS, a significant difference (P= 0.003) was noticeable as the roots of the WS treatment had more than double the length of that of the control (113.3 and 54.7 cm, respectively). The same pattern was also observed at 56 DAS at the following measurement, although the difference was not significant (P=0.082). The second drought cycle had no influence on RL of the topsoil layer.

In the middle soil layer, at 35-49 DAS the RL of the WS treatment was significantly higher from that of the control (P= 0.03)

In the bottom soil layer, at 63-70 DAS (second drought event), the RL of the WS plants was significantly higher than the control (P= 0.007).

Table 3 Root length (cm) of peas and barley [(n_{peas} =47-48, n_{barley}= 52-53) ± SEM] under different water treatments in three soil sections. Values are drawn from all root length measurements (peas: 12-61 DAS, barley: 14-70)

Plant species	**Water treatment**	**Soil section**		
		Top	**Middle**	**Bottom**
Peas	Control	77 ± 7	60 ± 8	25 ± 3
	Water stressed	87 ± 7	51 ± 5	33 ± 6
	Total	**82 ± 5**	**55 ± 5**	**29 ± 4**
Barley	Control	52 ± 3	32 ± 4	28 ± 3
	Water stressed	58 ± 5	37 ± 4	34.6 ± 4.0
	Total	**55 ± 3**	**35 ± 3**	**31.4 ± 2.5**

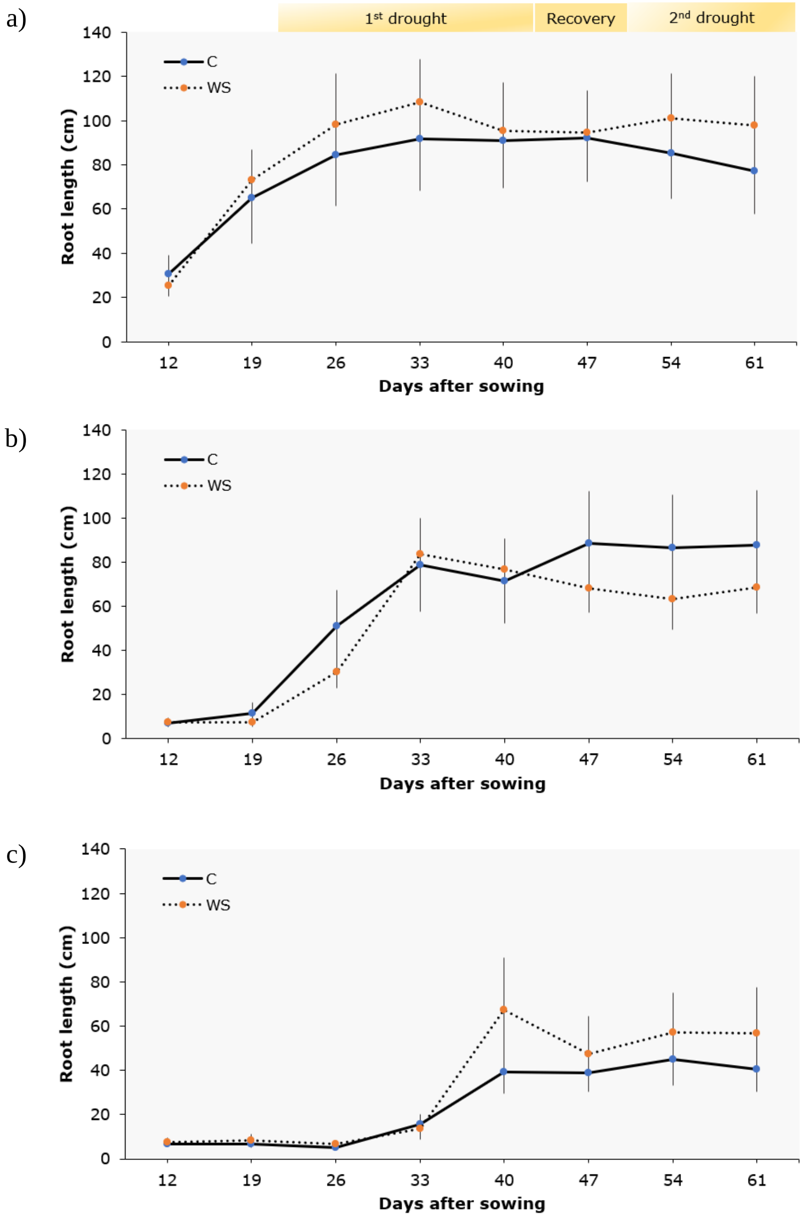
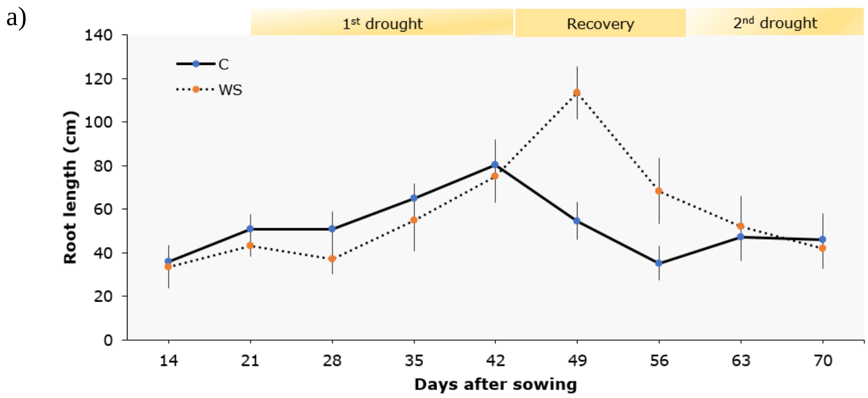


Figure 11 Root length (cm) of control (C) and water stressed (WS) peas in the a) topsoil layer, b) middle soil layer and the c) bottom soil layer (n= 6 ± SEM).



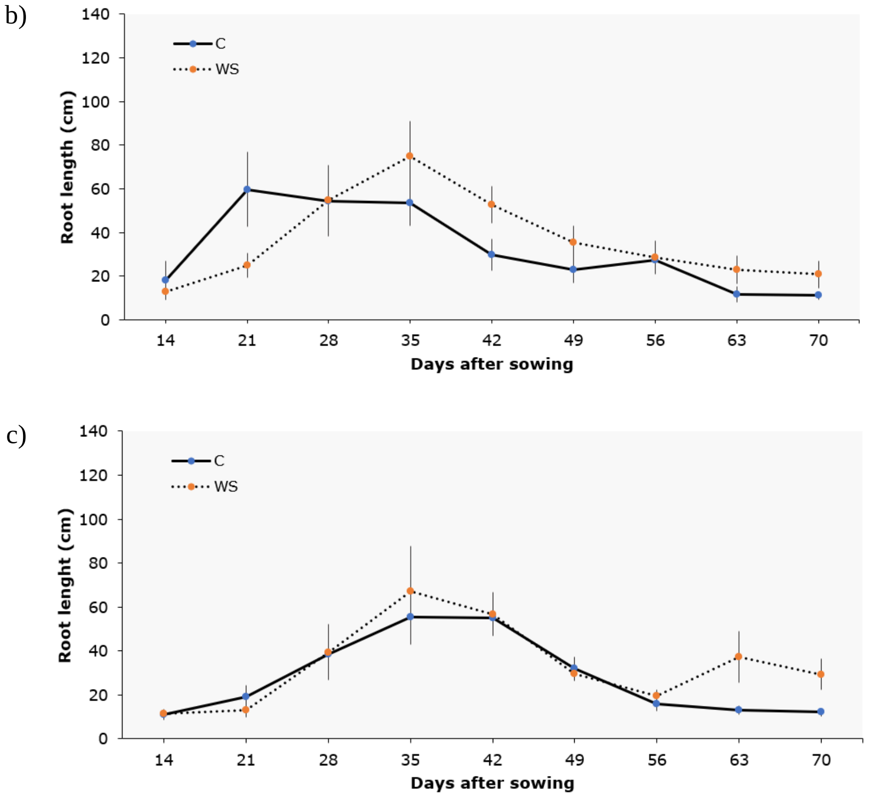


Figure 12 Root length (cm) of control (C) and water stressed (WS) barley in the a) topsoil layer, b) middle soil layer and the c) bottom soil layer (n= 6 ± SEM).

Stomatal conductance

Peas had higher stomatal conductance (gs) than barley: the control peas had an average gs of 733 mmol m^{-2} s^{-1} while the corresponding barley had 340 mmol m^{-2} s^{-1}, during the experimental period.

Peas

An effect of drought on gs was observed at 41 DAS (end of 1st drought), where WS plants had a 23.2 % lower gs than the control (P= 0.004). In the recovery phase, 50 DAS, gs was restored to a similar value (around 700 mmol m^{-2} s^{-1}) in both treatments. The second drought cycle was observed to affect gs at 64 DAS (P= 0.04).

Barley

The impact of water limitation on the gs was also noticeable towards the end of the first drought event, at 43 DAS (P= 0.005). During the recovery phase no differences between the treatments were observed. A noteworthy change occurred at the beginning of the second drought event: WS plants had higher gs than the control plants (P= 0.012). This pattern reversed as drought progressed and, at 73 DAS, WS plants had again lower gs than the control (P= 0.001).

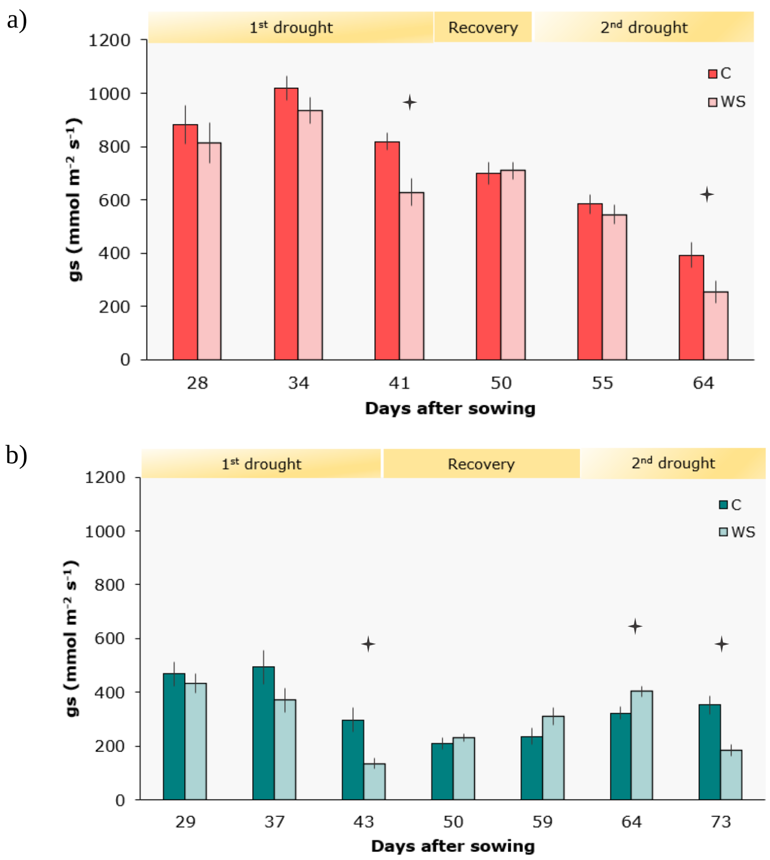


Figure 13 Stomatal conductance (gs) (mmol m^{-2} s^{-1}) of control (C) and water stressed (WS) a) peas and b) barley (n=6 ± SEM). Stars represent statistically significant differences between the C and the WS treatment.

Sap flow

Pea had higher daily sap flow rates than barley (Figure 14, 15). However, it should be noted that in each barley pot there were about 10 stems (5 per plant) as opposed to pea pots that contained 1-3 stems each.

Peas

The effect of terminal drought on the sap flow rate of pea was not evident. For most days shown in Figure 14a and 15a, the sap flow rates of the control plants slightly exceeded that of the WS plants. An exception to that, occurred at 60-61 and 69-70 DAS (Figure 14a) where the sap flow rates of the WS plants, was slightly higher. A downward trend, started at 58 DAS, was noticed in the control treatment until the sap flow rates became zero. Similarly, the same trend was observed in the WS treatment but with a lag time of 3 days.

Barley

In barley, there was an apparent impact of water limitation to the sap flow (Figure 14b and 15b). This was observed between 69-81 DAS, where the sap flow rates of the stressed plants were lower than the control and continued to decrease until 77 DAS to zero. In the control treatment, the sap flow rates started to fall later, at 76 DAS, and came close to zero at 82 DAS. However, at the beginning of the terminal drought, the WS treatment exhibited higher sap flow rates than the control. This is noted during a 3-day period at 62-65 DAS.

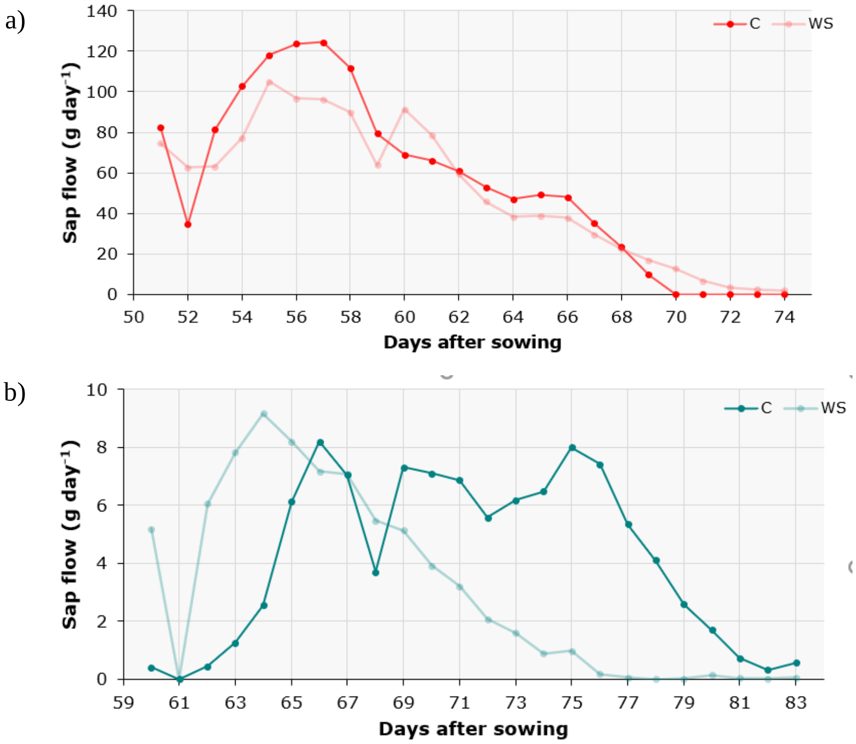


Figure 14 Accumulated sap flow rates (g day^{-1}) of control and WS a) pea and b) barley during terminal drought (n=2)

Hourly sap flow rates and stomatal conductance

Examples of hourly fluctuations in sap flow rates and gs within a day, during terminal drought, are shown in Figure 15 and 17&18, respectively. In both crops, the daily sap flow pattern was similar to that of solar radiation (Figure 16) as peaks and troughs in solar radiation are noted, shortly after, in the sap flow rates as well.

In both crops, between 9.00-15.00, gs did not fluctuate much (Figure 17,18). This was observed in both treatments, but it was not always in agreement with sap flow measurements: in peas, at 64 DAS, there is an apparent difference between the sap flow rates at 9.00 and 12.00, which is not reflected in gs. Similarly, in WS barley at 73 DAS, the difference in the flow between 12.00 and 15.00 is not seen in gs.

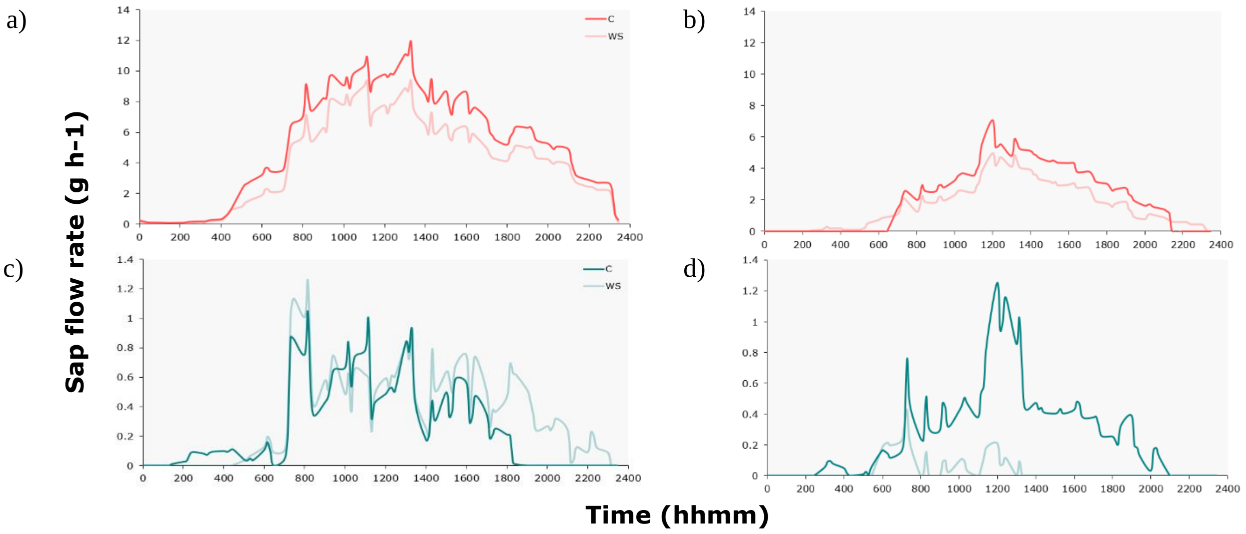


Figure 15 Sap flow rates (g h-^{1}) of a) peas at 55 DAS and b) peas at 64 DAS, c) barley at 64 DAS and d) barley at 73 DAS (n=2).

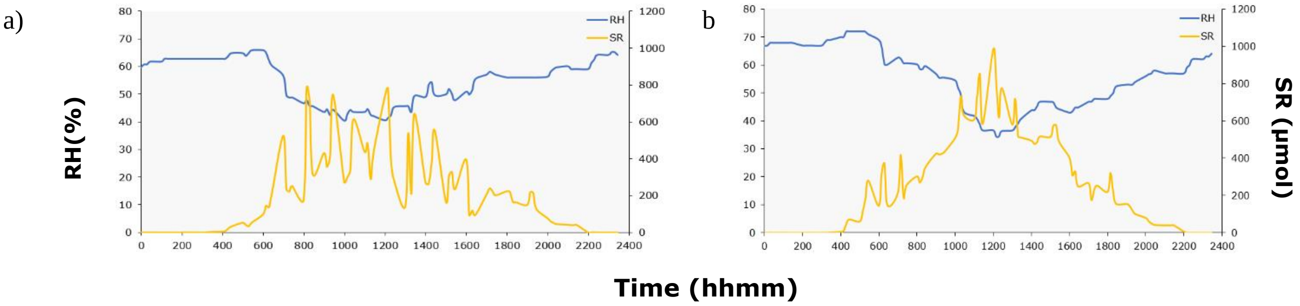


Figure 16 Relative humidity (RH) (%) and solar radiation (SR) (µmol) at a) 7/7 (peas=55, barley=64 DAS) and b) 16/7 (peas=64, barley=73 DAS).

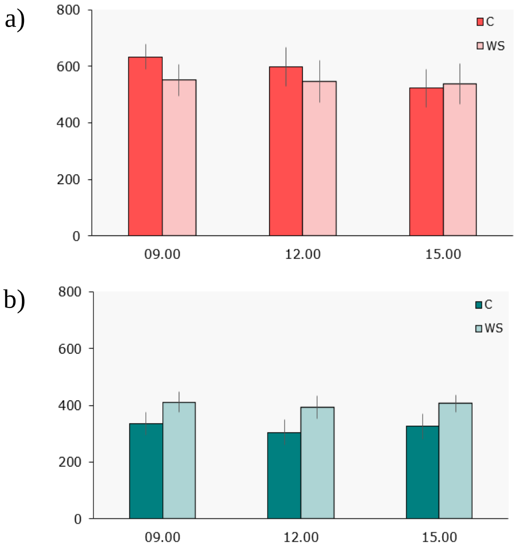


Figure 17 Stomatal conductance (gs) (mmol m^{-2} s^{-1}) of control and water stressed (WS) a) peas at 55 DAS and b) barley at 64 DAS, presented at three different times within a day (n=6 ± SEM).

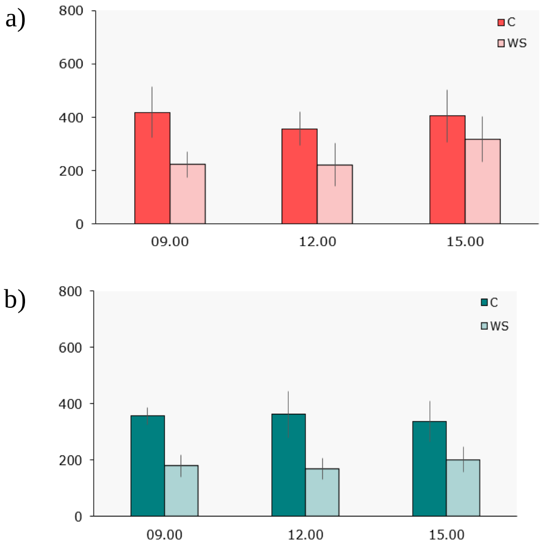


Figure 18 Stomatal conductance (gs) (mmol m^{-2} s^{-1}) of control and water stressed (WS) a) peas at 64 DAS and b) barley at 73 DAS, presented at three different times within a day (n=6 ± SEM).

Discussion

To cope with adverse water availability and to maintain their tissues hydrated, plants develop mechanisms to enhance water acquisition from the soil and limit water loss (Blum *et al*., 2005). Numerous studies have showed the benefits of deep and vigorous root systems in water acquisition under various types of drought (Uga *et al*., 2013; Kirkegaard *et al*., 2007; Manschandi *et al*., 2006). In the present study, we investigated whether two plant species, with different root systems, could exploit water deeper in the soil in response to topsoil drying, in order to alleviate water stress and yield penalties. The results show that, in pea, roots of the water stressed plants (WS) did not enhance their proliferation in lower soil layers in response to topsoil drying conditions whereas, in barley there was an increased root growth in the 36-62 cm and 71-97 layers when exposed to drought. Furthermore, sap flow measurements revealed differences in plant water use between the treatments and confirmed the role of enhanced root proliferation in water acquisition from the lowest soil layer.

Deep rootedness

The effect of water shortages on rooting depth was not evaluated in this study due to the limited size of the pots. The pots used were 100 cm long and since roots of both crops usually grow longer, we were not able to determine whether water stressed plants could explore deeper soil domains to acquire water when upper layers were drier. Instead, we studied whether roots that were already established in the lowest soil layers (36-62 and 71-97 cm) could proliferate more in response to drought in the upper layer.

Root length

When water shortages occur, an extensive root growth in the deep soil layers, where water is available, would be an advantage for sustaining relatively high yields (Comas *et al*., 2013). In this study, pea, showed a slight increase in RL in the bottom soil layer during the late stages of the intermittent drought and while the VWC of the upper layers (top and bottom) was less than 10%. After the topsoil layer got rewetted, RL in the bottom layer decreased and no difference between the treatments was noted. It can be speculated that this increase was a short term response to drought in the upper soil layers, as there was more water in the bottom to be exploited (Hoogenboom *et al*., 1987) but the data cannot confirm this, as the increase is not very evident and the variability in the data is big. Furthermore, it can be assumed that this increase did not alleviate stress, as at the time the increase was observed stressed peas partially closed their stomata (Figure 8). If the drought period extended longer or the stress was more intense, a further decrease in the VWC in the topsoil layer would allow for a clearer image of the root growth dynamics between the soil layers. Many authors have underlined the importance of an extensive root system in the deeper, moister soil layers, in grain legumes, when the upper layers are progressively drying. Early studies with soybean demonstrated increased RLD of water stressed plants during the reproductive phase in the 0.9-1.2 m (Garay and Wilhelm, 1983) and 1.2-1.4 m soil layer (Hoogenboom *et al*., 1987). On the contrary, Benjamin and Nielsen (2006), who also worked with soybean, found no effect of drought on total root weight in lower soil domains, when measured at the seed filling phase, but instead confirmed this for chickpea and field pea. Our findings in pea are not consistent with most of the aforementioned and the reasons behind this may reside in the severity of the drought imposed and the speed at which the soil water depleted. It is known that the extent to which plant growth and yield components will be affected by drought depends on the timing, duration and intensity of the prevailing drought (Farooq *et al*., 2017). In this study, pea, was subjected to a rather mild drought stress (Figure 1, 3a, 8a, 9a) and this might be one reason for a weaker response of the root growth to drought compared to other studies. Moreover, there is a lot of genotypic variation in root length density (Kashiwagi *et al*., 2006) which makes generalizations for single crops hard to make. Root system architecture is characterized by high levels of plasticity, meaning the ability of a genotype to generate numerous phenotypes depending on the ambient conditions (Miner *et al*., 2005). Consequently, root growth will respond differently in soils that are drying in a different manner, which is also affected by drought characteristics, soil texture etc. Soil texture has been shown to introduce variability in RL (Cai *et al*., 2018) and rooting depth (Kirkegaard *et al*., 2007; Tennant and Hall, 2001). In conclusion, various sources of variability can interfere with root growth patterns under drought conditions.

In barley, the increased RL of the WS treatment observed in the middle soil layer during the intermittent drought could also indicate a short-term response of roots to the drying topsoil conditions. This idea is further supported by the fact that the RL of the stressed plants are constantly higher than that of the control until topsoil gets rewetted. The enhanced root growth in deep soil layers has been demonstrated in cereals under receding soil moisture in the uppermost layers. Wheat (Kirkegaard *et al*., 2007; Manschadi *et al*., 2006), corn (Sharp and Davies, 1985) and rice (Uga *et al*., 2013) are some typical examples in which drought avoidance was achieved in that manner. In our experiment, it is difficult to conclude whether the increase in RL in the middle soil layer conferred stress alleviation but it is also noted that the plant partially closed its stomata right after the increase (Figure 13b) thus it is possible that the avoidance had a very short duration.

An increase in RL is also observed in the bottom soil layer, during terminal drought, but it is not clear whether it is a response to drought or a recovery response. It can be assumed that since water in the topsoil layer was gradually becoming limited, while it was >15% in the other layers, roots in the bottom layer responded with an increased growth. However, it must be taken into consideration that during this period where RL was increasing in the bottom layer (56-63 DAS) this layer got rewetted. Thus, the increase in RL could also be due to a regrowth response because water conditions became favorable again in the rhizosphere.

Compensatory root growth

In barley, a steep and significant increase in the RL of the WS treatment was observed in the topsoil layer following recovery. This is most likely attributed to the soil rewetting. Compensatory regrowth has been demonstrated in many studies where e.g. shoot growth (Huck *et al.*, 1983), soil bacteria populations (Meisner *et al.*, 2015) and root growth (Jupp and Newman, 1987) increased after water was re-introduced in a dry soil. Moreover, under drought conditions, there is a carbon cost for maintaining roots which can be avoided by root senescence and, when conditions become favorable, by creating new ones (Van Noordwijk *et al.*, 1998). This is true for very fine lateral roots with increased water uptake capacity (Eissenstat and Yanai, 2002). Thus, the plant can induce their senescence in response to drying soil and develop new ones after it is rewetted which will increase the total uptake. However, root senescence due to drought was not observed in barley, in this experiment. A rapid growth after a dry soil is rewetted can also have benefits for the yield (Comas *et al.*, 2013).

Overall response of root growth under receding soil moisture

Besides rooting patterns in specific soil layers during drought events, it must be pointed out that literature on the overall root growth along the soil profile is contrasting. Drought has usually been found to inhibit root growth (Gregory, 1988) and this is confirmed in a study with numerous annual species (Zhou *et al.*, 2018), in corn (Robertson *et al.*, 1980), common bean (Sofi *et al.*, 2018) and other crops. Yet, in chickpea (Ali *et al.*, 2005) total root growth was increased under water stress. Findings are sometimes contradictory, even within the same species as in e.g. soybean, where both an increase (Hoogenboom *et* al., 1987; Merrill *et al.*,

2002) and no effect (Robertson *et al*., 1980) on total root length was found under drought. As mentioned earlier, a source of variation in root traits is the drought characteristics (timing, duration, intensity) and this could also have affected the results, along with the specific growing conditions of the study (field, greenhouse, pot size, etc.).

An unexpected finding, contrary to numerous studies (Gregory, 1988; Hamblin and Tennant, 1987), was that the total RL of peas was greater than that of barley (Table 3). The difference is mostly evident in the topsoil layer.

Sap flow and stomatal conductance

To study plant water use, the rather novel approach of heat-balance method was used. This is one of the few studies where sap flow gauges were used on plants with such small stem diameter (2.1-3.5 mm).

It is noticed that in barley, the increased root growth observed in the bottom soil layer, during the terminal drought (56-63 DAS), coincided with a decrease in canopy cover (Figure 10b) and an increased transpiration rate, compared to the control (Figure 14b). Therefore, assimilates from the above-ground part were likely to have allocated to the roots in the bottom soil layer to allow for higher daily water uptake rates (Gowda *et al*., 2012). As mentioned earlier, the increase in RL during the terminal drought, in barley, could be attributed to drought or recovery. However, during prolonged drought events, anisohydric species (e.g. barley) tend to reduce their leaf area in an effort to maintain transpiration (Bodner *et al*., 2015). As this was also observed in this study, drought seems more likely to have triggered root proliferation.

A good agreement between the accumulated daily sap flow and the gs measurements (day averages) was observed, in both crops. In barley, at 64 DAS, the gs of the WS treatment was significantly higher than that of the control (Figure 13b) and similarly, the sap flow rates of the WS plants are much higher than those of the control (Figure 14b). The same trend is noted at 73 DAS yet, the WS treatment had lower gs and sap flow than the control. In peas, the agreement between the sap flow and the gs was only noticed at the early stages of the terminal drought, where at 55 DAS, control and WS peas did not differ in either of these traits. However, at 64 DAS, sap flow is similar in both treatments but gs in lower in the control. Studies on the relationship between sap flow and gs in herbaceous plants are rare and, in trees, a high correlation between these traits has found under well-watered and drought conditions (Sun *et al*., 2012).

Finally, it is confirmed that the greatest part of the energy applied on barley stems was partitioned to radial heat conduction, as expected from studies with cereals (Langensiepen *et al.*, 2014). It has been argued that under both field and greenhouse conditions low sap flow rates (<20 g h^{-1}) are the main cause (Zhang and Kirkham, 1995).

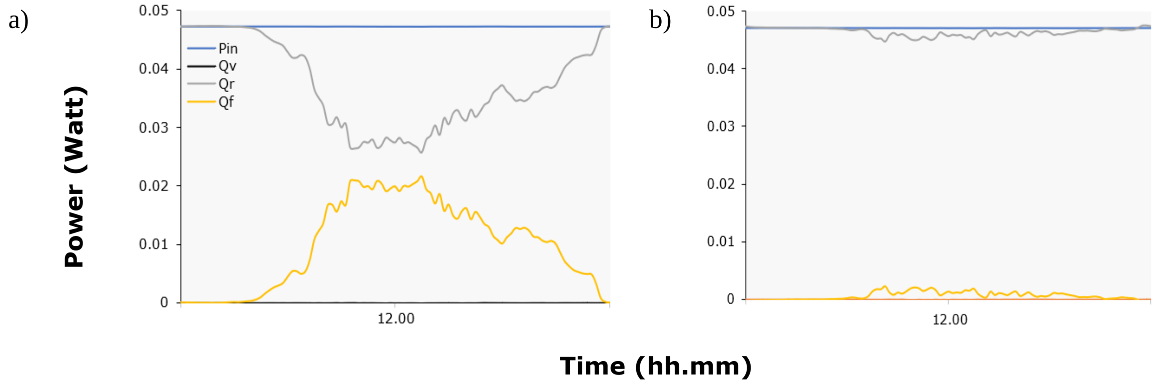


Figure 19 Energy fluxes of the heat-balance theory in a) peas at 55 DAS and b) barley at 64 DAS (n=1). P_{in}= power input from the heater, Q_f= energy convection carried by the sap flow, Q_v= axial heat conduction, Q_r= radial energy loss by conduction to the ambient

Water saving and water spending strategies

To cope with water stress, plants have developed three key strategies: drought escape, drought avoidance and drought tolerance (Verma *et al.*, 2018). Drought escape is achieved by rapid plant development in such a way that flowering, or maturation occurs before the onset of drought. By using this strategy plants finalize much of their development before adverse conditions arise. Although this is desirable for some types of plants (e.g. desert plants), it would not always be of an advantage for crops, as a diminished above-ground mass may not be able to meet the productivity demand. Drought escape is often found in native populations (e.g. crops in the Mediterranean region where terminal drought scenarios are frequent) and can also be manipulated by agricultural practices (early sowing). The intermittent drought in this study did not hasten the flowering time in any of the two crops. Drought avoidance involves a complex of mechanisms which allow the plant to keep its water status and is further classified into water saving and water spending strategies (Basu *et al.*, 2016). In this study, both crops have demonstrated mostly water saving strategies as, in an effort to minimize water loss, they reduced their leaf area (barley) and gs (peas and barley). The water spending strategy is utilized by crops to enhance water uptake from the soil. In this way, by maintaining relatively high transpiration rates under drought, plants might be able to accumulate more assimilates in the stem and relocate them in the grain (Lopes and Reynolds, 2010). In this experiment, barley has demonstrated an ability to utilize the water spending strategy as well. As mentioned earlier, barley roots proliferated more in the bottom soil layer during terminal drought, which was followed by increased sap flow rates. Thus, WS plants seemed to have utilized this strategy for increased water acquisition. Finally, the third strategy employed by plants for drought resistance is drought tolerance and involves osmotic regulation (solute accumulation in the cell), protection from oxidative stress and desiccation tolerance (Zhang, 2007) but it is out of the scope of this research.

Significance of findings and general considerations

In order to maintain relatively high yields under drought conditions, plants must have access to and utilize water from layers of soil that have not yet dried. Similarly, Lynch (2013) mentioned that for optimal water uptake "root foraging and resource availability should coincide in space and time". Several root traits have been proposed to confer drought tolerance (Watt *et al*., 2013) and in this study we focused mainly on the root length. It has been found that there is high variability for root length within plant species, and screening trials revealed drought tolerant genotypes with increased root proliferation at depths (Ali *et al*., 2005; Kashiwagi *et al*., 2006). Moreover, root length is highly heritable (Kashiwagi *et al*., 2006) which makes it a suitable trait for plant breeding. Thus, in drought-prone environments where plants mature while there is still water in depth, the approach of choosing the appropriate genotype that could reach and use this water could be adopted. From our experiment it was not fully understood whether these cultivars (*Pisum sativum* L. cv. Javlo and *Hordeum vulgare* L. cv. RGT Planet) would be suitable for drought-prone environments but future research, such as those of Kashiwagi *et al*. (2006), could focus on finding and developing new genotypes with enhanced water acquisition. Wild relatives have shown to be a gene pool of root traits valuable for drought tolerance (Johnson *et al*., 2000).

Crops that can forage for deep water under drought can be used in crop rotations with crops with shallower roots (Cutforth *et al*., 2013). As there is high variability among crops for total water depletion from the soil profile over the growing season, the amount of available water left for a crop in a rotation system will depend on the choice of the preceding crop. A shallow-rooted species (e.g. field pea) will leave water available in the subsoil for the following deep-rooted species (e.g. wheat) to use which can be beneficial, as mentioned earlier, especially in drought prone areas.

The study of deep roots involves many parameters that must be taken into consideration. The penetration and proliferation of roots in the subsoil can face several restrictions (Lynch and Wojciechowski, 2015) such as mineral toxicity, low temperatures and low oxygen levels. Moreover, plant resources are often stratified within the soil profile creating contrasting needs for root growth. A good example is phosphorus acquisition in drying soils as water may be located deeper in the soil while phosphorus resides in the upper most layers (Ho *et al*., 2005).

This study underlines the importance of genotype and drought characteristics (timing, duration, intensity) in drought avoidance.

Study limitations

A better understanding of the dynamics between soil water depletion and transpiration during the vegetative phase would have achieved if sap flow measurements were conducted then. The use of SGA2 gauges is only applicable to stems with diameter ranging from 2.1 to 3.5mm. In barley, this diameter range was reached relatively high in the stem during the recovery phase. The diameter of pea stems was, for the most part of the growth cycle, within the limits but in order to get a good signal from the gauges a substantial transpiring leaf area should exist above them. This was achieved during the recovery phase as well.

The use of longer pots would allow the comparison of rooting depth and the proliferation of roots to that depth between the control and the water stress treatment.

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