Physiological responses
to reduced water availability
in potato (*Solanum tuberosum*) crops

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Katharina Huntenburg

BSc Horticultural Science, Leibniz University Hannover, Germany
MSc International Horticulture, Humboldt University of Berlin,

Germany

Declaration

Except where references are made to other sources, I declare that the contents in this thesis are my own work and have not been previously submitted, in part or full, for the award of a higher degree elsewhere.

Katharina Huntenburg

Lancaster University

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Publications arising from this thesis

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Acknowledgements

Abstract

Irrigation is an important factor in potato crop management to maximise yield. Summers in the UK will be hotter and drier in the future due to climate change. To maintain crop productivity and use ground water resources sustainably, it is important to determine how potatoes regulate their water use and yield under reduced soil water availability. To better understand root-to-shoot signalling and water fluxes above and below-ground in potato under water-limiting conditions, this thesis presents data from the field and plants at specific phenological stages grown in controlled environments.

Potatoes were grown in the field in a factorial combination of soil compaction and deficit irrigation for an entire season, to understand responses to limited plant water availability. Although physiological parameters (stomatal conductance, photosynthesis rates, leaf tissue ABA) did not differ between treatments throughout the season, plant growth (ground cover, leaf length and number) and yield were reduced under both stresses. Shoot biomass mid-season was correlated with final tuber yield across all treatments.

A possible role of the tuber in regulating plant water relations has been little investigated. Magnetic resonance imaging (MRI) was used as a novel technology to monitor tuber volume growth and water content *in vivo* in well-watered plants and plants from which water was withheld for two days and then re-watered. Plant physiological parameters indicated drought stress prior to re-watering. Tuber volume growth varied diurnally with most growth occurring at night in well-watered plants. However, nocturnal tuber growth ceased immediately after withholding water. Re-watering on Day 3 recovered tuber volume of drought stressed plants to well-watered values.

Strigolactones (SL) have recently been identified as root-to-shoot signal under drought stress, inducing stomatal closure. However, stomatal conductance and leaf water potential of three transgenic lines of potato impaired in SL biosynthesis or signalling or hypersensitive to SL (ccd8, d14 and d53 respectively) were similar to the wildtype as the soil dried. Contrary to previous findings in tomato and *Lotus*, stomatal density was lower in the SL insensitive and SL deficient line and higher in the SL overexpressing line, but these differences did not alter water use.

In summary, this thesis employs a novel technique (MRI) and previously undescribed transgenic lines (d14, d53) in potato to better understand how drying soil affects physiological processes. While irrigation of the potato crop is important in early development and at tuber bulking stage, there may be scope to decrease irrigation at the tuber initiation and maturity stages. Further research is needed to understand the genotype x environment interactions of SL signalling in drying soil.

Contents

List of commonly used Abbreviations

ABA	Abscisic Acid
SL	Strigolactones
WT	Wildtype
Ψ_{root}	Root water potential
Ψ_{leaf}	Leaf water potential
$\Psi_{pre-dawn}$	Pre-dawn leaf water potential
ww	Well-watered
d	Drought stressed
g_s	Stomatal conductance
SLAC	Slow Anion Channel
DELLA	Protein (aspartic acid–glutamic acid–leucine–leucine–alanine)
PIP	Plasma membrane intrinsic protein
ABF	abscisic acid-responsive transcription factors
DREB	dehydration-responsive element-binding protein

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1. General Introduction

1.1. Potato agriculture

Potatoes are the 4th most important crop in the world (Obidiegwu et al., 2015). The yearly supply of potatoes worldwide has grown from 108 million tonnes in 1961 to 241 million tonnes in 2012 (Vinet & Zhedanov, 2011). Nevertheless, it is mainly produced for local markets as only 6 % of production enters the global market (FAO, 2009). The exported quantity of potatoes is even smaller in the United Kingdom (UK), where only 5 % of the produced yield is exported (Vinet & Zhedanov, 2011) and more potatoes are imported than exported (AHDB Potatoes, 2021) . This means that most of the potato yield is used within the country, and that local production must sustain demand.

In the UK, there are five to six million tonnes of potatoes produced every year (DEFRA, 2017). This quantity remains relatively stable even though production area halved from 1960 to 2016 (AHDB, 2017) (Figure 1.1.1). Thus, the productivity per hectare is increasing. However, the yields that farmers achieve fall short of yield potential achieved in breeders' trials under optimal conditions, the so-called yield gap. For example, the reported yield gap for potatoes grown for the processing industry in the Netherlands is ca. 30 % (Silva et al., 2017). For the UK, yield potentials of 105 – 115 t/ha have been estimated (Allen & Scott, 1980), while the actual agricultural yield ranges between 40 – 50 t/ha in the last ten years (DEFRA, 2012, 2017). That means current potato yield in the UK could double under optimal conditions.
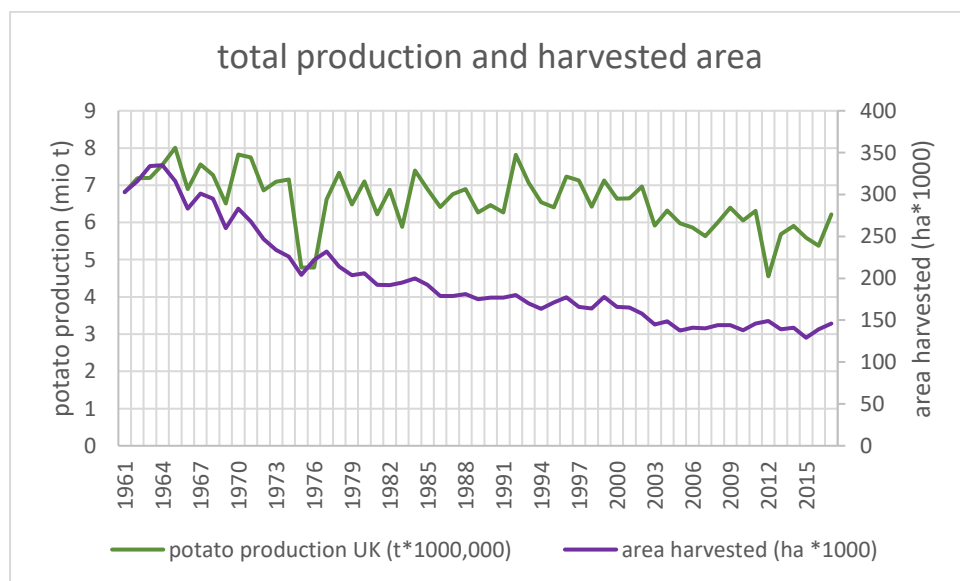
Figure 1.1.1: Development of total potato production (green) and planted/ harvested area of potatoes (purple) in the UK since 1961. Data: FAOSTAT, 2019

'Maris Piper' is the UK's most popular variety over the last ten years, with more than twice the area planted with Maris Piper than with the respective second ranked variety (AHDB Potatoes, 2021). Maris Piper has good cooking as well as frying properties and is well established with consumers in direct marketing and retail. Although maximum yields of *c.* 85 t/ha were reported for this cultivar almost 40 years ago (Allen & Scott, 1980), currently it averages 50 t/ha in the UK (Stalham, 2018, personal communication). Changes in yield can be closely related to different soil types or climates, but this maximum yield was achieved at two different places in two different years (Allen & Scott, 1980). Thus, it seems possible that improving the management of Maris Piper could increase yield by about 25 %. Water availability has a big impact on potato yield, because the tubers comprise ca. 83 % water (Saeed et al., 2008). Since retailers demand tubers with unblemished skins, and soil drying enhances development of the bacterial disease potato scab (*Streptomyces scabies*) causing corky lesions on the tubers, many growers regularly irrigate this crop and may sometimes over-irrigate it. This may cause misshapen tubers and promotes development of a fungal disease powdery scab (*Spongospora subterranea*) resulting in skin blemishes.

While over-irrigation restricts leaf gas exchange in solanaceous crops (Fiebig & Dodd, 2016), deficit irrigation (applying less water than crop evapotranspiration) also limits transpiration and photosynthesis (Li et al., 2021). Furthermore, low water availability decreases canopy size and light interception and therefore decreases the amount of carbohydrates available for tuber growth (Jefferies and Mackerron, 1987; Vos and Haverkort, 2007). Soil compaction reduces, among other factors, root length density and canopy expansion (Stalham *et al.*, 2007), which leads to reduced access to water with the above-mentioned consequences. Thus, this thesis focuses on the effect of reduced water availability caused by soil compaction and/or deficit irrigation, to help to develop recommendations for potato crop management in a changing climate.

1.2. Choice of variety

Different potato varieties are adapted to different conditions and can cope better or worse with a certain stress (Wishart et al., 2014; Obidiegwu et al., 2015). Thus, choosing the right variety for the typical climate and the distinct soil properties on the field can make a large difference to yield. In breeding terminology, drought tolerant varieties maintain yield under drought conditions (Obidiegwu et al., 2015). However, a physiological definition of drought tolerance differs (see section 1.6). A variety that copes better with drought stress could have a higher water use efficiency and therefore require less irrigation in the summer months to sustain yield or produce higher yields with the same

amount of water. For breeders to rapidly select tolerant varieties, it is crucial to have markers that indicate stress tolerance early in plant development. The capacity of a variety to produce long distance signals (see section 1.7) for a quick stress response could be such a marker. It is therefore important to understand the mechanisms by which plants sense drying soil and their long distance signalling responses to compaction and drought stress (Jin et al., 2013), to breed varieties suitable for likely field conditions.

This thesis is using 'Maris Piper' and 'Desiree', two well established potato varieties in the UK that are suitable for different uses from boiling to frying (AHDB, 2021). A better understanding of mechanisms in established varieties helps to identify in which areas improvements could be made to increase drought tolerance. This translates directly into breeding targets for new varieties.

1.3. The potato growing cycle

The growth of potato crops can be described in different stages (Figure 1.3.1). After planting seed tubers, Maris Piper takes approximately 35 days until emergence of the first shoots from the soil, depending on temperature (Stalham, 2018, personal communication). In the vegetative growth stage, shoot growth and canopy density increase (Figure 1.3.1, shoot growth). Approximately 4 weeks after emergence tubers and flowers are initiated and the plant subsequently goes into the tuber bulking stage and flowering (Figure 1.3.1), tuber initiation and bulking). The plant reaches maturity three to four months after emergence and senesces thereafter (Figure 1.3.1, shoot growth, tuber growth). After maturing the tubers are ready to be harvested. Abiotic stresses such as limited water availability can have different effects on yield depending on the developmental stage at which they occur (Ierna and Mauromicale, 2012; Pavlista, 2015) . Drought stress can result in darker fry colour of the tuber (Shock *et al.*, 1998; Pavlista, 2015), reduced starch content (Köhl *et al.*, 2021), increased incidents of common scab (*Streptomyces scabies*) (Pavlista, 2015)and a larger proportion of tubers in smaller tuber sizes (Aliche *et al.*, 2019). Thus, irrigation scheduling is an important component of potato crop management.
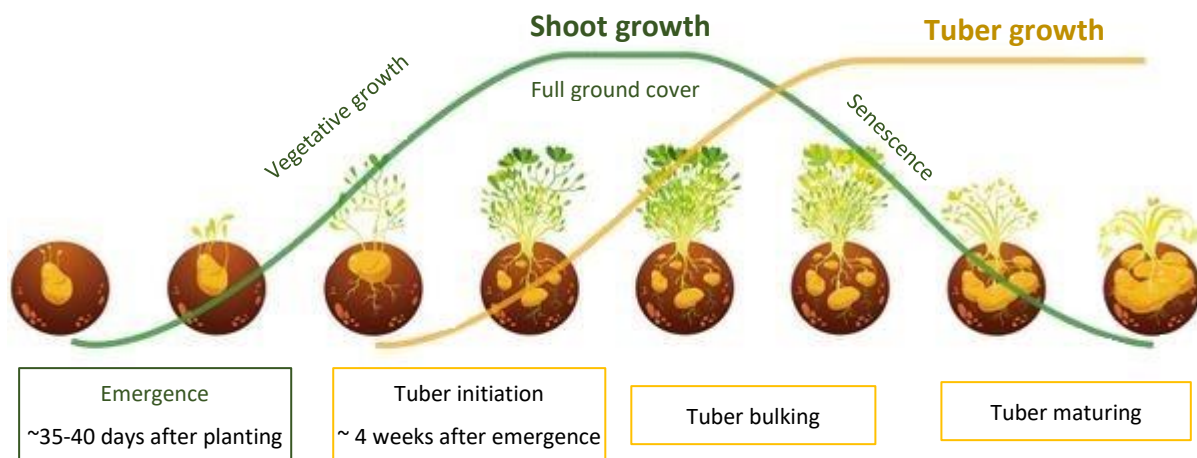
Figure 1.3.1: Growth cycle of potato crops. Maris Piper is usually planted in the UK in the middle of April and emerges after an average of 35 days. All other stages are reached accordingly.

Adapted from: Castlecor Potatoes

1.4. Irrigation in potato agriculture

In rainfed potato agriculture in the UK, drought stress is a major yield limiting factor (Haro-Monteagudo et al., 2017). The first half of the potato growing season (April to July, crop emergence to full ground cover) coincides with the driest period in the United Kingdom with less than 80 mm total rainfall per month typically occurring over eleven to twelve days (Met Office UK, 2021). Since low soil water availability can limit yield of potato crops (Vos & Haverkort, 2007) and potatoes are most susceptible to common scab (*Streptomyces scabies*) around tuber initiation and at low soil moistures (Braun et al., 2017), growers usually irrigate potato fields throughout the season. Water applications to potato crops accounted for over 50 % of the annual volume of irrigation water used in English agriculture over the last 30 years (Weatherhead, 2007). Therefore, optimizing water use in potato crops, while maintaining or increasing the yield is an important target to address sustainable water use as well as ensuring food security. Thus, it is necessary to better understand the physiological mechanisms regulating plant stress responses to better predict crop behaviour in distinct environmental conditions.

Reducing irrigation is not always detrimental to potato yield quality and quantity. After tuber initiation, up to 35 % less water can be applied without decreasing yield (Ahmadi et al., 2010b; Jensen et al., 2010) and in a UK study with deficit irrigation in two different potato cultivars, only 10 % of the tubers were affected by common scab (Puértolas et al., 2014). In addition, crop rotation choices, low

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soil pH and treatment of the soil with rapeseed meal or *Trichoderma viride* can help reduce infection with common scab (Charkowski et al., 2020; Hilton et al., 2006). That suggests there may be other measures to counteract scab and hence water saving irrigation techniques may be viable in UK potato crops without increasing the risk of yield losses due to scab. As mentioned above, deficit irrigation can have different effects on potato yield and quality depending on the developmental stage at which the crop is subjected to suboptimal water availability (Ierna and Mauromicale, 2012; Pavlista, 2015). In order to understand the impact of drought stress on the potato plant at different developmental stages, this thesis explores shoot water relations at the vegetative growth stage (Chapter 4) and tuber water relations at tuber bulking stage (Chapter 3).

1.5. Drought stress

Drought stress occurs when the plant is not able to extract enough water from the soil to support organ growth and functionality (e.g. photosynthesis, transpiration). The soil-plant-atmosphere continuum describes water movement from the soil through the plant into the surrounding air, driven by differences in water potential (Taiz & Zeiger, 2010). The air around the plant is drier than the air inside the leaf, so water diffuses through the stomatal pores into the air. As a consequence, the leaf water potential becomes more negative. Water is 'pulled' towards the lowest (i.e. most negative) water potential, and thus transported from the roots to the shoots against gravitational forces. Water movement from the roots to the shoot lowers root water potential, allowing water uptake from the surrounding soil. Thus, there is a continuous flow of water from the soil through the plant into the air.

The water potential (Ψ_w) of each cell and consequently every plant organ is the sum of the osmotic potential (Ψ_s), the turgor pressure (Ψ_p) and gravitiy (Ψ_g): $\Psi_w = \Psi_s + \Psi_p + \Psi_g$ (Taiz & Zeiger, 2010). Ψ_w and its components can be measured in different ways. Ψ_w and its components can be measured in different ways. A Scholander type pressure chamber increases the air pressure around a plant organ until external pressure is high enough to expel water from the cells, which appears as droplets on the cut surface. The (positive) air pressure needed to reach this point is equivalent to the (negative) water potential of the sum of cells in the organ (Taiz & Zeiger, 2010). Water evaporation cools the surface of plant tissues. This is used in a psychrometer, where vapour pressure in a cuvette is measured using a thermocouple. Lower water potential of the sampled tissue lowers the measured vapour pressure. This measurement can be conducted directly on the plant (*in vivo*) or on excised tissue to measure overall water potential. Measuring cellular sap provides the osmotic potential of the tissue (Scholander *et al.*, 1965). The osmotic potential of the sap can also be measured with different

methods either using the principle that the osmolyte content of a solution decreases the freezing temperature (cryoscopic osmometer) and decreases the refraction of a solution (refractometer) compared to water(Taiz & Zeiger, 2010). Cell turgor can be estimated by the sum of measured tissue water potential and osmotic potential. However, a pressure probe can directly measure cellular turgor by inserting a microcapillary into a cell, which leads to sap entering the capillary. The pressure needed to push the sap back into the cell is measured and equivalent to the turgor pressure of the cell (Hüsken *et al.*, 1978). Gravity is very similar in neighbouring cells but needs to be considered when water is transported between organs in the plant, especially in root to shoot transport of water against the gravitational force (-0.1 MPa m^{-1}). The gravitational component is usually ignored except in tall trees and lianas, and is of limited significance to potato crops.

Potato tubers are an additional organ to be considered when investigating plant water relations. To date, little is known about water fluxes to and from the tuber in response to changing water potentials in the soil, root or shoot. Directing the water flux towards the tuber during bulking phase could be an important breeding target in order to maximise tuber growth. However, to understand potato drought stress responses and effects on tuber growth, we have to understand the ability of the plant to acquire water from the soil and how it signals restrictions in water supply to limit transpirational water loss.

Leaf water deficit occurs when plant water requirements (determined largely by evaporative demand) exceed the capacity of the roots to extract water from the soil. Transpiration rate in tomato declined as the soil dried even when leaf water potential was maintained (Abdalla et al., 2021), indicating the stomata react to the water available to the leaf rather than the turgor pressure. Furthermore, intra-plant conductivity for water was not altered by drying soil (Abdalla et al., 2021). Thus, the capacity of the plant to supply water to the leaf depends firstly on the soil hydraulic conductivity and secondly on the water uptake into the root and root hydraulic conductivity (Cowan, 1965; Draye et al., 2010).

Soil hydraulic conductivity depends on capillary forces to distribute water upwards or horizontally. Ultramicropores (< 5 μm diameter) retain water very firmly (inaccessible to plants), Micro- and Mesopores (5 – 800 μm diameter) can hold and release water whereas macropores (> 800 μm diameter) are air-filled at field capacity and water drains by gravity (Weil & Brady, 2016). Soil type and soil management techniques determine pore size distribution and thus soil hydraulic conductivity. In addition, the soil hydraulic conductivity decreases as the soil dries out (Kramer & Boyer, 1995) which can occur around the root during the day due to plant transpiration (Figure 1.5.1; Cowan, 1965; Kramer and Boyer, 1995). In well-watered soils, the soil water content (and therefore hydraulic conductivity) around the roots is restored during the night (Draye et al., 2010).
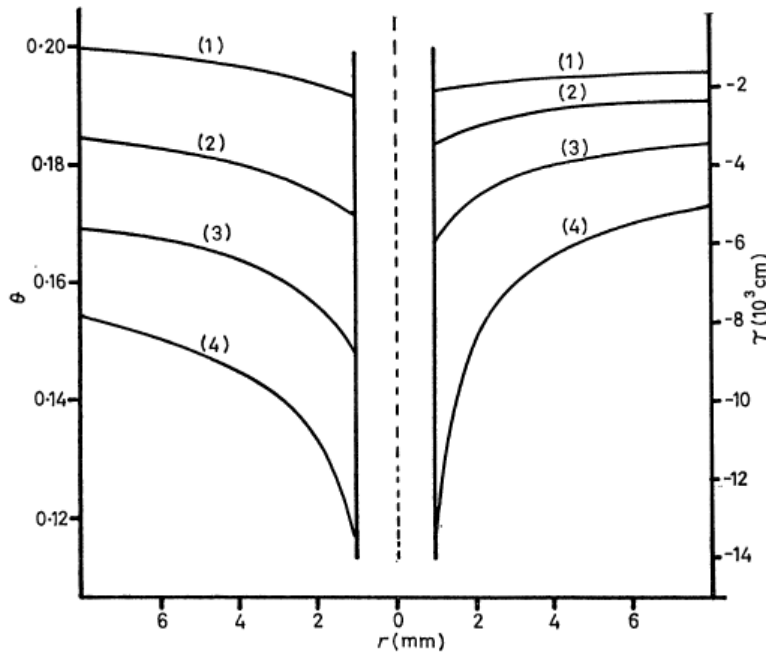
Figure 1.5.1: Variations of soil water concentration, θ, and the corresponding variations of matric potential, τ, with radial distance, r, from a cylindrical root, radius 1 mm, under conditions of steady state flow. The entry velocity of water at the root surface is 2.5 mm day^{-1}. Curve numbers refer to the following arbitrary levels of water concentration at a distance of 8 mm from the root: (1) 0.20; (2) 0.185; (3) 0.17; (4) 0.155 cm^3/ cm^3 soil.

Taken from: Cowan, 1965

The root hydraulic conductivity depends on water entering the roots (radial conductivity) and its transport from the point of entrance towards the xylem vessels for long distance transport to the shoots (axial conductivity). Water enters the root mainly at the root tip, and moves via different pathways (apoplast, symplast, transmembrane) through the cortex (Taiz & Zeiger, 2010; Chen, 2016). To enter the xylem vessels, water has to cross the Casparian strip, which is a ring of suberin around the endodermis cells that prevents water from passing through the apoplast (Taiz & Zeiger, 2010). Upon reaching the endodermis, water has to enter the cell to eventually contribute to shoot water supply. The transmembrane transport of water is facilitated by aquaporins. These are proteins in the cell membrane of animal and plant cells that transport water molecules into and out of cells and therefore enhance the water permeability of the membrane (Kaldenhoff et al., 1998). The aquaporins PIP1 and PIP2 play an important role in the hydraulic conductivity of plants (Kaldenhoff et al., 1998; Siefritz et al., 2002; Wang et al., 2017; Mahdieh et al., 2008) and gene expression of PIP1;1 and PIP2;1 in roots is downregulated in tobacco under drought stress (Mahdieh et al., 2008), suggesting root hydraulic conductivity is reduced under drought stress, to restrict water losses to the drying soil. In contrast, whole plant expression profiles in potato showed that some PIP genes are downregulated (PIP1;1, PIP1;5, PIP2;7, PIP2;8) and some were upregulated (PIP1;4, PIP2;1, PIP2;5, PIP2;6) in response

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to drought stress and ABA treatment (Venkatesh et al., 2013), showing that the role of aquaporins in drought stress response is complex. However, potatoes overexpressing PIP1 were more drought tolerant, which manifested in higher photosynthesis rates, higher water use efficiency, higher biomass production and higher yield under drought in PIP1 overexpressing lines compared to the wildtype at a constant soil moisture of 55-60 % field capacity (Wang et al., 2017). Thus, a higher number of aquaporins may be beneficial under short term drought conditions to better access water remaining in the soil.

The abundance of PIPs is regulated on the transcriptional level by environmental factors like drought stress and hormonal signals like ABA, while the activity of the channel is regulated by changes in cytosol pH and abundance of reactive oxygen species (Zargar et al., 2017). Drought stress elicits all of these signals, making aquaporins a key player in regulating plant water status. In summary, drying soil transports water less effectively to the roots and the uptake of water is hindered by the reduced number of aquaporins inserted into the cell walls or reduced activity of the aquaporins already present. These restrictions limit water transport into the leaf, leading to leaf water deficit. Excessive water loss from the leaves poses the risk of drying out. However, stomatal closure to prevent water loss from the leaves restricts gas exchange and therefore photosynthesis rates. Plants have different solutions to this, depending on species and severity of the stress.

1.6. Physiological response to drought stress

Plants use three different strategies to cope with drought stress: escape from drought, drought tolerance and drought avoidance. Drought escaping plants have only a short life cycle, quickly producing seeds before soil water availability becomes limiting. Drought tolerant plants sustain physiological activities such photosynthesis even though leaf water status declines (e.g. stomata are kept open despite decreasing leaf water potential). Drought avoiding plants maintain leaf water status, even at the expense of photosynthesis (e.g. stomata are closed despite reduced gas exchange) (Ludlow, 1989; Anithakumari, 2011; Obidiegwu et al., 2015). The same plant can adopt traits of the two latter strategies according to the intensity of soil drying (Ludlow, 1989).

Potato typically avoids drought by increasing root development to explore soil layers and expand in regions where sufficient water is available. Most root growth occurs in the upper 40 cm of the soil if plants are well watered, whereas under drought stress a greater proportion of roots can be found in deeper soil layers (60 − 100 cm, Stalham and Allen, 2004). This change in root architecture allows the plants to adapt to a certain extent to dry soil conditions. Under deficit irrigation the upper soil layers

gradually dry out between irrigation events, so water uptake from lower soil layers is even more important for the plant. Thus, potatoes grown in drying soil extract water from deeper soil regions than seen under well-watered conditions (Stalham & Allen, 2004).

In many agricultural crops, drying soil restricts shoot growth earlier than root growth (Kramer & Boyer, 1995). Thus, both root architecture and root-shoot ratio change to provide sufficient supply of water to the aerial plant parts. In this way the plant avoids drought stress under limited water supply. Moreover, stomatal closure can occur due to the physical consequence of reduced turgor in the whole leaf and therefore in guard cells (Davies et al., 2002). This physical reaction can be seen under severe water deficit conditions. However, soil drying can decrease stomatal aperture before leaf water potential is affected (Liu et al., 2005; Puértolas et al., 2014), indicating that chemical signals are sent from root to shoot to reduce water loss before hydraulic signals become important. For example, the concentration of abscisic acid (ABA) in the xylem sap increases in response to drought stress (Davies et al., 2002; Puértolas et al., 2014) and causes partial stomatal closure (Davies et al., 2002; de Ollas & Dodd, 2016). Thus, there is evidence for hormonal signalling under drought stress. However, it is not entirely understood how different hormones may interact.

1.7. Signalling under drought stress

A number of plant hormones are involved in long distance (root-to-shoot) signalling under drought stress, including ABA, strigolactones (SL) and others. ABA can be synthesized in both root and shoot according to their water status (Davies et al., 2002), which makes it a relevant candidate in drought stress signalling (Liu et al., 2005). Historically it was presumed that ABA was produced in the roots under drought stress (Davies et al., 2002) and subsequently transported to the leaves, where it induces stomatal closure (Liu et al., 2005). Interestingly, root xylem-ABA content continues to accumulate for days after maximum stomatal closure is achieved (Liu et al., 2005). This could mean that basipetal phloem transport of ABA to the roots recirculates ABA in the xylem (Slovik et al., 1995) independently of stomatal closure. Alternatively, ABA may continually be produced in drought-stressed roots at low levels, making it a possible signal to regulate other responses to drought stress like shoot growth inhibition (Zhang & Davies, 1989). Exogenous ABA partially restored vegetative growth of ABA-deficient tomato mutants even when the shoot water status did not change (Sharp, 2002; Sharp et al., 2000). However, ABA deficient maize mutants and wildtype maize treated with Fluridone (carotenoid biosynthesis inhibitor) growing at low water potentials (-0.3 MPa in vermiculite) had a higher shoot growth rate than the untreated wildtype at the same soil water potential (Saab et al., 1990), indicating

that ABA maintains shoot growth under well-watered conditions, but inhibits shoot growth under drought stress.

ABA is a drought stress signal that among others induces transcription factors such as ABF/ AREB, MYC, MYB and bZIP, which regulate drought stress responses in the plant (Lee *et al.*, 2010; Verma *et al.*, 2016; Hussain *et al.*, 2021). These transcription factors upregulate the expression of genes that lead to increased stress tolerance or increased ABA sensitivity (Verma et al., 2016). DREB also play an important role in drought stress responses, but different authors disagree whether they are induced by ABA (Verma et al., 2016) or independent of ABA (Agarwal *et al.*, 2006). DREB belong to the ERF-family of transcription factors, which are regulated by ethylene and ethylene and ABA interact via the DELLA proteins (Verma et al., 2016). Thus, ABA and ethylene crosstalk induces specific drought stress responses. Mild drought treatment (water loss > 80 ml was compensated by re-watering) increased both ABA and ethylene levels in wheat (Valluru et al., 2016). Elsewhere deficit irrigation based on soil water loss is described in more detail and resulted in 0.1 MPa difference in leaf water potential between well-watered and deficit irrigated plants (Puértolas et al., 2014). However, in maize seedlings increased ABA levels under drought stress prevented excessive ethylene production thereby maintaining root growth (Spollen et al., 2000). ABA-mediated suppression of ethylene synthesis under water deficit occurred in roots and shoots thereby maintaining root growth and transiently inhibiting shoot growth (Sharp, 2002). Thus, changes in root and shoot growth under drought stress could be mediated by the ratio between ABA and ethylene, which may vary between species.

Recent research questions this paradigm and suggests that ABA produced in the shoot is more important for shoot physiological responses than ABA produced in the roots (McAdam et al., 2016a; Thompson et al., 2007). Following this new model, a hydraulic signal from roots to the shoot (which cannot be detected with concurrent measurements of leaf water potential), induces ABA production in the shoot and subsequent stomatal closure (McAdam et al., 2016a; Merilo et al., 2018). This hydraulic signal is most likely increased xylem tension, which reduces water flow from the roots to the leaves and a consequent decrease in leaf water potential, similar to more severe drought stress conditions. However, statistical changes in leaf water potential (Ψ_{leaf}) between two populations of plants of < -0.1 MPa are unlikely to be detected with the pressure chamber used in this study (or other methods) since variation in Ψ_{leaf} within and between individual plants is likely of a similar magnitude.Redistribution of foliar ABA then results in accumulation in the roots (McAdam et al., 2016a). In isohydric plants that maintain leaf water potential as the soil dries, this hypothesis cannot be proven or contradicted until more sensitive instruments to measure leaf water potential are available.

However, xylem-ABA levels alone cannot explain stomatal closure in response to drying soil in potato (Liu et al., 2005; Ahmadi et al., 2010a). Other changes in xylem sap composition increase the sensitivity of the guard cells to ABA, or re-distribute ABA to the guard cells (Davies et al., 2002; de Ollas et al., 2018). Elevated xylem pH decreases the uptake of ABA by leaf mesophyll cells, which increases the amounts reaching the guard cells and thus promoting stomatal closure (Wilkinson & Davies, 1997). This mechanism increases ABA's effectiveness in the leaf, but this can only explain partly the lower stomatal sensitivity to synthetic ABA found in earlier studies (Munns & King, 1988). Furthermore, it is possible that a hormonal signal is sent from the root to the shoot under drought stress to trigger shoot ABA production. This signal could either be root-borne ABA that causes foliar ABA accumulation (Liu et al., 2005) or another hormone that is produced in the roots under drought stress, like strigolactones (SL).

Strigolactones (SL) are plant hormones that are derived from the same precursor as ABA, 9-cis-β-carotene (Lv et al., 2018) and can be produced in the root and the shoot (Ruyter-Spira et al., 2013). SL deficient plants show increased stomatal conductance (Visentin et al., 2016; Liu et al., 2015) and wider stomatal aperture (Lv et al., 2018) under well-watered conditions compared to the wildtype. However, studies using exogenous SL disagree, since exogenous SL decreased stomatal aperture in *Arabidopsis* (Lv et al., 2018), but did not alter whole plant transpiration (Kalliola et al., 2020). Interestingly, exogenous SL trigger the SLAC1 channel (Lv et al., 2018), a K^+ ion pump in the guard cell membrane that is also triggered by ABA (Grabov et al., 1997). Hence, it is possible that ABA and SL interact in stomatal closure signalling or are interdependent through feedback loops. In dry soil SL deficient plants of tomato and *Lotus* show stomatal closure similar to the wildtype (Liu et al., 2015; Visentin et al., 2016), indicating that SL are not necessary for stomatal closure under severe drought stress. Thus, a lack of root SL production in drying soil could result in increased biosynthesis of another long-distance signal eliciting stomatal closure, such as ABA.

Mild drought treatment (-0.08 MPa soil water potential) increased both ABA and ethylene production in wheat shoots (Valluru et al., 2016). However, roots of ABA deficient maize seedlings showed excessive production of ethylene (Spollen et al., 2000), indicating that ABA reduces ethylene production and thereby enables root growth under drought stress. In addition, shoot growth in ABA-deficient maize seedlings under water deficit (-0.3 MPa soil water potential) was similarly increased by exogenous ABA and the ethylene blocker silver thiosulphate (Sharp, 2002). That means that the previously stated changes in root and shoot growth under drought stress could be mediated by the ratio between ABA and ethylene. These ratios likely differ for roots and shoots in drying soil.

The majority of the hormonal signals described above were found in different agricultural crops such as wheat, maize or tomato. The harvested products from all these plants are the fruits or seeds produced from the pollinated flower above-ground. In contrast, potatoes have large storage organs below-ground and were bred to relocate considerable resources into these. This means that signalling mechanisms between the above-ground and the below-ground plant parts could differ from the other named crops. This thesis aims to understand more about the long-distance signalling and water fluxes in potatoes under water-limiting conditions.

1.8. Soil compaction in potato agriculture

Potatoes are usually planted in the United Kingdom between the end of March and the beginning of May (Stalham et al., 2007). Prior to planting, it is common practice to till and de-stone the field to a depth of 25 cm (AHDB Potatoes, 2013; Stalham et al., 2007). The soil is usually wet and close to field capacity when these soil management measures are undertaken (Stalham and Allison, 2019, personal communication). In these conditions, the soil is close to its plastic limit. That means traffic with heavy machinery will compress the soil particles, leading to compacted soil. Soil compaction decreases soil pore space, thus increasing the bulk density of the soil. This entails changes in physical properties as well as changes in chemical properties (Nawaz et al., 2013). These changes decrease root length and density (Stalham et al., 2007) and therefore the ability of the plant to extract water and nutrients from the soil. The susceptibility of a certain field to compaction depends not only on the soil type, but also on the current water content and the existing degree of compaction (Håkansson, 2005). This can also be seen in practice as shallow compaction in potato fields was frequently observed in wet springs (Stalham et al., 2007). Since the UK averages 12.3 rainfall days per month between March and May and the temperatures are not high enough to significantly dry the soil via evaporation (Met Office UK, 2021), potato fields are usually wet when they are prepared for potato sowing. Thus, compaction due to vehicle traffic on these fields is very likely and in a survey of over 600 fields over a period of 12 years, 65 % of commercial fields were severely compacted (≥ 3 MPa penetration resistance, Stalham *et al.*, 2007). Soil compaction decreased potato yield in Maris Piper by 40 % and in the same study deficit irrigation did not decrease yield further (Stalham et al., 2007). Hence, there seems to be an interaction between irrigation management and soil strength. Plant water availability is likely a key factor in this interaction. An experiment with a factorial combination of deficit irrigation and soil compaction is needed (Chapter 2) to disentangle the effects of the single stresses on plant physiology and yield.

1.9. Compaction

Soil compaction is a form of soil degradation (Nawaz et al., 2013) that changes pore size distribution, since the compaction process removes larger pores (> 30 µm) more than fine pore structures (Håkansson, 2005; Nawaz et al., 2013). The change in porosity lowers the gas permeability of the soil as well as the drainage capability (Wolkowski & Lowery, 2008) and can decrease soil hydraulic conductivity (Johansen et al., 2015) making water less accessible for the roots. These structural changes in the soil increase the likelihood of waterlogging under high irrigation amounts and excessive rainfalls and/or earlier drought stress under low irrigation amounts compared to uncompacted soil (Bengough et al., 2011). Additionally, compacted soil often restricts root growth due to higher soil resistance, which further reduces access to water and nutrient resources (Stalham et al., 2007). Soil compaction can also limit plant availability of important nutrients, such as nitrogen, phosphorus and potassium in the soil, because diffusion and mass flow are limited through smaller pore sizes and the resulting lower soil water content (Hamza & Anderson, 2005; Nawaz et al., 2013). Altogether, plants in compacted soil experience physical stresses such as mechanical impedance and chemical stresses such as low oxygen and nutrient availability. The physiological responses to these stresses may have an additive effect on crop yields.

1.10. Physiological responses to soil compaction

The higher bulk density and higher penetrometer resistance in compacted soil inhibits root growth (Nawaz et al., 2013), leading to shorter and thicker roots in many plant species (Materechera et al., 1991; Bengough et al., 2011; Nawaz et al., 2013; Jin et al., 2015; Colombi & Walter, 2016). Soils with a penetrometer resistance exceeding 1.5 - 2 MPa considerably restrict root growth of potato and other crops (Bengough et al., 2011; Stalham et al., 2007). A smaller root system limits water and nutrient capture, which can stunt plant growth. Additionally, limited soil oxygen availability can restrict plant growth (Ryan et al., 2016), trigger formation of aerenchyma, increase the thickness of the root cortex in nodal roots and decrease the root cortex thickness in tap roots (Colombi & Walter, 2016). Aerenchyma can provide oxygen to the root tip under anaerobic soil conditions, which can be present in compacted soils, and can cause thickening of the roots due to a thicker root cortex zone (Colombi & Walter, 2016).

Soil compaction usually decreases shoot growth and leaf area (Andrade et al., 1993; Wolkowski & Lowery, 2008; Mulholland et al., 1996; Hussain et al., 1999, 2000; Coelho Filho et al., 2013), often to a greater extent than root growth (Andrade et al., 1993). In maize and barley, soil compaction decreases stomatal conductance while leaf water potential is maintained (Tardieu et al., 1992; Mulholland et al., 1996). In young maize plants, leaf water potential is initially lower in compacted soil and xylem ABA levels are increased (Hartung et al., 1994), indicating a response to lower water availability. This reaction is also reported as response to drought stress (Section 2.2, above) with a similar signalling mechanism. These similarities indicate that plants grown in compacted soil also suffer from water deficit. However, there are also other long-distance signals under soil compaction that regulate plant responses.

1.11. Signalling under compaction

Stomatal closure under soil compaction is mediated via increased levels of ABA in the xylem sap of tomato (Hussain et al., 2000), However, wheat grown in compacted soil maintained leaf water potential without changes in xylem ABA content (Whalley et al., 2006). Thus, increased ABA levels may represent a signal of drought stress, because of limited water access to the roots, rather than a signal of increased soil strength *per se*. This distinction is somewhat academic since the stresses cannot be separated in the field. Under mild compaction (1.4 g cm^{-3}) increased ABA export from the roots maintained barley leaf area in WT plants, whereas this signal was insufficient to sustain leaf growth rates under severe compaction (1.7 g cm^{-3}, Mulholland *et al.*, 1996). In tomato, leaf expansion rates of the ABA deficient mutant *flacca* are lower than in the wild type under control conditions, but leaf expansion rate in both genotypes is similarly low under severe compaction (1.5 g cm^{-3}) (Mulholland et al., 1999), suggesting that ABA is needed to maintain leaf expansion under mild compaction, but not sufficient under severe compaction as other mechanisms limit growth. Furthermore, both findings suggest that other signals (than ABA) mediate leaf expansion under severe compaction stress, similar to the patterns of signalling in response to drought stress seen above.

Soil compaction increased leaf ethylene evolution up to 5-fold and decreased leaf area of wildtype tomato plants, with an attenuated response when plants were grown in split pots with half the roots in compact soil and the remainder in uncompacted soil. Transgenic plants with a reduced capacity for stress-induced ethylene synthesis (ACO1$_{AS}$) showed the same growth response under full compaction, whereas they maintained leaf expansion in the split pots (Hussain et al., 2000, 1999). Thus, ethylene signalling seems important in regulating growth when some roots are exposed to soil compaction, but

is overridden by other signals under severe compaction. Additionally, ethylene decreased root elongation but increased root radial growth under both water stress (Sharp, 2002) and under soil compaction (Nawaz et al., 2013). Furthermore, increased ethylene production of ABA-deficient tomato mutants can decrease leaf expansion, which can be restored by exogenous ABA (Sharp et al., 2000). Applying ABA to the compacted compartment of a split pot system decreased ethylene production and maintained shoot growth in both wild type and ABA deficient tomatoes, while $ACO1_{AS}$ plants were not affected by this treatment (Hussain et al., 2000). These findings suggest that ethylene and ABA interact under compaction stress to regulate shoot growth as with drought stressed plants. This means that root-sourced ABA could restrict root ethylene production or the sensitivity of tissues to ethylene in plants growing in compact soil.

Gibberellins (GA) generally promote root and shoot growth (Taiz & Zeiger, 2010). Decreased GA sensitivity and soil compaction (0.75 MPa) stunted root and shoot growth to a similar extent in wheat (Coelho Filho et al., 2013). Furthermore, supplying exogenous GA to plants grown in compacted soil promoted leaf elongation to a greater extent than GA supply to plants grown under normal conditions (Coelho Filho et al., 2013), suggesting that gibberellins are downregulated in plants under compaction. Similarly, soil compaction decreased foliar gibberellin levels (Donaldson, 2019). Thus, reduced GA synthesis under soil compaction could inhibit root and shoot growth.

The above-mentioned hormones likely interact to mediate plant responses to soil compaction. Gibberellin action under abiotic stress is often mediated by DELLA proteins, which act as growth repressors (Colebrook et al., 2014). Under salt stress and cold stress, ABA and ethylene regulate plant growth also through the function of DELLA proteins (Achard et al., 2006; Verma et al., 2016). Thus, these hormones may also interact via DELLA proteins under drought or compaction stress (Figure 1.11.1). In addition, the DREB1/CBF transcription factor family regulates the gibberellin metabolism under cold stress (Achard et al., 2006) and there are contradictory findings as to whether ABA induces these transcription factors under cold stress (Knight et al., 2004). More recent findings show that the transcription factors of the ABF family regulate ABA- and stress responses in the plant and that different members of the ABF family interact with members of the DREB family (Lee et al., 2010; Verma et al., 2016). Since transcription factors of the DREB family regulate gibberellin biosynthesis as well as ethylene signalling (Achard et al., 2006; Verma et al., 2016), crosstalk between these three hormones may involve several pathways. Ethylene and ABA also crosstalk in stress responses via the DELLA proteins (Verma *et al.*, 2016), so these proteins seem to have a crucial role in managing stress responses.

Taken together, drought stress and soil compaction induce similar physiological responses and long-distance signalling, probably since soil compaction also limits plant water access by decreasing root length. Hence, it is difficult to separate drought stress and soil compaction responses, but further understanding of both stresses, individually and in combination, is needed to suggest more precise crop management strategies and to identify breeding markers.
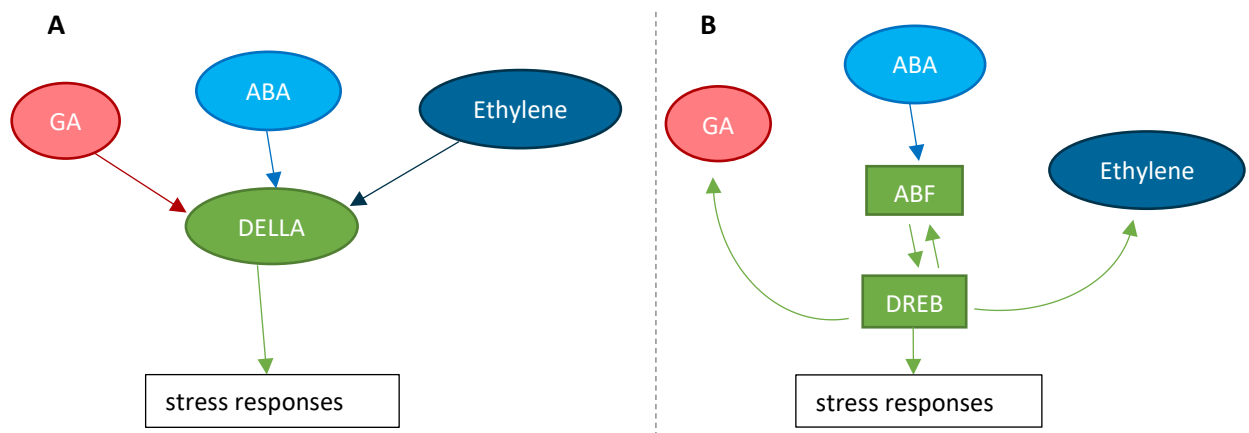
Figure 1.11.1: Possible interaction between gibberellins (GA), abscisic acid (ABA) an ethylene in signalling of abiotic stress. A) via DELLA proteins, B) via DREB and ABF transcription factors

1.12. Thesis structure

This thesis is aimed to understand the effect of limited water availability caused by drought stress and soil compaction on potato physiology and signalling through different stages of plant development. This was first investigated in a field experiment with the variety Maris Piper grown and monitored over a whole season in a factorial combination of drought stress and soil compaction (Chapter 2). In this experiment soil moisture was continuously monitored, shoot growth and physiological parameters were assessed weekly and correlations with yield were established to test the hypothesis that soil compaction and drought stress limit shoot growth, but only deficit irrigation induces foliar ABA accumulation and therefore restricts plant gas exchange and photosynthesis rates. The experiment was repeated on an adjacent field in the following year. However, penetrometer readings did not demonstrate significant soil compaction. Thus, the results from that year are not presented in the paper and this thesis. Specific mechanisms underlying the field responses found in the presented field experiment are further explored in the subsequent chapters.

In the field experiment, drought stressed plants had a large number of small tubers. To understand how tuber growth is affected by soil water availability and shoot physiological responses, an *in-vivo*

experiment was conducted using magnetic resonance imaging (MRI) to measure tuber volume and water content in the soil during tuber bulking stage (Chapter 3). These measurements were accompanied by soil moisture and shoot physiological measurements similar to those conducted in the field experiment, to relate tuber growth and water content to the previously discussed physiological effects. It is hypothesized that tubers take up water during the night and lose water throughout the day. This is expected to increase and decrease tuber growth rate respectively. Greater restriction of overnight growth rate in drought stressed plants than in well-watered plants could explain the smaller tubers under drought stress in the field.

Under long-term deficit irrigation in the field, potato plants restrict above-ground growth rather than gas exchange. However, in controlled environment experiments that withhold water, the stomata close before leaf water potential decreases. Both reactions maintain leaf water potential at different costs. To understand possible interactions or common causes between these responses, hormonal root-to-shoot signalling under drought stress was further examined (Chapter 4). The role of ABA in drought stress responses is well studied, but a newer group of hormones, strigolactones (SL), were reported to be long-distance signals under drought stress and to interact with ABA (Visentin et al., 2016; Liu et al., 2015; Ruyter-Spira et al., 2013). ABA levels did not differ between treatments in the field (Chapter 2) and changes in stomatal conductance could not entirely be explained by changes in leaf water potential in controlled environments (Huntenburg, preliminary data, Liu *et al.*, 2005), which suggests that an additional signal or several signals are involved in stomatal closure in drying soil. The role of SL in potato drought stress responses was investigated using transgenic lines deficient in SL biosynthesis and with increased and decreased sensitivity to SL during the primarily vegetative growth stage (Chapter 4). Based on previous literature, transgenics deficient in SL biosynthesis or insensitive to SL were expected to have a higher stomatal conductance than the wildtype under well-watered conditions, whereas the genotype hypersensitive to SL is expected to have a lower stomatal conductance than the wildtype. In drying soil, it is expected that all genotypes show similar levels of stomatal closure with higher ABA levels in the genotypes impaired in SL biosynthesis or signalling and lower ABA levels in the SL hypersensitive genotype compared to the wildtype.

2. Agronomic and physiological responses of potato subjected to soil compaction and/or drying

Graphical Abstract
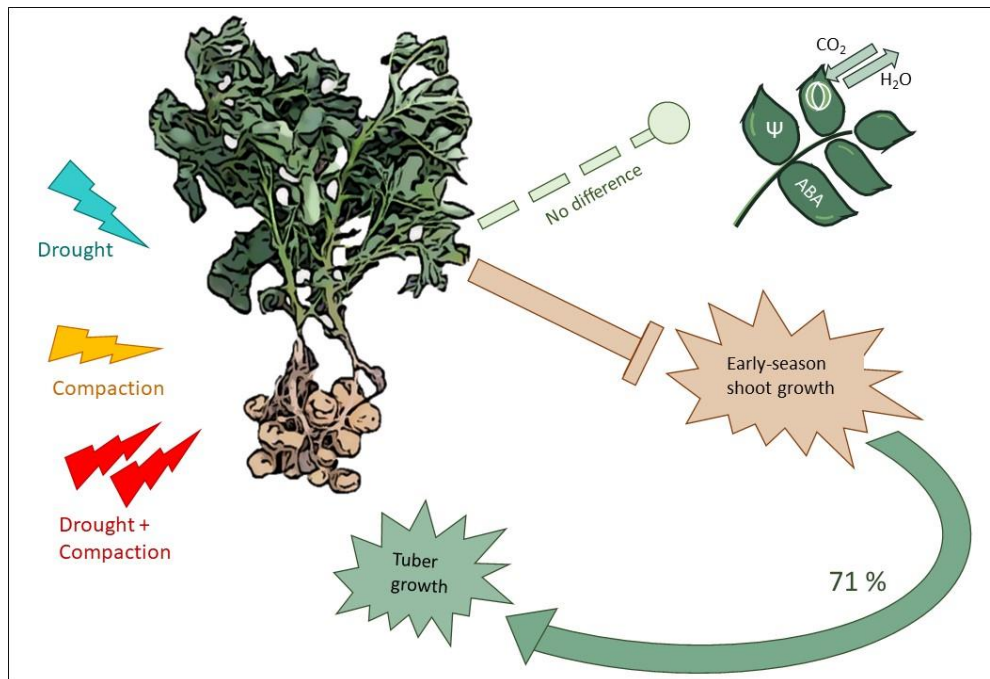
2.1. Introduction

Potato is the most produced non-cereal food crop worldwide (FAO, 2009), exceeding soybean production with 368 million tonnes of potatoes produced in 2018 (Vinet & Zhedanov, 2011). However, harvests can be threatened by abiotic stresses such as drought and soil compaction. In different climate change models, drought events are projected to decrease potato yields between 18 and 60 % globally, as well as on a regional scale (Obidiegwu et al., 2015; AHDB Potatoes, 2017). Trafficking heavy machinery on wet soils in autumn and spring causes soil compaction, a widespread issue in the UK with over 60 % of 800 tested commercial fields showing soil resistances that limit root growth rate (mm per day) and yield (Stalham et al., 2007). Moreover, soil drying can increase soil strength to the same extent as heavy trafficking (Whalley et al., 2006). During dry periods, roots growing in a compacted soil may be unable to overcome high soil resistance to grow into deeper soil layers to access water resources. Potatoes are sometimes regarded as being shallow-rooted (Obidiegwu et al., 2015; Stalham & Allen, 2004) which may constrain water uptake, even if there is considerable genetic variation in root growth between cultivars (Puertolas et al. 2014; Wishart et al. 2014). Hence, it is important to understand potato responses to low plant water availability (George et al., 2017), to develop strategies to minimise the impact of compaction and drought stresses on yield.

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Plant water availability is commonly measured as leaf water potential. In controlled environments, leaf water potential during the day ($\Psi_{daytime}$) was lower in deficit irrigated than well-watered potato plants (Puértolas et al., 2014) and pre-dawn leaf water potential ($\Psi_{pre-dawn}$) decreased as soil dried out (Whalley et al., 2006; Liu et al., 2005). Tissue water deficits stimulate production of the plant hormone abscisic acid (ABA), which closes the stomata thereby restricting plant water loss (and potentially plant carbon uptake). The sensitivity of transpiration and photosynthesis to stomatal closure varies between studies. Soil drying increased leaf xylem sap ABA concentration, thereby decreasing stomatal conductance and photosynthesis rates in containerised potato (Puértolas et al., 2014) and tomato (Thompson et al., 2007) plants. In field-grown potatoes, low plant water availability increased leaf ABA accumulation and reduced stomatal conductance, but photosynthesis rates were only reduced on some occasions (Liu et al., 2006). Hence, potato plants growing in drying soil restrict transpirational water loss by decreasing stomatal conductance, which can also decrease photosynthesis rates and therefore carbon gain per unit leaf area. In addition, soil drying restricted leaf expansion and initiation in potato (Fasan & Haverkort, 1991; Kawakami et al., 2006) thereby limiting total plant carbon gain by decreasing whole plant photosynthesis. Hence, less carbon is available for tuber growth and therefore yield. However, reports from controlled environment and field experiments show variability in the relationship between photosynthesis rates and stomatal conductance, while it remains unclear which mechanisms or signals limit shoot growth in drying soil. Understanding these mechanisms is important to identify drought-sensitive growth stages, allowing more precise and plant adapted irrigation scheduling.

Soil compaction restricts horizontal and vertical root growth of potatoes in the field, ultimately reducing tuber number and yield (Van Oijen et al., 1995; Stalham et al., 2007). Smaller root systems access less soil volume thereby limiting water and nutrient uptake, which results in smaller plants with less leaf area (Nawaz et al., 2013; Stalham et al., 2007). However, the mechanisms by which soil compaction restricts plant water status and gas exchange remain unclear. Compaction decreased leaf water potential in maize (Tardieu et al., 1992) and wheat (Whalley et al., 2006), but not in sunflower (Andrade et al., 1993). Potatoes grown in compacted soil reached full ground cover later and had lower photosynthesis rates than those grown in loose soil (Van Oijen et al., 1995), and therefore produced lower yields. Soil compaction increased root xylem sap ABA levels in tomato, thereby reducing stomatal conductance (Hussain et al., 2000), and possibly photosynthesis, although ABA levels may have increased simply because sap was collected at low flow rates from plants growing in compacted soil, in the absence of any change in $\Psi_{daytime}$. Shoot and leaf ABA concentrations of tomato did not change across a similar range of soil bulk densities (Tracy et al., 2015). Moreover, well-watered maize plants grown in the field at different bulk densities showed no differences in stomatal conductance or

leaf xylem ABA concentration (Tardieu et al., 1992). Hence drought and soil compaction can decrease plant water availability individually, thereby limiting total biomass and tuber dry weight of field-grown potatoes (Kawakami et al., 2006; Shock et al., 1998; Stalham et al., 2007). However, little is known about the impact of combined drought stress and soil compaction on potato growth and physiology. Since heavy machinery may compact soil in wet spring months and rainfed crops commonly experience drought stress later in summer in the UK, it is important to understand the effect of both stresses combined and individually to design appropriate management strategies.

Irrigation increased potato yield to a greater extent in loose than in compact soils (Stalham et al., 2007), indicating that different mechanisms may co-ordinate plant responses to the two stresses. When a factorial combination of soil drying and soil compaction was applied to field-grown maize (Tardieu et al., 1992) and wheat (Whalley et al., 2006) plants, soil drying, but not soil compaction, decreased stomatal conductance in both species without the two factors interacting. Soil compaction, but not deficit irrigation, decreased root and shoot biomass in wheat (Whalley et al., 2006). However, to our knowledge no study has investigated the physiological responses of a dicotyledon to a factorial combination of the two stresses. As dicotyledonous leaf growth may be more sensitive to leaf water deficit as the growing tissues are exposed to the atmosphere and not enclosed in older leaves as in monocotyledons (Radin, 1983), potatoes may be highly susceptible to these stresses. Thus, the current experiment comprehensively evaluated physiological ($\Psi_{pre-dawn}$, $\Psi_{dayitme}$, leaf ABA concentration, stomatal conductance, photosynthesis rate) and agronomic (ground cover, shoot biomass, total yield, tuber size distribution) responses to a factorial combination of drought and soil compaction in a field grown potato crop. We hypothesize that both stresses limit shoot growth, but only deficit irrigation induces foliar ABA accumulation to restrict plant gas exchange and photosynthesis rates.

2.2. Material and Methods

Field and Crop Management

A 2x2 factorial combination of drought and compaction stress was set up at NIAB, Cambridge (0°05'58.8'' E and 42°14'06.1'' N) in a randomized block design with four replicates. Plots consisted of four harvest rows and two guard rows, with inter-row spacing of 0.75 m. Plant spacing was 0.3 m with 30 plants per row. On 14th March 2018, prior to planting, organic matter (municipal compost, Amey PLC, Cambridge, UK) was incorporated (25 cm depth) into the sandy loam. On 19th April 2018, the compaction treatment was imposed by driving a John Deere 6120R tractor with rear-mounted plot drill and fronted-mounted disc roller packer (total laden weight 7570 kg). The tractor ran on

340/85R/48 rear tyres at 25 PSI pressure and 340/85R/28 front tyres at 15 PSI over the entire area of the plot, so that by driving and reversing across the plot, each tyre compressed the soil twice. The soil was close to field capacity at plough depth at this stage (by irrigating to saturation prior to compaction treatment). With the soil water content and bulk density at the time of compaction, the Terranimo soil compaction model (www.terranimo.dk, Aarhus University, Denmark) indicated severe compaction to 55 cm depth, but with a lesser effect to 70 cm. Following compaction, the area was spring-tined to a depth of 10-12 cm and then roto-ridged into ridges with a Rumptstad rototiller on 20th April 2018.

Seed tubers of *Solanum tuberosum* variety 'Maris Piper' were planted on 25th April 2018 into the pre-formed ridges. All treatments reached 90 % plant emergence at 34 days after planting. An overhead irrigation boom, running with a speed of 30 m/h and nozzles hanging 1.5 m above ground, irrigated the crop. Irrigation was scheduled according to soil moisture deficit (SMD) as explained by Stalham et al. (2007) with a threshold to irrigate at 25 mm SMD for well-watered treatments and at 60 mm SMD (allowing potato evapotranspiration of 1-2 mm per day on the same soil as the present study - Stalham & Allen, 2004) for droughted treatments. Well-watered plots were first irrigated on 12th June, while drought stressed plots were first irrigated on 2nd July. Irrigation intervals averaged 5–10 days thereafter, depending on weather conditions. Soil moisture was continuously monitored by Theta-probes (Delta-T, Cambridge, UK) installed 25 cm below the top of the ridge in two blocks with two probes per plot (Fig. 1). Daily weather data was obtained from a weather station on NIAB research grounds in close proximity to the experiment site. Soil strength was assessed at the beginning, middle and end of the season (26th April, 12th June and 9th October respectively) using a penetrometer with a 1 cm^2 surface cone (Penetrograph, Eijkelkamp Soil & Water, Giesbeek, NL), to a depth of 1 m from the top of the ridge (Fig. 2). There was no irrigation in the weeks before the measurements in spring and autumn, while in summer the plots were irrigated 5 days before measuring soil strength. Protective spraying against blight was carried out when necessary.

Measurements

Ground cover was assessed weekly in the two middle rows of each plot using a 75 x 60 cm grid. Leaf number and leaf width were assessed weekly on the same three plants per plot. The width of the tenth leaf (numbering from the base of the plant) was measured in weeks 24 – 29 and on a young leaf with approximately 10 cm length from week 30. Pre-dawn leaf water potential ($\Psi_{\text{pre-dawn}}$) was measured 1-2 days after an irrigation event with a Scholander-type pressure chamber. Leaf gas exchange was measured on one plant per plot and using an infrared gas analyser (LI-6400 Portable Photosynthesis System, LI-COR, Lincoln, USA) with 1500 µmol PAR (10 % blue), 400 ppm CO_2,

300μmol/s flow rate, a Block Temperature of 25°C and ambient humidity inside the cuvette. Leaf water potential (Ψ_{leaf}) of that same leaf was determined with a Scholander-type pressure chamber on the same days that pre-dawn water potential was measured. Gas exchange and Ψ_{leaf} were measured between 4 hours after sunrise and ending no later than 3 hours before sunset, with time of day being implemented as a random effect in the linear models where necessary (see section 2.3.) From the same plant, tissue samples of young, developing leaves (entire leaf) were taken, directly put into liquid nitrogen (calendar weeks 26 to 32) or kept on dry ice (calendar weeks 34 and 35) until storage at -80 °C. Leaf ABA concentration was determined by radioimmunoassay (Quarrie et al., 1988). Freeze dried and ground leaves were extracted in de-ionised water at a ratio of 1 : 50 (leaf tissue (μg) : water (μl)) overnight and then kept frozen at – 20 °C until measured. At final harvest, 2.5 m² in the middle of each plot were harvested (10 plants). Tubers were graded into size classes in 10 mm increments (from 10 – 20 mm to 80 – 90 mm) and for each size class tuber number and total weight was taken. Total yield contained all size classes, while marketable yield only considers tubers larger than 40 mm.

Data analysis and statistics

Statistics were carried out with the software R version 4.0.3 (R Core Team, 2020, Vienna, Austria). Three-way repeated measures ANOVAs were carried out to evaluate the effects of compaction, irrigation and calendar week on weekly measured variables and compaction, irrigation and soil depth on soil strength. Assumptions of independent and identically distributed (as Normal) data and sphericity were tested (Shapiro-Wilk Normality test, Mauchly's test) and accounted for if required. Due to missing data some weeks had to be excluded from repeated measures analysis. As a result, the repeated measures ANOVA was carried out using weeks 29-31 and 35 for stomatal conductance and photosynthesis rates and weeks 26 – 28, 32 and 35 for leaf ABA concentration. The analysis of the penetrometer resistance in September is truncated to depths of 0 – 30 cm for the same reason.

Two-way ANOVAs (for main effects of drought, compaction & their interaction) were carried out separately on each measurement occasion to highlight when the differences found in repeated measures ANOVA occurred, as well as for tuber yield and size. Least significant difference (LSD) values at the 5% level of significance are given in the figures where interactions are significant, otherwise error bars indicate standard errors. Regression lines were estimated using linear models.

2.3. Results

Environmental conditions and treatments

The irrigation schedule was calculated according to soil moisture deficit (SMD), a method successfully applied to several potato experiments in the same soil type on NIAB trial grounds (Stalham & Allen, 2004). Soil moisture at 25 cm was generally higher in well-watered treatments than in drought-stressed treatments (

Figure 2.3.1). Before calendar week 28, compacted plots were wetter than uncompacted plots, probably due to lower water use of smaller canopies in the compacted treatments (cf.

Figure 2.3.1, Figure 2.3.3). Soil moisture in all treatments increased notably after heavy rainfall in week 32 (> 20 mm in 2 days) in all treatments. Irrigation was stopped after week 34 to encourage crop senescence. Altogether, the irrigation schedule created clear differences in soil moisture between the well-watered and drought-stressed treatments. Rainfall events in the second half of the season changed overall soil moisture levels, but did not change the difference between irrigation treatments (
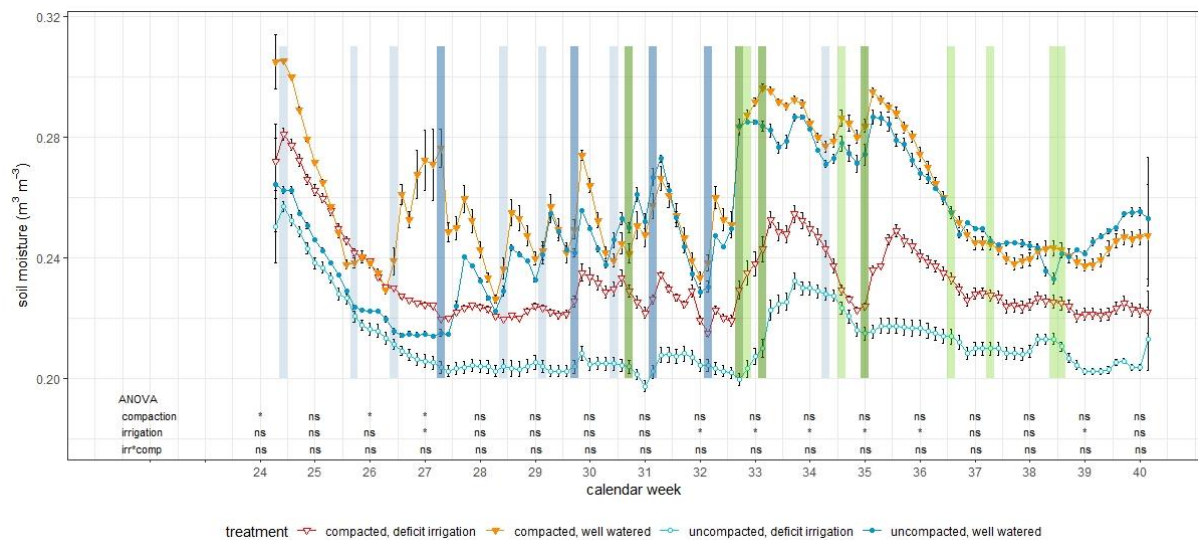
Figure 2.3.1).

Figure 2.3.1: Soil moisture at 25 cm depth over the season 2018. Means ± SE of four Theta-probes per treatment) with residual degrees of freedom df = 4. Blue vertical bars indicate irrigation days, with light blue being well-watered plots only and dark blue being all plots irrigated. Green vertical bars are rainfall events with light green > 3mm rainfall per day and dark green > 10 mm per day. Statistical significance of irrigation, compaction and their interaction reported for mean values of each week with: ns = not significant $P > 0.05$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Penetrometer resistance below 20 cm depth was higher in compacted than uncompacted soils at the beginning and the end of the season. In July, the differences between treatments were small and overall soil resistance lower because the soil had recently (2 days previously) been irrigated and was therefore softer (

Figure 2.3.2). In September, the drought stressed treatments had higher soil resistance than their respective well-watered treatments, but statistical analysis was only possible at 0-30 cm depths due to limited replication in strong soil below those depths. Overall, compacted treatments showed a higher soil resistance than uncompacted treatments at depths between 20 cm and 35 cm, Soil resistance below 15 cm depth increased between April and September in all treatments.
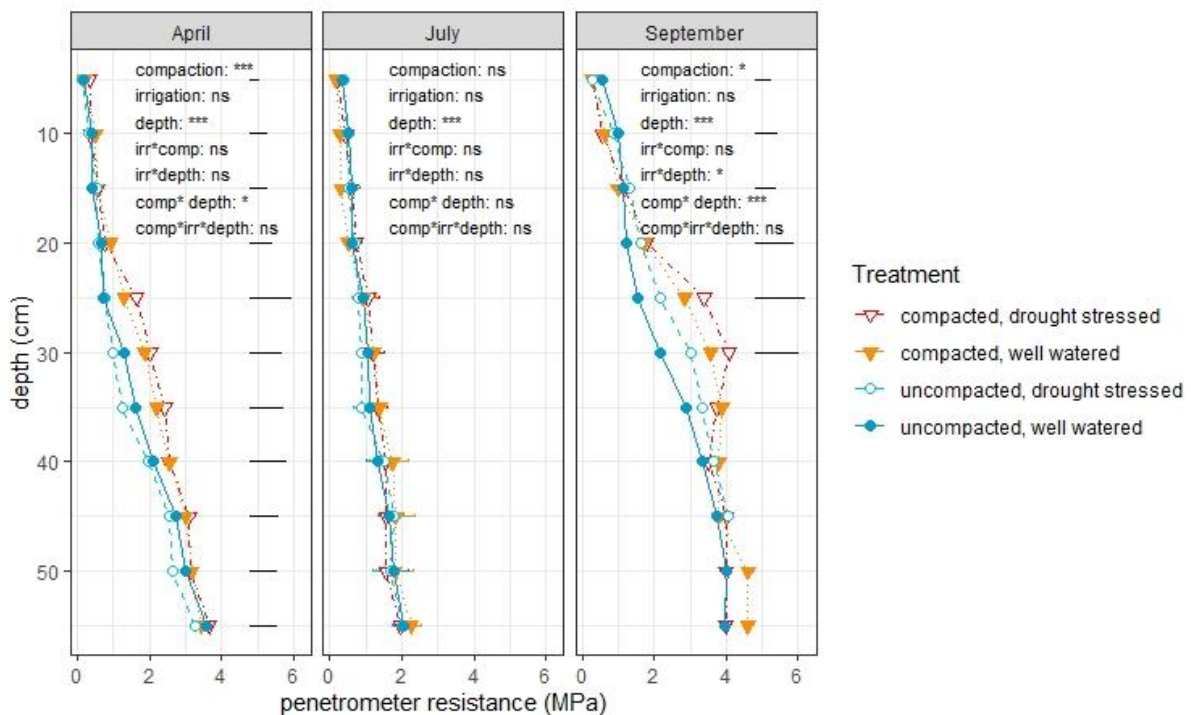
Figure 2.3.2: Soil resistance of all treatments in the beginning (April), middle (July) and end (September) of the season. Means of 4 plots per treatment and time point with 3 technical replicates per plot with SE for all depths where no significant interaction between treatments was found. LSD (5%) given for each depth (black horizontal lines) where interactions were significant with residual degrees of freedom df = 11 in April and July and df = 12 in September.). In September statistical analysis could not be carried out for soil layers from 35 – 55 cm depth due to missing values (too high resistivity to take measurements). Measurements in July were taken 2 days after irrigation, while measurements in April and September were taken before irrigation started and after irrigation stopped, respectively. Statistical significance of irrigation, compaction, depth and their interaction reported each measurement time point, with: ns = not significant P > 0.05; * P < 0.05; ** P < 0.01, *** P <0.001.

Plant growth

Plants emerged 3 days earlier in uncompacted soil (49 days after planting) than compacted soil (52 days). Ground cover differed between compaction treatments at an early growth stage (from calendar

week 24), with plants in uncompacted soil growing faster and more rapidly reaching full ground cover than plants in compacted soil. After full canopy cover was reached, plants in uncompacted soil senesced more quickly than in compacted soil (Figure 2.3.3). Irrigation treatments commenced from calendar week 24 and plant growth in the well-watered plants remained rapid, while growth of the deficit-irrigated plots was greatly restricted in the first weeks after emergence and ground cover never reached the absolute values of well-watered plants (Figure 2.3.3). This confirms that the timing of irrigation of the well-watered treatments was adequate to maintain optimal growth. Taken together, soil compaction and drought stress reduced the duration of full ground cover and therefore time of maximum light interception.
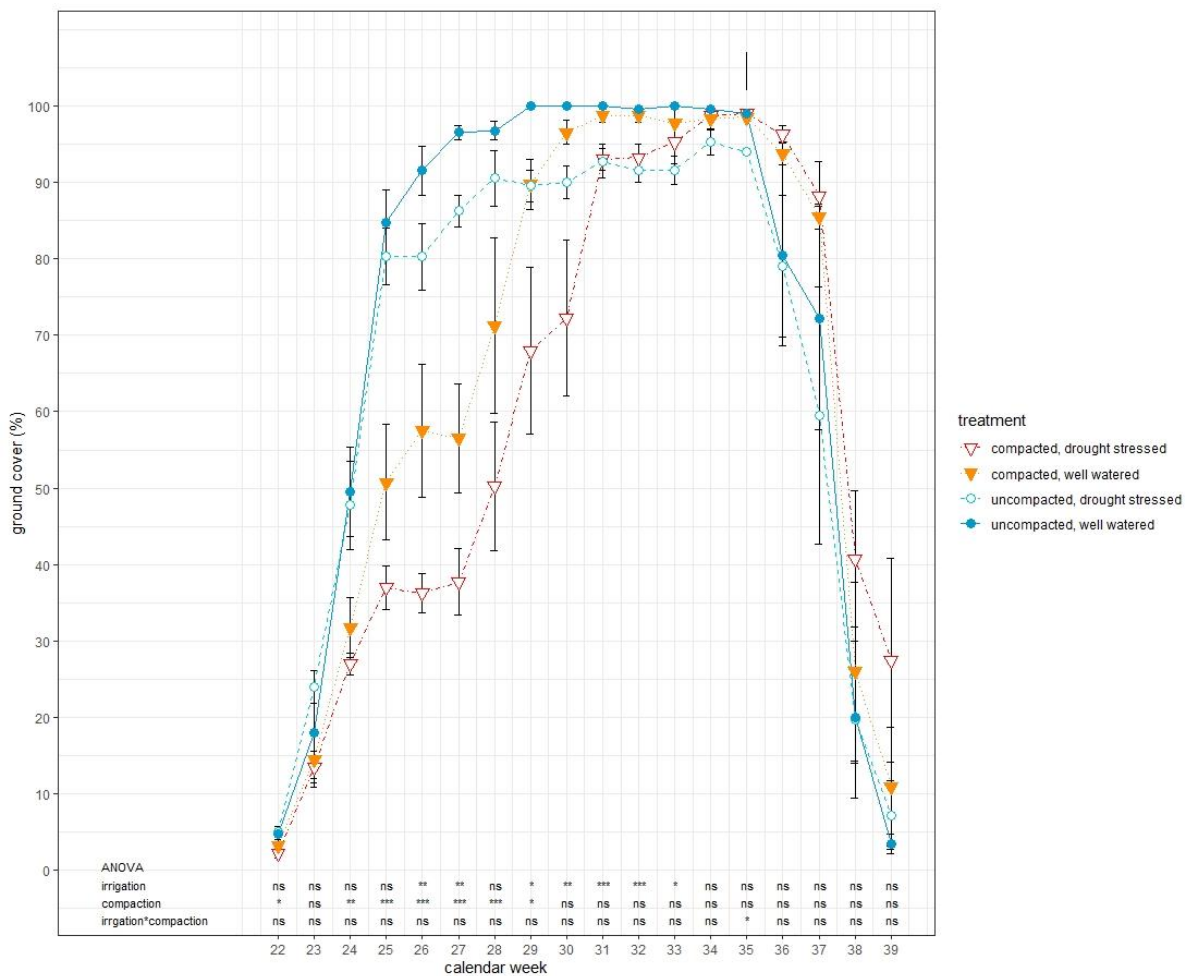
Figure 2.3.3: Weekly ground cover of field grown 'Maris Piper'. Means ± SE of four plots per treatment with residual degrees of freedom df = 12. LSD (5%) for week 35 is given (black vertical line), because of significant interaction in this week. Statistical significance of irrigation, compaction and their interaction reported each week with: ns = not significant $P > 0.05$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Leaf expansion only differed between treatments until calendar week 29, with the two individual stresses reducing leaf expansion compared to the control and the combined stresses reducing growth rate further (Figure 2.3.4A and B). At the beginning of the season, soil compaction limited leaf initiation

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while after week 28 deficit irrigation had a greater effect. By week 33, few new leaves developed (Figure 2.3.4C). Thus, leaf expansion and leaf initiation showed similar treatment responses to overall canopy cover.
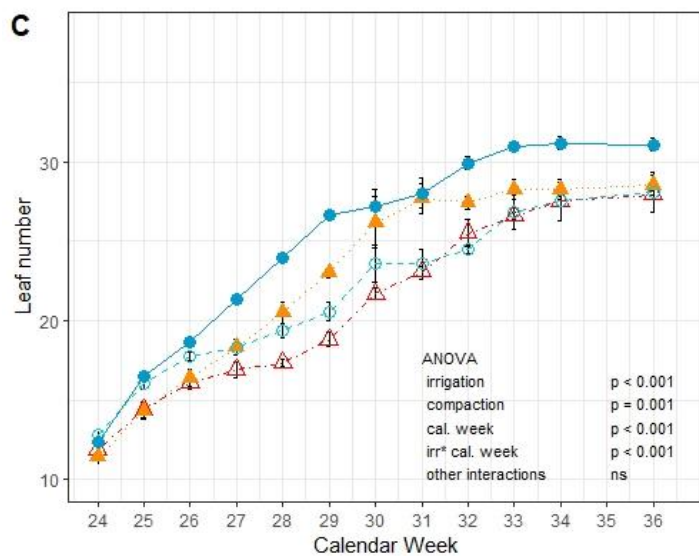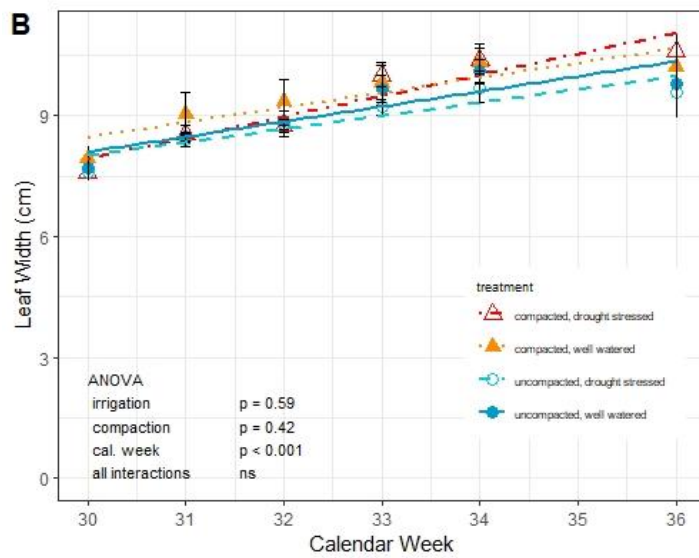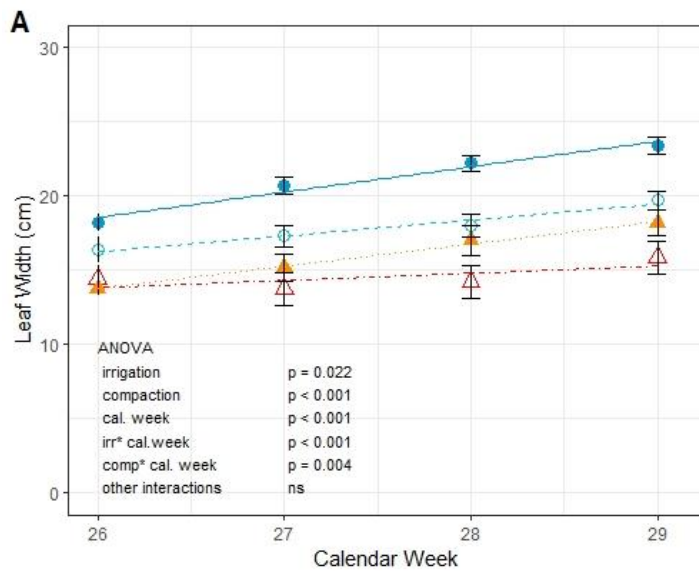
Figure 2.3.4: leaf width in the first half (A) and the second half (B) of the season and weekly leaf number (C) of field grown 'Maris Piper'. Means ± SE of 12 plants per treatment (and measurement date) with residual degrees of freedom df = 12. Regression lines were fit using linear models.

Plant physiological parameters

To investigate whether differences in carbon gain (photosynthesis) affect shoot growth, leaf gas exchange was measured. Surprisingly, stomatal conductance and photosynthesis rates did not differ between treatments on most measurement dates (Figure 2.3.5A and B). Stomatal conductance (to water vapour) is linearly correlated with transpiration per unit leaf area. Irrigation as a main effect only had a significant impact in week 29, with deficit-irrigated plants having lower stomatal conductance and photosynthesis rates than well-watered plants ($p < 0.05$ for both parameters, Figure 2.3.5A and B). At this time, irrigation had been suspended for 17 days in the deficit irrigation treatments (

Figure 2.3.1). In the last measurement week, both stomatal conductance and photosynthesis rates approximately halved as the canopy senesced. Thus, adverse soil conditions restricted shoot growth, but photosynthesis and transpiration per unit leaf area (stomatal conductance) were only affected after a prolonged period without irrigation.

As leaf gas exchange did not differ between treatments, plant water status was also measured. For pre-dawn and daytime leaf water potential, generally there were no treatment differences (Figure 2.3.6). In weeks 26, 29 and 30, pre-dawn water potential of well-watered plants was higher than in drought-stressed plants (Figure 2.3.6). In week 26, pre-dawn leaf water potential was measured the night before and the night after irrigating the well-watered plots. Before irrigation, pre-dawn water potentials were similar, but after irrigation the pre-dawn water potential of the well-watered plants was significantly higher ($p < 0.0001$, $F_{(1,10)} = 33.96$), by 0.05 MPa than of the drought-stressed plants. In week 29, drought-stressed plants had not been irrigated for 17 days when measured pre-dawn and soil moisture had been low for this period in drought-stressed treatments (

Figure 2.3.1). Hence, the difference in pre-dawn water potential between irrigation treatments in this week is a result of low water availability in drought stressed plants. In week 30, all plots were irrigated the day before measurements. Thus, there was an immediate response in pre-dawn water potential to an irrigation event in well-watered plants, but a decrease in drought stressed plants only became apparent after a prolonged period at low soil moisture. In week 32, compacted treatments had higher pre-dawn water potentials than uncompacted treatments. Daytime leaf water potential did not differ between treatments throughout the whole season, with values between -0.9 and -1.4 MPa (Figure 2.3.6B). Thus, pre-dawn leaf water potential better discriminated the treatments than daytime leaf water potential.
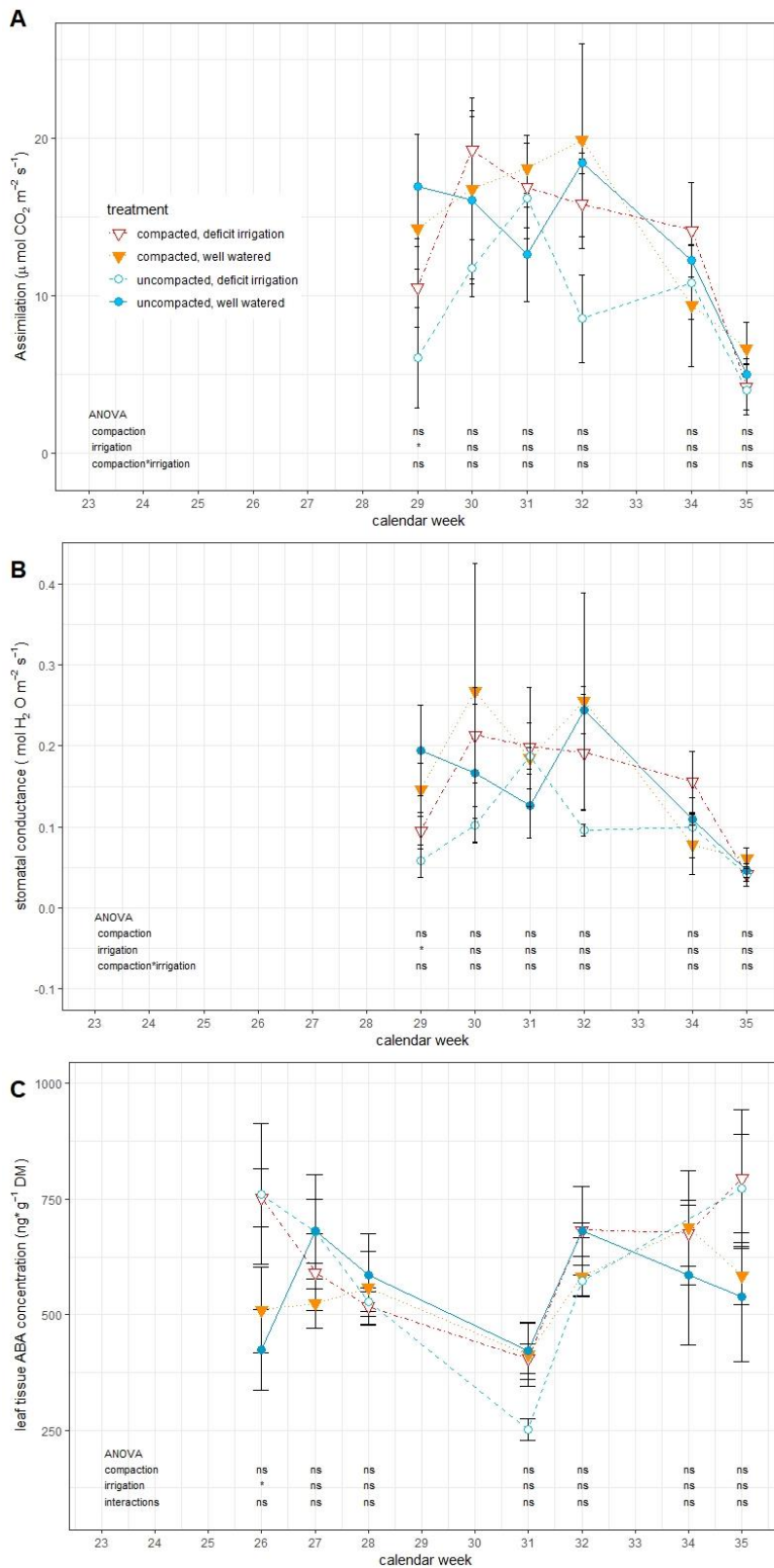
Figure 2.3.5: Stomatal conductance (A), photosynthesis rates (B) and leaf tissue ABA levels (C) measured in field grown Maris Piper under different compaction and irrigation treatments. Means ± SE of 4 plants per treatment and measurement day with residual degrees of freedom df = 8 for stomatal conductance and photosynthesis rate and df = 6 for leaf tissue ABA levels. Asterisks indicate significant differences between treatments on a distinct day with: ns = not significant P > 0.05; * P < 0.05, ** P < 0.01, *** P <0.001.
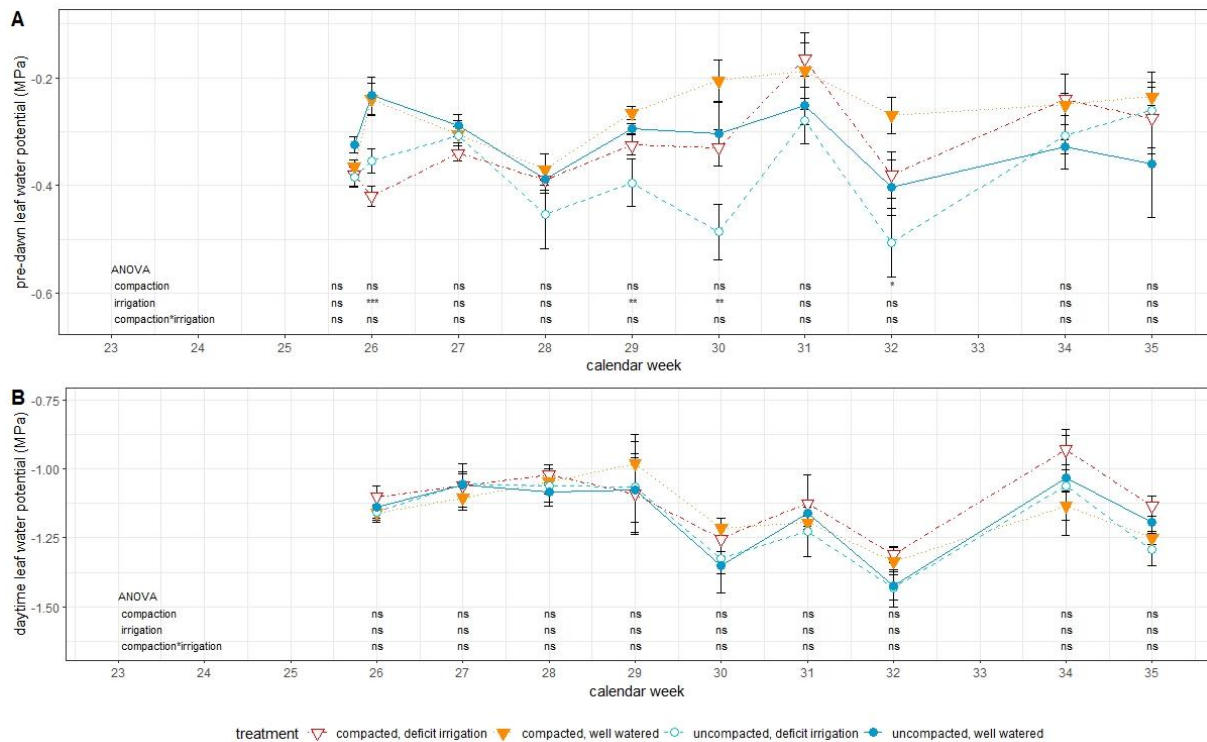
Figure 2.3.6: Development of pre-dawn (A) and daytime (B) leaf water potential of potato 'Maris Piper'. Means ± SE of 4 plants per treatment and measurement day with residual degrees of freedom df = 10. Asterisks indicate significant differences between treatments on a distinct day with: ns = not significant P > 0.05; * P < 0.05, ** P < 0.01, *** P <0.001.

To understand hormonal responses, leaf tissue ABA levels were measured from samples taken directly after gas exchange measurements. In week 26, samples were taken the day after well-watered plants were irrigated for the first time and ABA levels in drought stressed plants were 60 % higher than in well-watered plants with mean values of 758 ng ABA*g^{-1} DM and 473 ng ABA*g^{-1} DM for drought-stressed and well-watered treatments, respectively (Figure 2.3.5C). On all following measurement dates, no treatment differences were detected (Figure 2.3.5C). Drought-stressed treatments had the highest values in weeks 26 and 35. Comparing the treatments via repeated measures ANOVA (Supplementary Table 2.1) showed no significant impact of any factor, likely due to the high variability of the data. Thus, any treatment differences in leaf ABA concentration were transient and not maintained through the growing season.

Tuber yield

Reduced plant growth and therefore lower biomass at full ground cover was correlated with lower tuber yield per hectare (Figure 2.3.7). Soil compaction and drought decreased the yield by 31 % and

had synergistic effect of co-occurring stresses (Figure 2.3.8A). These findings illustrate that drought stress and soil compaction substantially decrease yield and that a large proportion of this variation can be explained by canopy growth (R^2 = 0.71, Figure 2.3.7).
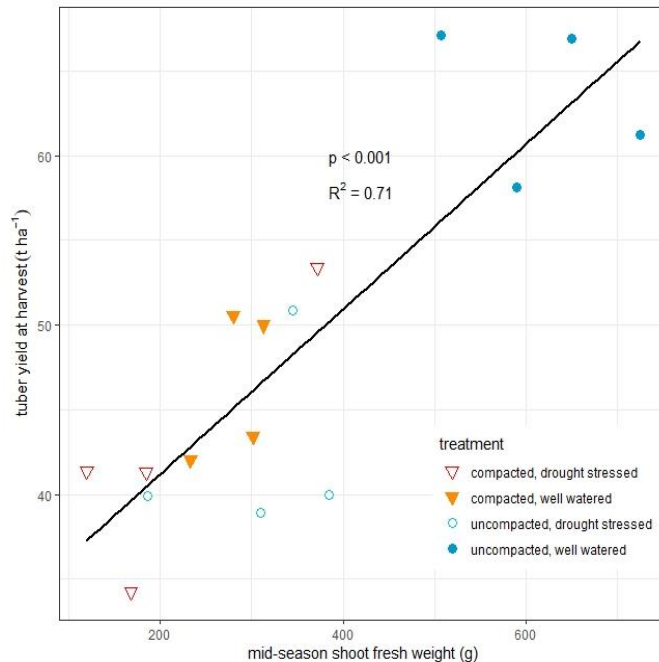
Figure 2.3.7: Relation between biomass at full ground cover (measured in calendar week 31) and final yield for field-grown 'Maris Piper'. Each data point represents one plot with three plants harvested for above ground biomass and yield calculated as in from 10 plants harvested per plot (2.5m²). Regression line was calculated using a linear model (y = 0.05 (± 0.0008) x + 31.42 (± 3.24)). Error bars omitted for clarity, residual standard error = 5.7 on 14 degrees of freedom.

Interestingly, significant differences in tuber size distribution were observed between irrigation treatments, but not between compaction treatments. Drought-stressed plots had more tubers between 30 mm and 50 mm and fewer tubers >60 mm ($p < 0.05$ for all comparisons) than well-watered plots (Figure 2.3.8B). Total tuber number was also higher in drought-stressed treatments than in well-watered treatments (28 tubers per m² vs. 24 m² tubers respectively, p = 0.036, $F_{(1,12)}$ = 5.57). Hence, lower yield of the drought-stressed treatments resulted from smaller, rather than fewer, tubers.
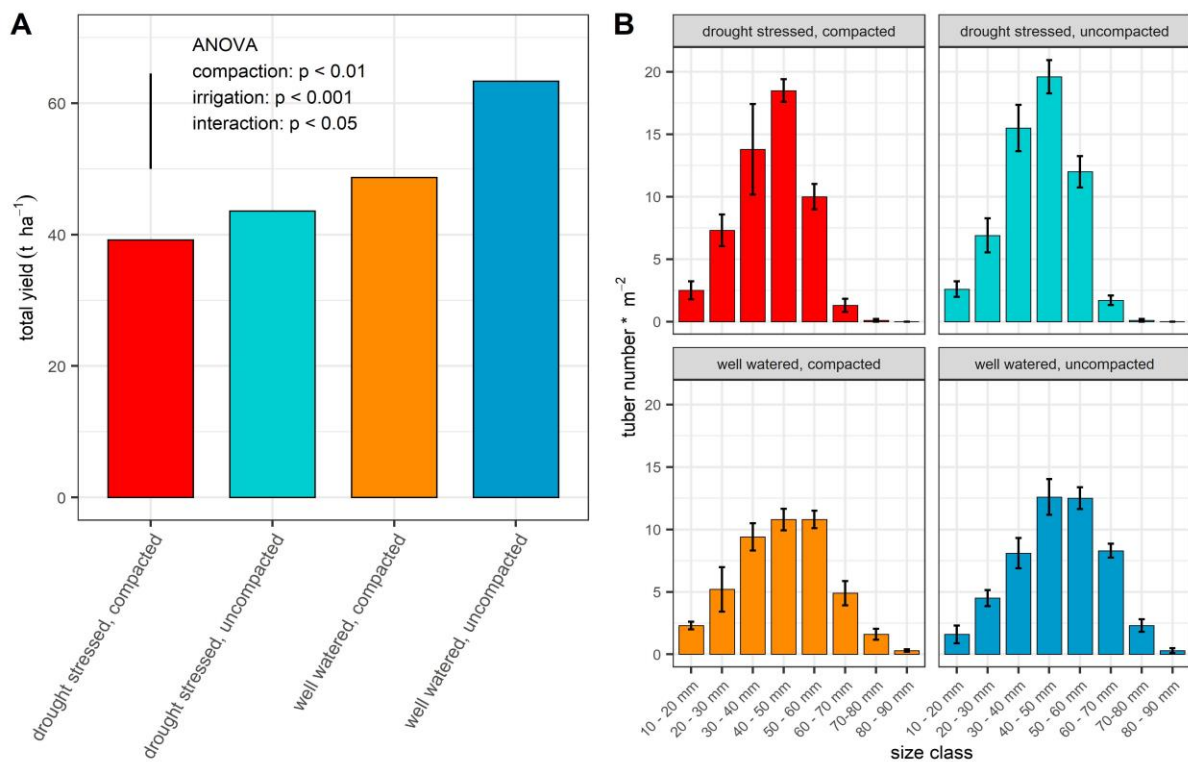
Figure 2.3.8: Total yield (A) and tuber size distribution (B) of field grown 'Maris Piper'. Means (± SE) of four plots per treatment, yield calculated and tubers counted of 10 plants per plot (2.5m²). Vertical line in (A) shows LSD (5%), residual degrees of freedom = 12.

2.4. Discussion

This is the first study to measure canopy cover and leaf gas exchange of a field grown crop weekly under a factorial combination of drought and soil compaction. Ground cover quickly increased under optimal conditions, but was limited by deficit irrigation and especially soil compaction (Figure 2.3.3), whereas leaf gas exchange was similar in all treatments throughout the growing season. Slower leaf growth rate and leaf initiation in the beginning of the season reduced leaf area and thus light interception, thereby diminishing whole plant carbon gain and therefore total yield. In potato, the duration for which full ground cover is maintained explains a high percentage of yield differences (74 – 87 %) among cultivars and treatments (Boyd et al., 2002). Since there were only small differences in late-season senescence between treatments (Figure 2.3.3), the time to reach full ground cover seems more important. Indeed, early-season biomass explained 71 % of the variation in final yield, with soil compaction, deficit irrigation and their combination decreasing yields similarly by 31 % (Figure 2.3.7). It has been established that the duration of light interception determines final yield (Haverkort & Struik, 2015) and full irrigation is necessary until after tuber initiation phase to ensure high yield (Jensen et al., 2010). While this indicates the importance of early season shoot development

in yield formation, to our knowledge the correlation between biomass at full ground cover and yield has not been reported before in potato. This finding could have an important impact on irrigation scheduling and crop management throughout the season to save water resources and maintain or increase yield per hectare. Therefore, it is important to understand the physiological mechanisms regulating canopy expansion and leaf gas exchange.

Decreased canopy growth can result from fewer leaves or reduced leaf expansion or both. Drought decreased leaf number in potato (Fasan & Haverkort, 1991;Figure 2.4) while compaction inhibited tillering and thus leaf initiation in wheat (Jin et al., 2015). Moreover, leaf expansion was inhibited by drought in potato (Obidiegwu et al., 2015) and by soil compaction in sunflower (Andrade et al. 1993). Before full ground cover was reached, leaf expansion rate decreased in the order: control > the two single stress treatments > the combined soil compaction and drought stress treatment (Figure 2.3.4A). This reflects ground cover measurements over the same period (calendar week 24 to 29). However, ground cover curves for the different treatments diverge further after irrigation treatments were imposed in week 24 (Figure 2.3.3). This is probably because the deficit-irrigated treatments produced fewer new leaves after week 26. Differences in ground cover development before week 31 (when maximum ground cover was reached) resulted from differences in leaf expansion and number of leaves; thereafter leaf expansion rates did not differ between treatments (Figure 2.3.4B). Leaf number increased until week 33 and then remained constant (Figure 2.3.4C). After reaching maximum ground cover, the canopy continues to develop, which may enhance light interception slightly, but not considerably.

Restricted shoot growth under drought stress and soil compaction has often been associated with increased ABA levels (Mulholland et al., 1996; Sharp, 2002), but ABA-deficient mutants show less growth especially under these conditions (Hussain et al., 2000; Aroca et al., 2008). In potato, deficit irrigation only increased leaf tissue ABA levels in one week (week 26, Figure 2.3.5C), thus an impact of ABA levels on plant growth in this experiment is unlikely. Furthermore, ABA limits synthesis of another growth inhibitor, ethylene (Sharp, 2002; Hussain et al., 2000). Subjecting part of the root system to either soil drying (Sobeih et al., 2004) or soil compaction (Hussain et al., 2000) increased ethylene biosynthesis and limited leaf growth rates in tomato, but not in a transgenic tomato (ACO1$_{AS}$) with low stress-induced ethylene biosynthesis, but similar ABA levels as wildtype plants. While these observations indicate the importance of ethylene, reduced gibberellin (GA) biosynthesis limits shoot growth under soil compaction (Coelho Filho et al., 2013) and drought stress decreases expression of GA biosynthesis genes while increasing expression of GA deactivation genes (Colebrook et al., 2014). Hence, it is possible that ABA, ethylene and GA all interact to regulate shoot growth when plants grow in dry and/or compact soil.

Together with light interception, plant gas exchange is important for total plant carbon gain and therefore the plant's capacity to grow tubers. Deficit irrigation, but not soil compaction, decreased stomatal conductance (g_s) in factorial experiments with wheat (Whalley et al., 2006) and maize (Tardieu et al., 1992). Potato responded similarly, with deficit irrigation only significantly decreasing g_s and assimilation rate (both $p < 0.05$, F-Test, calendar week 29) after 17 days without irrigation (Figure 2.3.5B), confirming previous experiments (Liu et al., 2006; Ahmadi et al., 2010a). Overall, leaf gas exchange was similar between treatments throughout most of the season and leaf-level carbon gain was thus similar in all treatments. Nevertheless, g_s was relatively low (generally < 0.4 mol m^{-2} s^{-1} (Fig. 5B) compared to other studies that report values above 0.5 mol m^{-2} s^{-1} for well-watered plants (Liu et al., 2006; Ahmadi et al., 2010a; Puértolas et al., 2014). When vapour pressure deficit (VPD) increased from 0.7 to 1.5 kPa around potato leaves (McAdam et al., 2016b), g_s rapidly declined to approximately the same values reported here. In the present study, although VPD was high (0.9 – 1.3 kPa) on most measurement days, it only explained 2 % of the variance in g_s ($R^2 = 0.02$, $p = 0.004$). Since measurements within the same VPD range showed no effect on potato gas exchange (Ahmadi et al., 2010a), other factors such as plant hormones may influence g_s under low plant water availability.

Increased leaf xylem sap ABA levels correlated with decreased g_s in potato (Liu et al., 2005), tomato (Thompson et al., 2007) and soybean (Castro et al., 2019) in controlled environments. However, no such correlation occurred in field-grown potatoes (Ahmadi et al., 2010a). Similarly, leaf tissue ABA levels and g_s were not correlated ($R^2 = 0.06$, $p = 0.07$), with similar ABA accumulation between treatments due to higher ABA accumulation in the well-watered plants or limited ABA accumulation in deficit-irrigated plants. Leaf xylem sap ABA concentration correlates with soil moisture in pot-grown tomato (Dodd, 2007) and sunflower (Dodd et al., 2008). However, preferential water uptake from moister parts of the soil profile attenuates any effect of localised root ABA accumulation in potato, thereby minimising or eliminating root-to-shoot ABA-signalling (Puértolas et al., 2015). Here, soil moisture was measured at 25 cm depth, but the roots of field-grown potatoes can grow as deep as 80 cm to access water (Stalham & Allen, 2004; Puertolas et al. 2014). Thus, plants likely accessed water at deeper layers and therefore the measured soil moisture does not reflect total plant water availability. Alternatively, high VPD (1.5 kPa) can stimulate foliar ABA accumulation in well-watered plants (McAdam & Brodribb, 2015), but there was no correlation between VPD and leaf ABA levels in the present study ($R^2 = 0.02$, $p = 0.26$). Alternatively, stability of ABA levels in deficit irrigated plants suggests that (deeper) roots acquired sufficient water to prevent leaf water deficit (Fig. 6).

Leaf water potential is highest before dawn and with plants in the dark for several hours at considerably decreased transpiration rates (Ramírez et al., 2018) pre-dawn leaf water potential measurements indicate soil water availability. In contrast to the relative stability of daytime leaf water

potential (Ψ_{day}), deficit irrigation decreased pre-dawn leaf water potential ($\Psi_{pre-dawn}$) only after prolonged times without irrigation (Figure 2.3.6) as in maize (Tardieu et al., 1992). Decreasing $\Psi_{pre-dawn}$ correlates with stomatal closure in different crops. However, potato showed small changes in stomatal conductance (0.12 mol m^{-2} s^{-1} g_s difference with $\Psi_{pre-dawn}$ between -0.4 and -0.1 MPa) compared to crops such as soybean (0.44 mol m^{-2} s^{-1} g_s difference over a similar $\Psi_{pre-dawn}$ range) and sunflower (0.54 mol m^{-2} s^{-1} g_s difference with $\Psi_{pre-dawn}$ between -0.68 and -0.25 MPa) (Granier & Tardieu, 1999). Thus, decreased $\Psi_{pre-dawn}$ in potato does not necessarily result in measurable stomatal closure. Taken together, while treatment differences in leaf gas exchange were not detected, understanding the substantial growth differences requires further investigations of plant water relations and hormone signalling effects.

Under drought stress, more potatoes fell into small size grades than under well-watered conditions, as in a study of 103 potato cultivars (Aliche et al., 2019). Since drought, but not soil compaction, affected tuber size distribution (Figure 2.3.8B), tuber development seemed to respond to systemic stress signals rather than local soil conditions. However, soil resistance did not differ between treatments in the top 20 cm, where most potatoes grow (

Figure 2.3.2), so the direct impact of high soil resistance on tuber growth could not be examined. When drought stress restricted canopy growth, the available assimilates were distributed between a larger number of tubers (Figure 2.3.8B), producing a skewed tuber size distribution. Some potato cultivars undergo a second phase of tuber initiation under well-watered conditions (Walworth & Carling, 2002), which might have occurred in the second half of the season, when soil moisture increased following rainfall (week 33 and thereafter). The time until harvest would have been shorter for these tubers, hence the final tuber size of potatoes initiated in the second wave was smaller, leading to many small tubers in deficit-irrigated plants. In addition, an interaction between ABA and GA has been suggested to regulate tuberization and therefore tuber size distribution under drought stress (Jensen et al., 2010). Further research is needed to understand how tuber size is regulated.

To conclude, soil compaction and drought stress applied individually decreased shoot growth and yield, but both stresses occurring simultaneously had synergistic effect on yield (Figure 2.3.8A). Shoot biomass at full ground cover adequately predicted final yield ($R^2 = 0.71$, $p < 0.001$), indicating that vegetative growth in the first half of the growing season is critical in ensuring yield. This finding can be of great importance for crop management and irrigation scheduling. Since leaf gas exchange was not correlated with yield, leaf water status or ABA status, we conclude that plants under restricted water availability grow deeper roots to access water in deeper layers. Moreover, hormonal signals from the root system are postulated to restrict shoot growth sufficiently to ensure it can be sustained according to the available water supply. Further research is needed to test these hypotheses.

3. Moderate water stress impairs potato tuber growth, which resumes upon re-watering

This content is under embargo and will be published in 2023

4. Do strigolactones mediate stomatal drought stress responses?

Graphical Abstract
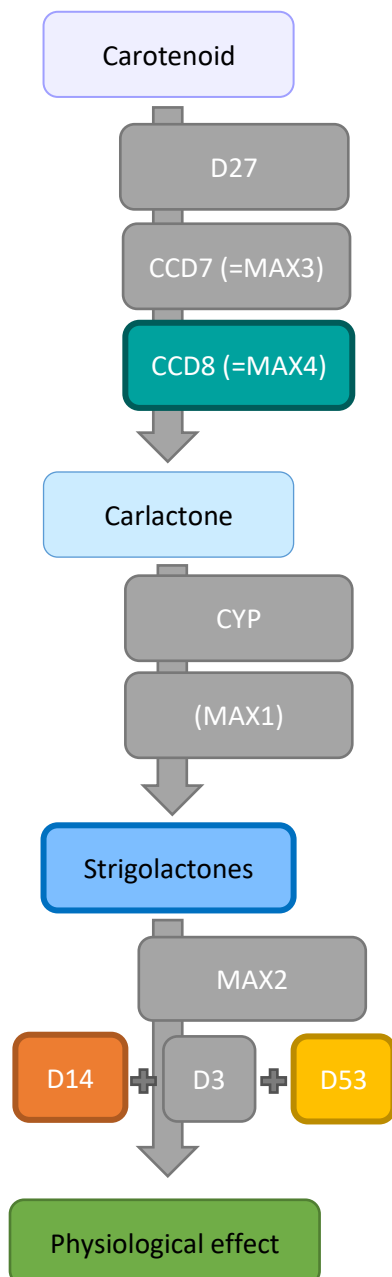
4.1. Introduction

Strigolactones (SL) are phytohormones that are released into the rhizosphere by a host plant and facilitate germination of parasitic weeds as well as symbiosis with arbuscular mycorrhiza (Akiyama et al., 2005; Saeed et al., 2017; Cook et al., 1966). SL can be produced in the root and the shoot (Cook et al., 1966; Ruyter-Spira et al., 2013; Arite et al., 2007) although expression of the SL-biosynthesis genes CCD7 and CCD8 (carotenoid cleavage deoxygenases) was much higher in tomato roots than in shoots (Visentin et al., 2016), suggesting that the root is the main site of SL-biosynthesis. In addition to being root-borne and active in the rhizosphere, SL are xylem mobile (Kohlen et al., 2011) and have an impact on shoot architecture and physiology (Saeed et al., 2017). SL-deficiency increases shoot branching in *Arabidopsis*, pea, potato and rice (Gomez-Roldan et al., 2008; Pasare et al., 2013; Umehara et al., 2010), increases stomatal conductance in tomato and *Lotus* (Visentin et al., 2016; Liu et al., 2015) and increases stomatal density in *Arabidopsis* (Ha et al.,

Figure 4.1.1: Strigolactone biosynthesis and signalling pathway with important enzymes. Adapted from Ruyter-Spira et al., 2013 and Zhou et al., 2013. MAX3 and 4 were identified in Arabidopsis and are homologues to CCD7 and CCD8.

2014) under non-stress conditions. These changes in SL-deficient mutants increase transpirational water loss. Furthermore, applying exogenous SL (GR24) lead to stomatal closure in excised *Arabidopsis* wildtype leaves (Lv et al., 2018). Since SL are mainly synthesized in the roots, but reduce transpiration through stomatal closure, they could play a role in root-to-shoot signalling under drought.

SL are derived from a ß-carotene that is transformed through enzyme activity (D27, CCD7, CCD8) into carlactone and then further converted into 5-deoxystrigol (a precursor for all other strigolactones) by cytochrome P450 (CYP) and possibly MAX1 (Ruyter-Spira *et al.*, 2013, Figure 4.1). Strigolactone signalling is then mediated by MAX2, D14, D3 and D53, leading to the morphological and physiological phenotypes (Ruyter-Spira *et al.*, 2013; Zhou *et al.*, 2013, Figure 4.1). The present study uses genotypes with a knock-down in SL biosynthesis (ccd8) (Pasare et al., 2013), a knock-out in SL signalling (d14) (Arite et al., 2009) and a knock-down in the D53 enzyme (d53), which causes increased sensitivity to SL (Jiang et al., 2013).

In recent studies, the impact of ABA on SL biosynthesis and SL signalling was investigated using hormone mutants and biosynthesis inhibitors, concluding that drought stress induces transcription of SL biosynthesis genes (Ha et al., 2014) and SL are needed for stomatal closure under drought stress or in response to ABA (Bu et al., 2014; Ha et al., 2014). An ABA-deficient tomato mutant (*notabilis*) had decreased SL levels in root tissue (López-Ráez et al., 2010) and a SL deficient mutant (*Slccd8*) showed decreased ABA levels in leaf tissue (Torres-Vera et al., 2014), suggesting interdependence between ABA and SL biosynthesis.

In contrast, different authors disagree whether SL act in an ABA-dependent or ABA-independent manner. Detached leaf assays in tomato and *Lotus* showed that SL-deficient plants (*Slccd7* and *Ljccd7*) treated with ABA (leafy twig in water with added ABA) closed their stomata less or later than the corresponding wildtype (Liu et al., 2015; Visentin et al., 2016), suggesting that SL increase stomatal sensitivity to ABA. According to these results, SL-related decrease in stomatal conductance seems ABA-dependent. However, ABA-deficient *Arabidopsis thaliana* mutants (max1, max3, max4) showed a stomatal closure similar to the wildtype when treated with GR24, a synthetic SL (Lv et al., 2018). Thus, the impact of SL on stomatal aperture can also be independent of ABA. Treating *Arabidopsis* leaves with both GR24 and ABA caused greater stomatal closure than either hormone applied in isolation (Lv et al., 2018), suggesting an additive effect of the two hormones on stomatal closure. The authors suggested independent signal transduction pathways for SL and ABA, but both eventually influence the slow-type anion channel (SLAC1), a K^+-ion pump that influences water efflux from the guard cells (Lv et al., 2018). While this hypothesis explains signalling responses to ABA, SL and their combination, it does not consider any possible evolutionary benefit of developing two independent

signalling systems with signalling molecules derived from the same precursor, which ultimately target the same ion channel to trigger stomatal closure. Further research is needed to understand the interaction between SL and ABA especially under drought stress conditions.

Few studies have considered the role of SL under drought stress in whole plants, with contradictory results (Visentin et al., 2016; Liu et al., 2015; Marzec et al., 2020). SL-deficient plants of *Lotus* and tomato have higher stomatal conductance and a longer response time to ABA compared to their wildtype under non-stressed conditions. As shoot water potential decreased due to drying soil, the stomatal conductance in the ccd7-silenced line in *Lotus* decreased gradually at a similar rate to the wildtype (Liu et al., 2015), while in tomato the stomatal conductance of the ccd7-silenced line decreased more rapidly than in the wildtype over a similar shoot water potential range (Visentin et al., 2016). Both studies only provide one data point at maximum shoot water potential and stomatal conductance declines with decreasing shoot water potential. Hence, it is not possible to clearly separate the hydraulic effect on stomatal closure from a possible signalling effect. In barley, stomatal conductance in the SL-insensitive mutant (d14) did not differ from the wild-type under well-watered conditions and decreased with drying soil. Stomata of the d14 mutant closed more slowly than the wildtype (Marzec et al., 2020) as the soil dried, but the values were so low (2 - 7 mmol m^{-2} s^{-1}) that their accuracy might be compromised due to the measurement range of the instrument (5 - 1200 mmol m^{-2} s^{-1}). Thus, the role of SL in drought stress responses is ambiguous among different species.

Even within one species, effects of SL on transpiration differ between studies (Bu et al., 2014; Ha et al., 2014). In excised leaves of *Arabidopsis,* the water loss in ccd7 (max3) and ccd8 (max4) mutants was similar to the wildtype, while the max2 mutant lost more water in the same time (Bu et al., 2014). However, in excised rosettes relative water content of all mutants (ccd7, ccd8, max2) decreased quicker than in the wildtype (Ha et al., 2014). This could be due to a higher stomatal density in the mutants or a lower responsiveness of the stomata to ABA in that study (Ha et al., 2014). In summary, it is unclear whether SL affect drought stress responses by inducing stomatal closure directly, or indirectly by altering stomatal density and thus affecting the rate of water loss, or by interacting with the ABA biosynthesis or signalling pathway. It is possible that species differ in the magnitude of these effects. Hence, the present study examines responses of transgenic potato (altered in SL biosynthesis or signalling) to drying soil to bridge the gap between knowledge gained from drought stress experiments in whole plants and additional insights from stomatal density measurements in *Arabidopsis* studies.

In potato, like in many other plants, root water potential decreases much earlier under drought stress than leaf water potential (Liu et al., 2005) and therefore root water potential is a more sensitive

indicator of reduced soil water availability. To understand whether the intensity (mild or severe drought) of soil drying affects the involvement of ABA and SL in drought stress responses, an experiment with slow depletion of soil moisture by whole plants and daily sampling is needed. Measuring leaf and root water potential as well as ABA and SL levels regularly throughout the drying cycle will help understand the physiological significance of hormone accumulation. Recent studies strongly suggest that drought stress signalling affects ABA and SL interactions and stomatal response (López-Ráez et al., 2008; Ha et al., 2014; Bu et al., 2014). However, to date no study has examined the impact of SL levels on ABA biosynthesis. The present study aims to determine the impact of SL on stomatal conductance and ABA levels using transgenic lines altered in SL-biosynthesis and SL-signalling. The potato cultivar 'Desiree' and the corresponding transgenic lines ccd8 (impaired SL biosynthesis, described for potato in Pasare *et al.*, 2013), d14 (impaired in SL-signalling, described for barley in Marzec *et al.*, 2020) and d53 (increased SL signalling, described for rice in Zhou *et al.*, 2013) were monitored under well-watered conditions and after withholding water to determine the impact of SL on stomatal conductance as the soil dries. We hypothesise that compromised SL biosynthesis or signalling enhances transpiration rates compared to the wildtype as the soil dries, while increased SL signalling decreases stomatal conductance under control conditions and drought stress. Whether these postulated differences in transpiration were caused by changes in stomatal density or changes in hormonal signals (such as ABA accumulation) was also assessed.

4.2. Material and Methods

Plant Material and growing conditions

Seed potatoes (*Solanum tuberosum* L.) of the variety 'Desiree' (hereafter called wild-type, WT) and its transgenic lines were kindly provided by Colin Turnbull of Imperial College London. The used lines were a DWARF14 knock-out obtained using a CRISPR-Cas9 construct (hereafter called d14), a CCD8-silenced line using a RNAi construct (hereafter called ccd8, Pasare *et al.*, 2013) with approximately 80 % reduction in gene expression (Pasare et al., 2013) and a DWARF53- silenced line using a RNAi
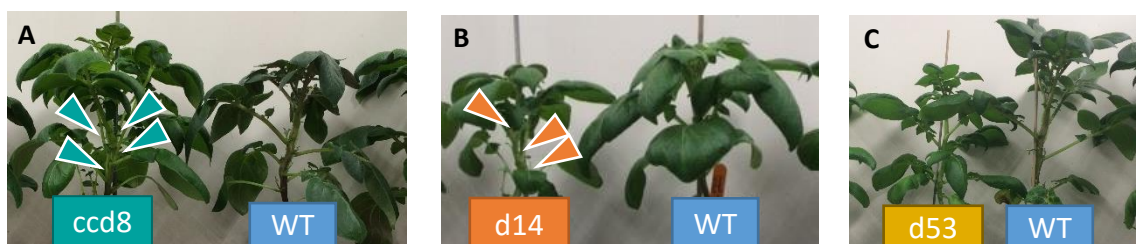
Figure 4.2.1: Phenotypes of strigolactone mutants in potato 'Desiree' and the wildtype (WT) in each of the experiments. Arrows indicate axillary outgrowth. (A) ccd8 mutant with axillary outgrowth, WT without outgrowth, (B) d14 mutant with axillary outgrowth, WT without outgrowth, (C) d53 mutant and WT with axillary outgrowth.

construct (hereafter called d53) with approximately 60 % reduction in gene expression. The transgenic lines were compared to the WT with the respective empty vector construct. In a preliminary experiment the WT and the two empty vectors behaved similarly.

Three experiments under similar conditions were carried out. In each experiment, one transgenic line was compared to the WT (Figure 4.2.1). Tubers were planted at 5 cm depth into cylindrical pots (9 cm diameter x 25 cm height) filled with standard potting compost (John Innes No. 2, Westland Horticulture Ltd, Huntingdon, United Kingdom). Plants were then grown in a controlled environment at 14 h daylength (LED, B100 Valoya, Helsinki, Finland, 250-300 µmol m^{-2} s^{-1}) and 18/22 C (night/ day temperature). The first stem that emerged was retained, but all subsequent stems were excised to ensure uniform, single-stemmed plants that could be inserted in a pressure chamber to measure root water potential. All pots were watered every second day (with tap water) until emergence. After emergence, plants were watered daily with nutrient solution (Miracle grow, half strength, Scotts Miracle-Gro Company LLC, Marysville, USA) to field capacity. When the plants reached 5-7 leaf stage (approximately four weeks after planting), water was withheld from half the plants (drought stressed, d) for seven days while the remainder were watered as described above (well-watered, ww).

Plant physiological measurements and hormone assays

Whole plant evapotranspiration was estimated by placing the pot on a balance at 1 h intervals prior to other measurements and soil moisture was measured concurrently with plant physiological measurements (ML3 Theta-Probe, DeltaT Devices, Burwell, UK) and the average of the top and bottom of each pot was used. Stomatal conductance (g_s) of the abaxial surface of the youngest fully expanded leaf of four plants per treatment and day was measured using a transient time porometer (Model AP4, Delta-T Devices, Burwell, UK). Subsequently a young, still expanding leaf of the same plant was harvested and immediately frozen in liquid nitrogen for hormone analysis. The same four plants per treatment were harvested each day (10am – 5pm) to measure leaf and root water potential (Ψ_{leaf} and Ψ_{root}, respectively) using a Scholander-type pressure chamber, as well as leaf area and fresh mass of the above ground plant parts. After measuring root water potential, 0.3 MPa additional pressure was applied to the root system to collect root xylem sap for 2 minutes.

Abscisic acid (ABA) concentration of root xylem sap was determined by radioimmunoassay (Quarrie et al., 1988). The xylem sap of *S. tuberosum* does not present nonspecific interference in the assay (Liu et al., 2005).

Stomatal density was measured from leaf imprints from leaflets of the youngest fully expanded leaves as previously described (Weyers & Johansen, 1985) using a Microscope with a 10x magnification ocular

lens and 40x magnification objective lens (field of view = 0.0063 mm^2). Means were calculated for n = 5 plants using 5 leaflets per plant and 3 fields of view per leaflet.

Statistical analysis

Statistical analysis was carried out with the software R version 4.0.3 (R Core Team, 2020, Vienna, Austria). Three-way ANOVAs (for main effects of genotype, treatment, measurement day and their interactions) were carried out for each experiment (individual comparison of each genotype with the wildtype). For stomatal density a one-way ANOVA with subsequent Tukey test were carried out to assess differences between genotypes. Regression lines were estimated using linear models.

4.3. Results
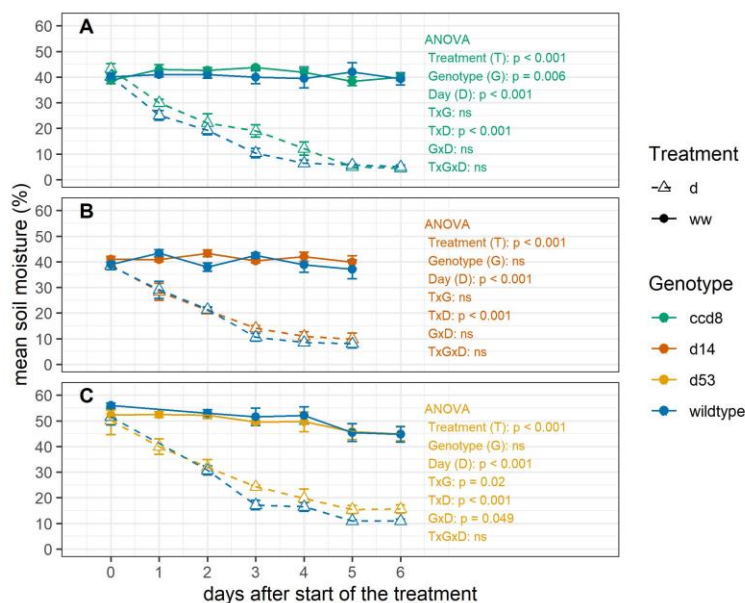
Figure 4.3.1: Soil moisture of well-watered (filled circle) and drought stressed (open triangle) potato plants in three strigolactone (SL) mutants compared to the wildtype. Mean ± SE of 4 plants per treatment, genotype and measurement day.

Daily irrigation maintained mean soil moisture at high levels in well-watered plants. Withholding water continuously decreased soil moisture throughout the measurement period in drought stressed plants of all genotypes (Figure 4.3.1). The ccd8 line had a higher soil moisture on day 3 after the start of the treatment, however the values did not significantly differ between genotypes in the following days (Figure 4.3.1A). The d14 line did not show any differences in soil drying to the wildtype (Figure 4.3.1B). The drought stressed plants of d53 showed a higher soil moisture on day 3 after the start of the treatment than the drought stressed plants of the WT, but values were similar in both genotypes before and after this day (Figure 4.3.1C). Thus, genotypes that were compromised in either SL biosynthesis or signalling dried the soil at comparable rates to the WT.

Stomatal conductance decreased linearly with soil moisture in all genotypes (Figure 4.3.2A). Leaf water potential was maintained at -0.4 MPa at 20 - 50 % soil moisture (Ψ_{soil} = -0.3 - -0.1 MPa, Supplementary Figure 4) and declined rapidly at < 20 % soil moisture (Ψ_{soil} < -0.3 MPa, Supplementary Figure 4) in all genotypes (Figure 4.3.2B). Stomatal conductance declined with leaf water potential as the soil dried, but similarly in the transgenic lines and the WT (p > 0.05 for all individual comparisons). In well-watered plants (Ψ_{leaf} > -0.4 MPa), stomatal conductance was not correlated with leaf water potential (Figure 4.3.2C, R^2 = 0.13). Stomatal conductance decreased linearly with Ψ_{leaf} at < -0.45 MPa in all genotypes (Figure 4.3.2C, R^2 = 0.52). There was no significant difference between genotypes in the response of stomatal conductance to leaf water potential (p > 0.05 for all individual comparisons). Thus, leaf water relations of all genotypes showed consistent response to soil drying.
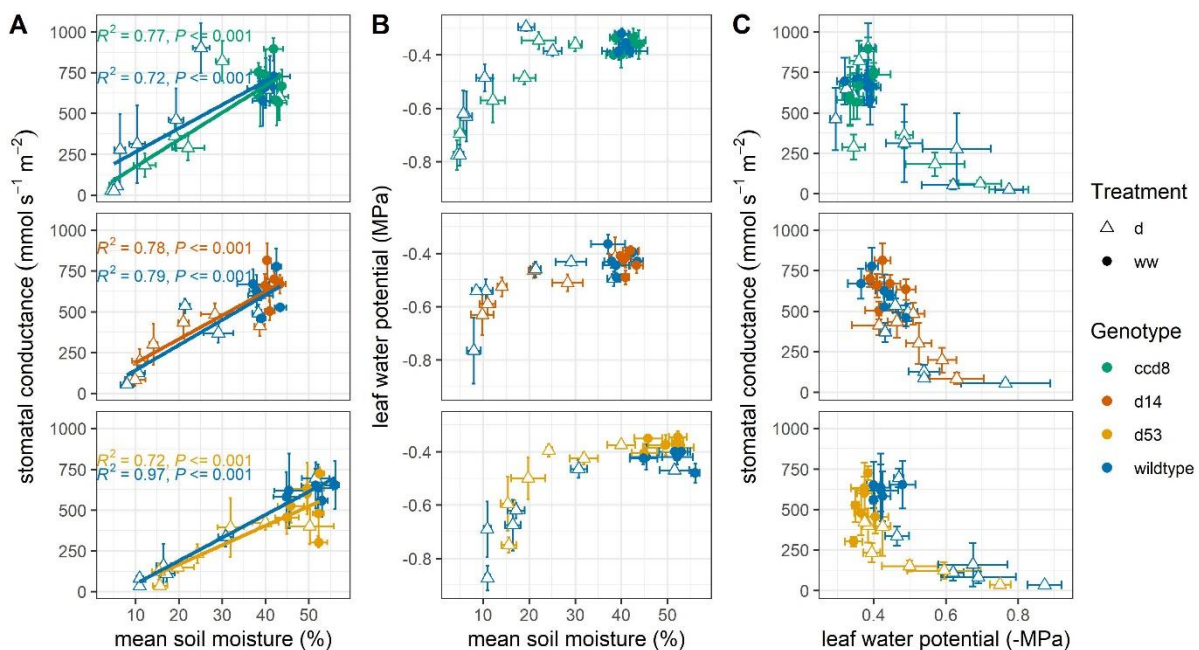
Figure 4.3.2: Correlation between stomatal conductance and soil moisture (A), leaf water potential and soil moisture (B) and stomatal conductance with negative leaf water potential (C) for well-watered (filled circle) and drought stressed (open triangle) potato plants in three strigolactone (SL) mutants compared to the wildtype. Each point represents Mean ± SE of 4 plants per treatment, genotype and measurement day.

Stomatal density on the abaxial side of the leaf was similar in the wildtype and the SL hypersensitive genotype (d53), but lower than the WT in the SL deficient (ccd8) and insensitive (d14) genotypes (Figure 4.3.3A). Adaxial stomatal density was similar between the WT and the SL deficient (ccd8) and SL insensitive (d14) genotypes (Tukey test, p < 0.01), while the hypersensitive genotype (d53) showed a higher stomatal density than all three other genotypes (Figure 4.3.3B). However, adaxial stomatal density was 78 % lower than the abaxial stomatal density for the WT and d53 and 85 % lower than the

abaxial stomatal density for ccd8 and d14, respectively. Thus, abaxial stomatal density has a stronger effect on whole plant transpiration.
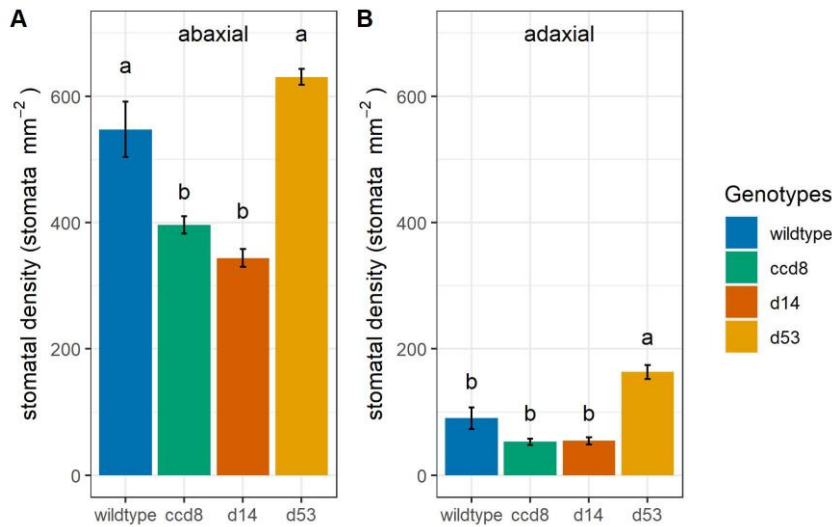
Figure 4.3.3: Number of stomata on the abaxial (A) and adaxial (B) side of the leaf. Means ± SE of 5 plants (n = 5) with 5 leaflets per plants measured at 3 different points. Different letters represent significant differences according to Tukey Test (p < 0.01).

Root xylem sap ABA concentration was similar for the ccd8 silenced line and the wildtype (p > 0.05). In both genotypes the logarithm of the ABA content in the xylem sap increased linearly with decreasing soil moisture without difference between the slopes (Figure 4.3.1A). Thus, there was no difference in ABA export from the roots between the SL deficient line (ccd8) and the WT. Stomatal conductance declined with increasing ABA content (presented in log(pmol ml^{-1}) for clarity at lower concentrations) (Figure 4.3.1B). Slopes of the regression lines did not differ between genotypes (p > 0.05, Figure 4.3.1B). Hence, stomatal response to increased ABA levels is similar in the SL deficient line (ccd8) and the WT.
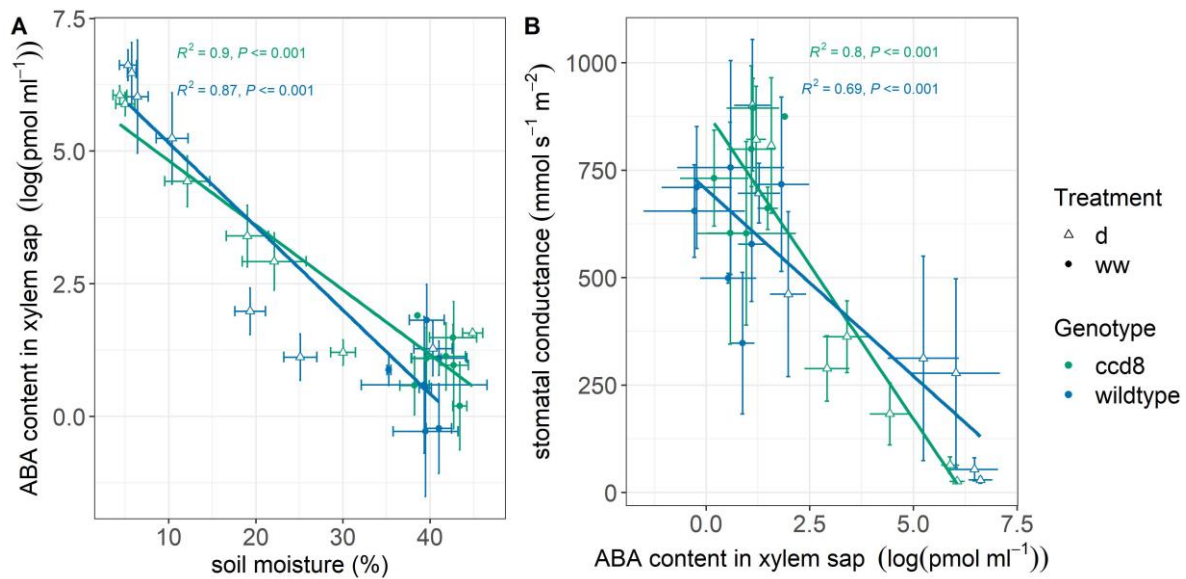
Figure 4.3.1: Relation between (A) soil moisture and logarithm of root xylem sap ABA concentration and (B) logarithm of root xylem sap ABA concentration and stomatal conductance in well-watered (closed circle) and drought stressed (open triangle) plants of the wildtype (blue) and the ccd8 silenced line (green). Each point represents Mean ± SE of 2-4 plants per treatment, genotype and measurement day.

4.4. Discussion

Strigolactones have previously been suggested to be involved in regulating stomatal conductance as the soil dries in various species (Visentin et al., 2016; Liu et al., 2015; Ha et al., 2014; Marzec et al., 2020). *Arabidopsis* mutants impaired in SL biosynthesis or signalling for example, lost water faster than the WT in excised leaf or excised plant assays (Ha et al., 2014; Bu et al., 2014). However, whole plants of a tomato ccd7-silenced line closed stomata more rapidly in response to drying soil than the WT, while whole plants of a *Lotus* ccd7-silenced line subjected to drying soil closed stomata at a similar rate to the WT (Visentin et al., 2016; Liu et al., 2015). Thus, it is not clear to what extent SL are involved in stomatal regulation under drought stress and what other factors (e.g. environment or species) might influence the effect of SL. The present study is the first to explore the effect of strigolactones on stomatal conductance in potatoes under drought stress. There was surprising consistency in the leaf water relations of three genetically modified lines (impaired in SL biosynthesis (ccd8), SL insensitive (d14) or SL hypersensitive (d53)) in response to drying soil, suggesting that SL may have a limited role in regulating water status in potatoes.

The SL deficient and insensitive lines expressed the previously described bushy phenotype (Pasare et al., 2013) with increased axillary shoot outgrowth, while the SL hypersensitive line showed no axillary outgrowth (Figure 4.2.1). These phenotypes confirmed the known genetic alterations in the SL biosynthesis or signalling pathway. In potato, stomatal response to drying soil was similar in WT plants

and genotypes impaired in SL biosynthesis or hypersensitive/ insensitive to SL (Figure 4.3.2A). Soil moisture gradually decreased in all experiments throughout the measurement period (Figure 4.3.1). Thus, all genotypes were subjected to comparable levels of water availability on respective days in the experiment.

Stomatal closure in drying soil is mediated by leaf water status and signals from the root (Tardieu & Davies, 1992). In the present study, stomatal conductance decreased linearly with decreasing soil moisture, while leaf water potential is maintained between 20 – 50 % soil moisture and only decreases linearly at <20 % soil moisture (Figure 4.3.2A and B), suggesting that stomatal conductance may be regulated by leaf water status at <20 % soil moisture and <-0.4 MPa leaf water potential (Figure 4.3.2C). However, stomatal conductance ranged from 250 to 800 mmol m^{-2} s^{-1} at mild drought stress (soil moisture > 20 %, leaf water potential > -0.4 MPa) in these experiments and therefore must be regulated by a different signal. Under rapid soil drying leaf or shoot water potential explains > 90 % of variation in stomatal conductance in ccd7 silenced lines and WT of tomato and *Lotus* respectively (Liu et al., 2015; Visentin et al., 2016), suggesting that stomatal conductance is regulated by leaf water relations. Under well-watered conditions (water potentials close to zero), stomatal conductance in the ccd7-silenced line (impaired SL biosynthesis) was higher than in the WT (Liu et al., 2015; Visentin et al., 2016). *Lotus* maintains this difference between genotypes in drying soil (Liu et al., 2015). In contrast, stomata of the tomato ccd7-silenced line close rapidly as leaf water potential decreases, resulting in stomatal conductance similar to the wildtype (Visentin et al., 2016). Surprisingly, these genotypic differences could not be confirmed for potato, despite being a close relative to tomato. The stomatal conductance of well-watered plants is similar to previously reported ranges of potato stomatal conductance in controlled environment and in the field (300 – 1200 mmol m^{-2} s^{-1}, (Liu *et al.*, 2006). Thus, the control plants were not suffering from water deficit and a similar response to those reported in tomato and *Lotus* (Liu et al., 2015; Visentin et al., 2016) could be expected. Stomatal conductance of all three tested transgenic lines was similar to the wildtype under well-watered conditions and decreased linearly for all genotypes in drying soil without significant differences in the slope of the linear regression. (Figure 4.3.2A). This suggests that SL may not be involved in stomatal closure under drying soil in potato.

At least three possible reasons may account for the results of the present study:

i.   genotypic differences in stomatal density could explain variation in stomatal conductance in other studies,

ii.   SL and other signals for stomatal closure are tightly connected via feedback loops, so that variation in SL biosynthesis/ signalling alters other signals to elicit stomatal closure, which results in a similar stomatal conductance phenotype (Figure 4.4.2),

iii.   SL are not involved in stomatal closure in potatoes under drought stress.

Stomatal density (abaxial and adaxial) was higher in SL deficient mutants than in the WT of *Arabidopsis* (Ha et al., 2014) and stomatal conductance in the same mutants was higher than in the WT (Kalliola et al., 2020), suggesting that the increased stomatal conductance of SL-deficient plants at least partly results from higher stomatal density. In contrast, SL-deficient/-insensitive potato had lower abaxial stomatal density than the WT, with adaxial stomatal density higher in the SL-hypersensitive genotype than in the WT (Figure 4.3.3). Hence, different species seem to express different phenotypes in response to genetically altering the SL pathway and assumptions made from experiments in the model plant *Arabidopsis* may not be valid in other plants. Thus, reporting stomatal densities alongside stomatal conductances is vital for meaningful comparisons. Further studies on a wide range of species are needed to understand relationships between SL and stomatal density and possibly cluster plants into different response groups.

Despite the differences in stomatal density between potato genotypes, no differences in stomatal conductance were observed (Figure 4.3.2). Thus, genotypes with lower stomatal density must have had a wider stomatal aperture (and vice versa), leading to a similar transpiration rate per unit leaf area (= stomatal conductance) in all genotypes. This suggests that internal signals regulate stomatal aperture to prevent excessive water loss. Studies that found higher stomatal conductance in SL-depleted plants under well-watered conditions (Visentin et al., 2016; Liu et al., 2015) may have worked with genotypes that expressed higher stomatal density than the WT and compared the plants under luxury water consumption, with all stomata at maximum aperture. Under these conditions it is possible to observe higher stomatal conductance of the SL-depleted lines, which is then rapidly reduced as the soil dries.

Nevertheless, the regulation of stomatal conductance seems to be closely related to soil water availability, implying long-distance root to shoot signalling. ABA is a long-distance signal that decreases stomatal conductance in potato under drying soil (Liu et al., 2005). Similarly, stomatal conductance was inversely related to root ABA concentration in *Lotus*, with higher stomatal conductance in the ccd7-silenced line compared to the WT at the same root ABA concentration (Liu et al., 2015), indicating that stomata of the SL deficient line are more sensitive to endogenous ABA than the WT. Stomatal conductance in potato decreased with increasing root xylem sap ABA concentration (Figure 4.3.1B), but in contrast to the mentioned result in *Lotus*, response of the potato ccd8-silenced line did not
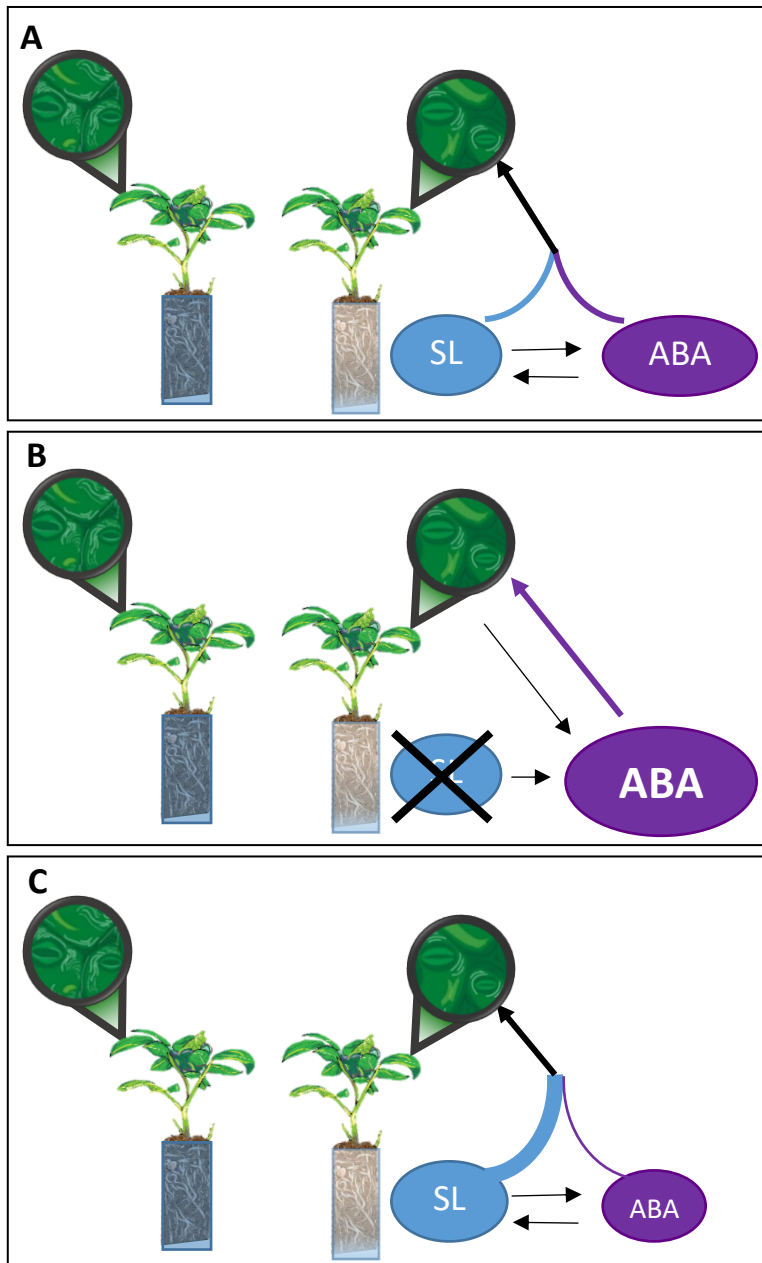
Figure 4.4.2: Hypothetical interactions between SL and ABA in regulating stomatal closure in drying soil in WT (A), SL deficient or insensitive plants (B) and SL hypersensitive plants (C).

differ from the WT. The speed of soil drying and consequently the level of drought stress experienced by the plant differed between the studies with rapid soil drying and severe stress in *Lotus* (Liu et al., 2015) and slow soil drying and initially mild drought stress in potato (Figure 4.3.1), which could explain different responses of SL-deficient genotypes. Moreover, SL-deficient genotypes in different species seem to respond differently to drying soil as discussed in the first paragraph of this section. Note that different carotenoid cleavage deoxygenase genes were silenced in the compared studies (CCD7 in *Lotus*, CCD8 in potato). In SL biosynthesis, these enzymes are both involved in the step of forming carlactone from 9-cis-ß-carotene (Ruyter-Spira *et al.*, 2013, Figure 4.1). However, silencing CCD7 or CCD8 could have different effects on plant responses. For example, CCD7 may be essential in SL biosynthesis while CCD8 may be substituted by another enzyme with a similar function. Alternatively, the by-products of one cleavage step may have an impact on stomatal closure or stomatal sensitivity to ABA, in which case SL as an end product may be of minor importance in drought stress signalling.

ABA and SL derive from the same precursor (ß-carotene) and target the same ion-channel (SLAC1) (López-Ráez et al., 2010; Lv et al., 2018) (Figure 4.4.3). Thus, it is possible that in WT plants both

hormones reduce stomatal conductance under soil drying. In genotypes impaired in SL biosynthesis, there is hypothetically less competition for the precursor ß-carotene and consequently more ABA may be produced, which would lead to stomatal closure and a phenotype similar to the WT (Figure 4.4.2A and B). In plants that are insensitive to SL, SL are still produced, but do not have the physiological effect. Thus, in drying soil the stomata do not close as quickly. This could increase ABA biosynthesis via feedback mechanisms, which may lead to stomatal closure similar to the wildtype (Figure 4.4.2B). This is supported by higher ABA content in excised drying leaves of *Arabidopsis* mutants impaired in SL signalling compared to the WT (Piisilä et al., 2015) (Figure 4.4.3, arrow 1). However, in potato the SL deficient line (ccd8) had similar ABA levels in the root xylem sap as the wildtype (Figure 4.3.1). Thus, for this specific line the hypothesis of compensating SL deficiency by increased ABA export cannot be verified. The reverse mechanism may still be true for SL hypersensitive plants (Figure 4.4.2C). These postulated interactions between SL and ABA assume that substrate competition and feedback from the stomata are regulating the ABA/SL ratio and that ABA and SL have an additive effect on stomatal closure, but can also act singularly. This hypothesis could not be confirmed for ABA levels in root xylem sap. However, it is necessary to investigate ABA levels in root xylem sap of genotypes with impaired or enhanced SL signalling as well and in leaf tissue of all genotypes to best determine whether SL biosynthesis and signalling interact with the biosynthesis of ABA.

In contrast to the above findings, some recent studies found a synergistic interaction between SL and ABA. Compared to the wildtype, *Arabidopsis* mutants impaired in SL biosynthesis or SL signalling showed reduced stomatal closure in response to exogenous ABA (Ha et al., 2014; Lv et al., 2018; Piisilä et al., 2015; Bu et al., 2014) (Figure 4.4.3, arrows 2 and 3), suggesting that SL enhance stomatal sensitivity to ABA. In addition, roots of ABA deficient tomato mutants (*notabilis, flacca, sitiens*) showed reduced transcription of SL biosynthesis genes (CCD7, CCD8) and therefore reduced SL content and reduced ABA content compared to the WT (López-Ráez et al., 2008) (Figure 4.4.3, arrow 4), indicating that ABA biosynthesis is needed for SL biosynthesis. These findings suggest that ABA promotes SL biosynthesis and SL promote ABA signalling efficacy. Analysing SL and ABA concentration of root and shoot tissues of the transgenic lines and WT used in this study could help to determine whether SL biosynthesis or sensitivity affect ABA and SL production in potato in drying soil. Furthermore, supplying detached leaves of these genotypes with ABA through the transpiration stream could confirm if stomatal sensitivity to ABA is altered in SL-depleted, SL-insensitive or SL-hypersensitive potato plants. These suggested studies could clarify the mechanism behind the responses described here.
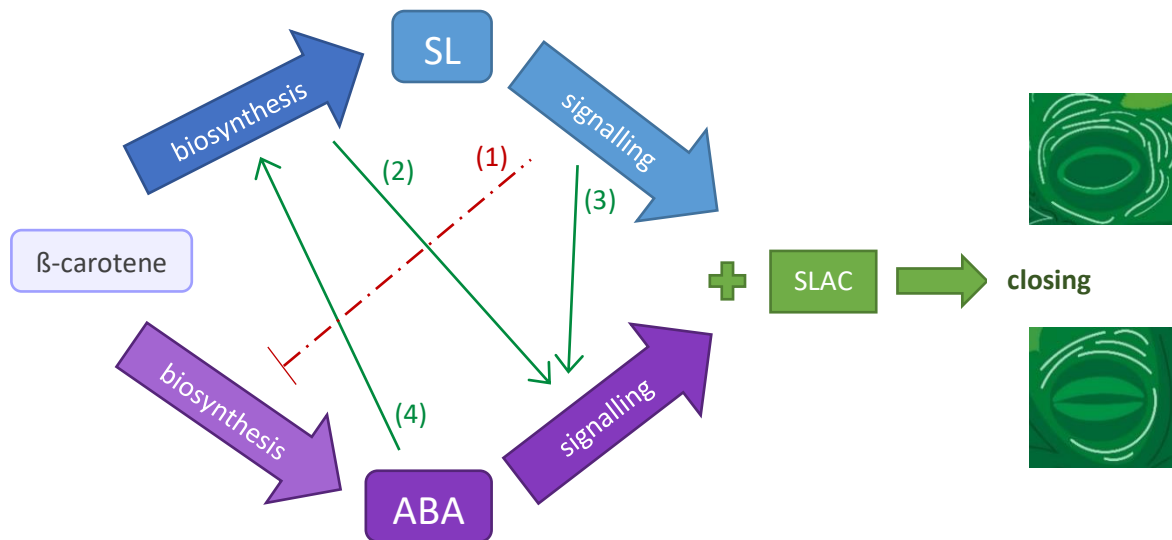
Figure 4.4.3: Previously proven interactions between strigolactone (SL) and abscisic acid (ABA) biosynthesis and signalling pathways. Red bar at 90° angle indicates negative relation (1), green arrows indicate positive relation (2, 3, 4).

(1) Arabidopsis mutants impaired in SL signalling (max2) accumulate more ABA in excised leaves as they dry out than the WT (Piisilä et al., 2015).

(2) Arabidopsis mutants impaired in SL biosynthesis (ccd7 and ccd8) close stomata less than the WT when treated with ABA (Ha et al., 2014; Lv et al., 2018).

(3) Arabidopsis mutants impaired in SL signalling show reduced sensitivity to ABA - stomata wider open than the WT when treated with ABA (Piisilä et al., 2015; Bu et al., 2014, Lv et al., 2018).

(4) Roots of ABA-deficient tomato mutants (notabilis, flacca, sitiens) showed reduced CCD7 and CCD8 transcription (Lopéz-Ráez et al., 2010).

4.5. Conclusions

Three independent experiments showed that stomatal conductance of potato in drying soil is neither altered by the ability to produce SL nor by the sensitivity to SL. However, impaired SL biosynthesis or signalling decreases stomatal density and SL hypersensitivity increases stomatal density. It is unclear whether this is the result of more stomatal cells per epidermis cell or due to slower epidermal cell expansion. These results contradict previous findings in other species and suggest that the involvement of SL in regulating stomatal conductance in drying soil may be more complex than previously assumed. The responses shown for potato differ markedly from those shown for tomato, *Lotus* and *Arabidopsis*. This indicates that SL responses are either species dependent or dependent on other environmental factors that unintentionally differed between different studies. Further studies with several species grown under the same environmental conditions or different genotypes of the same species grown in a range of different environmental conditions could help to understand the interaction Genotype x Environment x Hormone response.

5. General discussion

Reduced plant water availability affects potato crops differently at individual developmental stages (Jensen et al., 2010). In the first month after emergence, reduced water availability restricts potato shoot growth (Figure 2.3.3), but not root growth (Stalham & Allen, 2004). After tuber initiation (see Figure 1.3.1), irrigation may be reduced by 30 % until canopy senescence without yield penalties (Jensen et al., 2010). This thesis examined the effect of drought stress and soil compaction on field-grown potatoes throughout a whole season and on final yield (Chapter 2), with plants under deficit irrigation producing more, but significantly smaller, tubers (Figure 2.3.8). These field results stimulated *in-vivo* measurements of tuber volume growth and water fluxes at bulking stage in relation to shoot and soil water status using magnetic resonance imaging (MRI) (Chapter 3). Water influx overnight allowed tuber volume growth, which ceased the first night that water was withdrawn (Chapter 3). Despite changes in shoot growth and yield, plant gas exchange and photosynthesis rates did not differ between treatments in the field for most of the season (Figure 2.3.5), with homeostasis of leaf water potential and foliar ABA concentration which have traditionally been associated with stomatal closure (Figure 2.3.5, Figure 2.3.6). Thus, it was explored whether the hormone group strigolactones (SL) are involved in regulating stomatal conductance when drought stress was imposed in controlled environment conditions (Chapter 4).

5.1. Isohydric potatoes:

mild soil drying restricted leaf gas exchange to maintain leaf water potential

Soil compaction and deficit irrigation reduce plant water availability by restricting root growth (Stalham et al., 2007) or decreasing soil water content (

Figure 2.3.1) respectively. In field-grown potato 'Maris Piper', both stresses delayed canopy closure by reducing leaf initiation and decreasing leaf expansion rates in the vegetative growth phase (Chapter 2). A smaller leaf area decreases whole plant water loss allowing adaptation to reduced water availability. Reduced leaf growth can either be due to reduced turgor and hence reduced cell elongation or due to hormonal imbalance inhibiting shoot growth. In just-germinated maize seedlings, increased ABA levels under drying soil reduced shoot elongation compared to ABA deficient mutants (Saab et al., 1990). However, uncoupling the hormonal effect from the water deficit effect by using specific maize mutants showed that leaf elongation rates decreased linearly with pre-dawn Ψ_{leaf} with little impact of genotypic variation in ABA status (Voisin et al., 2006). Thus, in drying soil reduced cell turgor and hence reduced cell expansion rates limit leaf growth, which delays canopy closure. The

duration of full light interception determines yield (Haverkort & Struik, 2015) and accordingly treatments with later canopy closure produced a lower tuber fresh mass (Chapter 2).

Stomatal closure can also limit photosynthesis rates and therefore plant carbon gain (Ahmadi et al., 2010b). Potato exhibits an isohydric strategy in drying soil with homeostasis of leaf water potential (Obidiegwu et al., 2015; Liu et al., 2005). Hence chemical and hydraulic signals from root to shoot strongly regulate transpirational water loss as the soil dries (Limpus, 2009). Leaf water potential in potato is maintained by early stomatal closure under mild drought stress (Figure 4.3.2A-C). It has been suggested that drought stress elicits a hydraulic root-to shoot signal to induce stomatal closure, but currently available methods are not able to detect the change in shoot water potential due to a lack of sensitivity (McAdam et al., 2016b; Merilo et al., 2018). In view of marginal decreases in water potential (Chapter 4, Figure 4B), root-derived hormones like ABA are more likely to be the main drivers of stomatal closure (Thompson et al., 2007; McAdam et al., 2016b) (see also Chapter 1, Section 1.2). In the absence of more sensitive measurement methods for leaf water potential, these considerations remain hypothetical. Since early stomatal closure in response to mild soil drying is not correlated with a measurable decrease in leaf water potential in the present study (Figure 4.3.2A and B), a hormonal root-to-shoot signal is likely to induce stomatal closure.

ABA is a long established hormonal signal for stomatal closure (Zeevaart & Creelman, 1988) and stomatal conductance of potato is inversely correlated with the concentration of ABA in the root xylem sap as the soil dries (Liu et al., 2005). Furthermore, the ABA deficient potato mutant *droopy* had a higher stomatal conductance than the wildtype even under high VPD and spraying the leaves with ABA or feeding detached leaves with ABA solution via the transpiration stream restored wildtype behaviour (Quarrie, 1982). Thus, ABA can induce stomatal closure in potato. However, stomatal closure in drying soil cannot fully be explained by root xylem sap ABA concentration ($R^2 = 0.86$ for relation between stomatal conductance and xylem sap ABA, Liu *et al.*, 2005), suggesting that other signals may be involved.

Strigolactones were previously identified to be involved in drought-induced stomatal closure (Visentin et al., 2016; Liu et al., 2015; Ha et al., 2014). A SL depleted tomato mutant *Slccd7* had increased root ABA content and showed a stronger decrease in stomatal conductance than the WT as the soil dried (Visentin et al. 2016), indicating that SL may interact with ABA in early drought stress signalling. In contrast, barley seedlings impaired in SL signalling (*Hvd14*) accumulated lower levels of ABA in a rapid dehydration assay compared to the WT (Marzec *et al.*, 2020), suggesting the opposite interaction between SL and ABA under severe stress. However, three strigolactone mutants (ccd8, d14, d53) of the potato variety 'Desiree' did not differ from the wildtype in stomatal responses to drying soil (Figure

4.3.2). ABA levels of the SL deficient line (ccd8) were similar to the wildtype as the soil dried out (Figure 4.3.1). Hence, no influence of SL on stomatal conductance and ABA levels in drying soil was found and previous findings in other species could not be confirmed. Further research is needed to understand potato long-distance signalling in drying soil especially at mild water deficits.

In the field, stomatal conductance only decreased in drought-stressed treatments compared to well-watered treatments on the day after deficit irrigation was first imposed (Figure 2.3.5A). On later measurement dates, the standard deviation of the gas exchange measurements was large (Figure 2.3.5B), decreasing the likelihood to find statistical differences between treatments. During the time course of a day stomatal conductance of potato plants initially increases and then decreases again (Wheeler *et al.*, 2019). These diurnal fluctuations could be the cause for the large standard deviations, since measurements were taken over a whole day. In the field light intensity and vapour pressure deficit (VPD) change throughout the day depending on the angle of the sun and the air temperature. Both parameters cause increased variability in stomatal conductance measurements. However, no clear pattern in mean values is visible with drought stressed plants seemingly having higher stomatal conductance than well-watered plants in the beginning of the season and a lower stomatal conductance later in the season (Figure 2.3.5B).In summary, there is no obvious trend in stomatal conductance after the first watering event. Thus, stomatal closure seems to be an early response to deficit irrigation in the field, augmenting other adaptive mechanisms such as shoot growth inhibition and reduced tuber bulking as soil water deficit continues (Figure 2.3.4, Figure 2.3.8). To uncouple the latter two effects, tuber volume changes were examined over three days of drought stress (Chapter 3). During this time, controlled environment experiments showed no effect of drought stress on shoot growth or leaf area of potato (Supplementary Figure 5).

5.2. Regular water supply sustains tuber volume growth at bulking stage

Potato tubers form around 70 % of the total plant fresh weight at harvest (Saeed et al., 2008), and drought stress reduced final yield (Stalham & Allen, 2004; Saeed et al., 2008). However, very few studies investigated short-term changes in tuber growth when plants were grown in drying soil (Gandar & Tanner, 1976; Baker & Moorby, 1969). Using magnetic resonance imaging, this thesis presents the first *in-vivo* measurements of tuber volume and water content over a drying cycle of three days and nights, with subsequent re-watering (Chapter 3). All tubers of a plant were imaged individually every four hours to regularly measure tuber volume and water content fluctuations.

Tubers are generally regarded as a sink organ, with carbohydrates being allocated to them via the phloem (Aliche et al., 2020b), especially during tuber bulking phase. Nevertheless, the MRI measurements showed a diurnal pattern of water influx (night) and efflux (day) from the tuber (**Error! Reference source not found.**A). In drying soil this pattern resulted in a longer duration of water efflux in the day and consequently lower total water uptake. These water flux patterns lead to an increase in tuber volume overnight in well-watered plants, but withholding water caused tuber growth to cease on the first night of the treatment (**Error! Reference source not found.**B). However, volume growth recovered to values of the well-watered plants after re-watering after 2 days and nights of drought stress. Leaves of *Arabidopsis* grow more during the day than at night, but daytime leaf expansion rates show a stronger decrease under mild drought stress (Dubois et al., 2017; Pantin et al., 2013). More detailed data from *Ricinus communis* shows that leaf growth under well-watered conditions mainly occurs pre-dawn and decreases throughout the day. Increasing drought stress diminishes this pre-dawn peak in growth rates until growth rates are similarly low during the day and night (Schurr et al., 2000). Transferring these results to potatoes, leaves grow predominantly pre-dawn and in the early morning hours when leaf water potential is highest, while tuber growth starts as soon as stomata are closed (and transpirational water loss is limited) or additional water becomes available through irrigation. This indicates that both leaves and tubers rely on hydraulic pressure for cell expansion. Furthermore, the decrease of leaf growth and complete halt of tuber growth under drought suggests that leaf growth may be prioritised over tuber growth, but confirming this suggestion would require concurrent near-continuous measurements of diurnal leaf and tuber growth. Such measurements could better understand possible competition for water resources between above- and below-ground plant organs in potato.

The ability to recover tuber volume upon re-watering is very important for irrigation scheduling. Soil water availability and evaporative demand determine the stress experienced by the plant. The results in this thesis show that re-watering the crop every third day under the prevailing evaporative demand maintained maximum tuber growth rates. Further research is needed to define the severity of drought stress that can be tolerated (lowest soil moisture at a given evaporative demand or highest evaporative demand at given soil moisture) with a full recovery of tuber volume upon re-watering to tailor irrigation strategies in the critical tuber bulking stage. The ability to grow deeper roots and thus access water resources in deeper soil layers or to regulate transpiration through stomatal closure will influence the capacity of a variety to tolerate drought stress conditions. Thus, an experiment with sequential drought stress and re-watering, comparing varieties that differ in root length and stomatal response to drying soil, may provide valuable data for direct application in the field.

Another interesting observation was the hydraulic lift when drought stressed plants were re-watered from the base of the pot (Chapter 3). Increased soil water content in the top layer of the pot (which contained the potatoes) immediately after re-watering was confirmed by the increase in tuber water content in the MRI measurement 3 h after re-watering. However, the soil in the middle layer of the pot remained relatively dry (**Error! Reference source not found.**). Investigating the osmotic potential of the tubers during bulking stage and under drying soil would help to understand driving forces of water flow under these circumstances.

Taken together, the results presented in this thesis suggest that diurnal water fluxes in and out of the tuber allow tuber growth overnight. Furthermore, potatoes of the variety 'Maris Piper' can tolerate mild soil water deficit (in this case for two days and nights) and thereafter recover tuber growth when re-watered. Possible changes in cell wall properties may interact with cellular turgor to determine threshold limits of soil drying, below which tuber growth may not recover after re-watering.

5.3. Early shoot growth determines final yield

Radiation interception is an important factor in potato crop performance and total intercepted radiation correlates linearly with tuber yield (Haverkort & Harris, 1986; Allen & Scott, 1980). During the vegetative growth phase, increased ground cover is linearly correlated with increased canopy light interception until about 80% groundcover, when canopy light interception reaches its maximum (Firman & Allen, 1989). Thus, the duration of full ground cover plays a major role in determining final yield (Haverkort & Struik, 2015). The maturity type of the variety largely determines the timing of crop senescence (CPVO, 2017). However, growers may choose to defoliate the crop prior to natural canopy senescence to advance the date of skin set and therefore reduce damage at harvest (Firman & Allen, 2007). Therefore, increasing the duration of light interception is best achieved by reaching full ground cover early in the season. Indeed, the field experiment described in this thesis supports this finding, with mid-season shoot biomass adequately predicting final yield (Figure 2.3.7). Both investigated stresses (soil compaction and deficit irrigation) reduced shoot growth in the vegetative growth phase and reduced the maximum ground cover value and/ or delayed when this was reached (Figure 2.3.3). Hence, reduced plant water availability (by either restricting root growth or decreasing soil water availability) reduced total light interception and therefore plant carbon gain and hence the capacity to bulk tubers. In consequence, it is important to monitor soil moisture and evaporative demand in potato crops in the vegetative growth stage and tailor irrigation schedules to these parameters in order to achieve early canopy closure.

Interestingly, plants in the deficit irrigated treatments produced smaller, but a larger number of tubers (Figure 2.3.8B). Similarly, a large study of over 100 varieties indicated that overall tuber size distribution was skewed towards a higher number and smaller tubers under non-irrigated conditions compared to the well-watered control (Aliche et al., 2019). These findings suggest that tuber number might be increased by imposing drought stress at tuber initiation stage. Nevertheless, water supply must be resumed at the tuber bulking stage as determined in Chapter 3 to ensure a higher number of tubers of a marketable size (> 40 mm diameter). Note that in the present study two heavy rainfall events in the second half of the season increased soil moisture in the drought stressed and compacted treatment to levels similar to well-watered treatments before the rainfall and remained high for several days (Figure 2.3.1). Soil moisture in the single drought stress treatment increased, but remained lower than well-watered values before the rainfall (Figure 2.3.1). As discussed in Chapter 3, this increased water availability most likely promoted tuber growth. Thus, the rainfall did not exaggerate, but possibly minimise, the differences between treatments. However, the timing of drought stress was not as important as the overall degree of stress in determining drought tolerance of 34 potato varieties (Sprenger *et al.*, 2015). As discussed in Chapter 3, the increased water availability in the second half of the season in the present study most likely promoted tuber growth. Thus, the rainfall did not exaggerate, but possibly minimise, the differences between treatments. Nevertheless, drought stress still decreased tuber size compared to well-watered treatments (Figure 2.3.8). Further research is needed to investigate whether deficit irrigation can be timed to enhance tuber initiation, without limiting shoot growth to attain early canopy closure.

Since the tendency to produce more tubers under drought stress was highly dependent on the genotype (Aliche et al., 2019), screening commercially available genotypes is necessary to provide tailored recommendations for short-term drought treatment at tuber initiation. Furthermore, the intended marketing of the potato tubers should be considered. The variety Maris Piper is grown for many purposes including chipping, oven baking and boiled or mashed potatoes. Chipping and baking potatoes usually require large tuber sizes, so a treatment that produces more, but slightly smaller tubers may not be desirable. In contrast, cooking potatoes in retail often are at the smaller end of the size spectrum and the proposed short-term drought treatment could result in a larger percentage of the yield being marketable for this purpose. In summary, ensuring rapid vegetative growth early in the season by sufficient irrigation is important to achieve maximum yield. However, short-term drought stress treatment at tuber initiation stage could increase tuber number and still provide enough resources for satisfactory tuber bulking. During tuber bulking, irrigation needs to be sufficient to meet plant water requirements again.

5.4. Conclusion

The response of one potato genotype to water deficit can markedly differ between pot grown plants and field experiments. For example, starch yield was generally lower in plants grown in pots with horticultural substrate than in the field, but there was also a significant interaction between the type of experiment (pot or field) and the genotype (Köhl et al., 2021), indicating that the soil type and possibly the environmental conditions can have an effect on the genotypic variability. In hoping to generate results with practical impacts, this thesis started with a field experiment to understand plant physiological responses, then used controlled environment experiments to investigate further questions arising, while using the same variety where possible. Shoot physiological measurements adopted consistent techniques where possible, to ensure comparisons between experiments in plant perceptions of drought stress.

This thesis set out to understand potato physiology and signalling at limited water availability in different phenological stages. This was achieved by

- investigating the role of strigolactones (SL) on stomatal closure under drought stress in vegetative growth stage by comparing transgenic lines impaired in SL biosynthesis or signalling or hypersensitive to SL, compared to the wildtype (Chapter 4)
- using magnetic resonance imaging (MRI) to reveal diurnal changes in tuber volume growth and tuber water content at tuber bulking stage (Chapter 3)
- monitoring field-grown potatoes throughout a whole season from emergence to harvest (Chapter 2) to determine how the plant integrates different processes to determine yields.

Sufficient soil water availability was important in the vegetative growth stage to ensure maximum canopy growth rates and therefore maximum light interception. Avoiding drought stress during tuber volume growth (tuber bulking stage) was also important, although short periods of decreased soil water availability can be tolerated with subsequent irrigation recovering tuber volume. Although potatoes show an isohydric response to mild drought stress, root-derived strigolactones do not seem to be involved in regulating this response. Further research is needed to understand the mechanisms by which mild soil water deficit elicits stomatal closure in potato.

In conclusion, irrigation scheduling needs to be tailored to different developmental stages to achieve maximum yield. A possible irrigation scheme has been outlined here, based on crop phenological stages and evaporative demand, which can be adapted to different varieties and environmental conditions (Figure 5.4.1). This makes the suggestions made here more universally applicable and more flexible than fractions of full irrigation given elsewhere (Jensen et al., 2010).
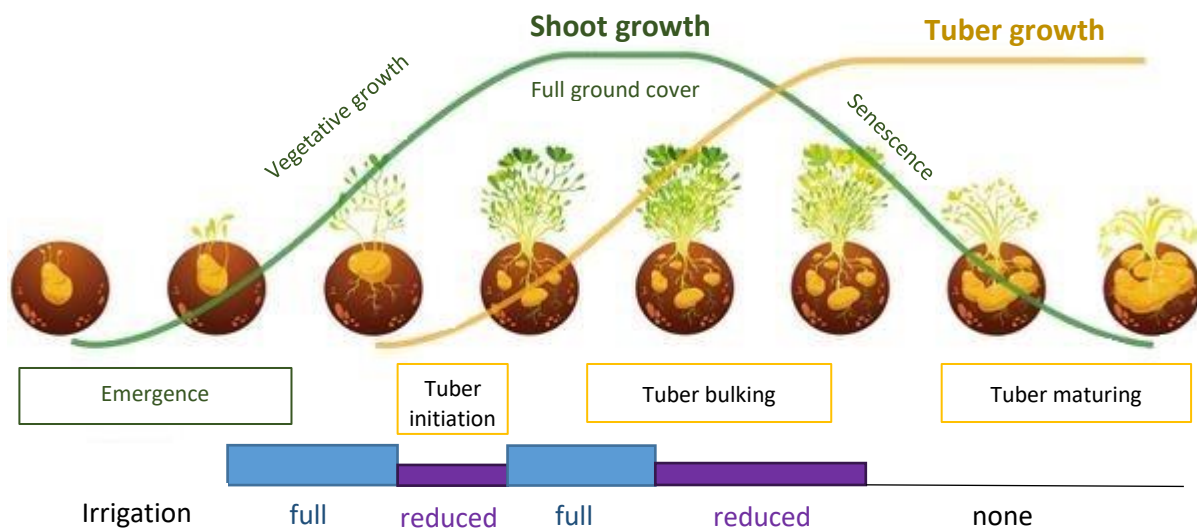
Figure 5.4.1: Growth cycle of potato crops with a possible irrigation scheme for water saving crop management. This schematic is only approximate and needs to be adjusted according to evaporative demand in the specific season.

Adapted from: Castlecor Potatoes

It is particularly important to compare different genotypes in future studies, as there is wide cultivar variation in potato drought stress responses (Aliche et al., 2018) and national organisations such as the UK's Agriculture & Horticulture Development Board recommend specific varieties to growers (https://varieties.ahdb.org.uk/). Institutional networks such as the International Plant Phenotyping Platform (IPPN) can allow access to novel technologies such as MRI (Chapter 3) and be of great value to compare genotypes in the future. Understanding the mechanisms of water fluxes in the potato plant *in situ* will be valuable in comparing varieties of differing drought tolerance. However, these experiments should always be accompanied by larger field experiments to determine if the effect seen in controlled environment also occurs in the field. Furthermore, it is important to conduct controlled environment experiments in conditions that resemble the field as closely as possible to mitigate unwanted side effects and genotype x environment interactions (Köhl et al., 2021).

This thesis encompasses field experiments and measuring plant biochemical / hormonal processes to reveal mechanisms of water use in the potato plant. By enhancing our physiological understanding of this crop, this approach ensures that the results from model systems are relevant to the reality of potato farming, which is demonstrated by practical suggestions for potato farming and further research.

6. References

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7. Supplementary Data to Chapter 2

Supplementary Table 2.1: Supplementary Table 1: outcome of repeated measures ANOVAs for the measured plant physiological parameters. F-values and p-values are reported and printed bold where $p < 0.05$. P-values have been adjusted by Greenhouse-Geisser correction for the main factor 'week' and all interactions with the factor 'week' involved in the parameters leaf width (both time intervals), leaf number, leaf water potential (pre-dawn and daytime) and stomatal conductance.

outcome of repeated measures ANOVA	compaction	irrigation	calendar week	compaction: irrigation	compaction: cal. week	irrigation: cal. week	compaction: irrigation: cal. week	weeks used in the analysis
soil moisture	$F_{(1, 4)} = 1.61$, $p = 0.27$	**$F_{(1, 4)} = 8.11$, $p = 0.047$**	**$F_{(16, 64)} = 11.42$, $p < 0.001$**	$F_{(1, 4)} = 0.23$, $p = 0.66$	$F_{(16, 64)} = 1.21$ $p = 0.29$	$F_{(16, 64)} = 1.32$ $p = 0.21$	$F_{(16, 64)} = 1.20$ $p = 0.29$	24 – 40
ground cover	**$F_{(1, 12)} = 21.11$, $p < 0.001$**	**$F_{(1, 12)} = 11.47$, $p = 0.005$**	**$F_{(17, 204)} = 135.20$, $p < 0.001$**	$F_{(1, 12)} = 0.04$, $p = 0.84$	**$F_{(17, 204)} = 12.87$, $p < 0.001$**	**$F_{(17, 204)} = 2.07$, $p = 0.009$**	$F_{(17, 204)} = 0.76$, $p = 0.74$	22 – 39
leaf width first half (Fig. 4A)	**$F_{(1, 12)} = 37.46$, $p < 0.001$**	**$F_{(1, 12)} = 6.91$, $p = 0.022$**	**$F_{(6, 72)} = 124.03$, $p < 0.001$**	$F_{(1, 12)} = 1.24$, $p = 0.29$	**$F_{(6, 72)} = 6.22$, $p = 0.004$**	**$F_{(6, 72)} = 8.64$, $p < 0.001$**	$F_{(6, 72)} = 1.11$, $p = 0.35$	26 – 29
leaf width second half (Fig 4B)	$F_{(1, 12)} = 0.70$, $p = 0.42$	$F_{(1, 12)} = 0.30$, $p = 0.59$	**$F_{(5, 60)} = 62.03$, $p < 0.001$**	$F_{(1, 12)} = 0.07$, $p = 0.80$	$F_{(5, 60)} = 0.61$, $p = 0.59$	$F_{(5, 60)} = 0.38$, $p = 0.73$	$F_{(5, 60)} = 0.67$, $p = 0.55$	30 - 36
leaf number	**$F_{(1, 12)} = 18.55$, $p = 0.001$**	**$F_{(1, 12)} = 46.84$, $p < 0.001$**	**$F_{(11, 132)} = 461.06$, $p < 0.001$**	$F_{(1, 12)} = 3.27$, $p = 0.96$	$F_{(11, 132)} = 1.88$, $p = 0.16$	**$F_{(11, 132)} = 11.56$, $p < 0.001$**	$F_{(11, 132)} = 1.64$, $p = 0.21$	26 - 36
pre-dawn leaf water potential	**$F_{(1, 10)} = 7.72$ $p = 0.02$**	**$F_{(1, 10)} = 12.96$ $p = 0.005$**	**$F_{(9.90)} = 5.85$, $p = 0.002$**	$F_{(1, 10)} = 0.28$, $p = 0.61$	$F_{(9.90)} = 1.96$, $p = 0.13$	$F_{(9.90)} = 2.41$, $p = 0.08$	$F_{(9.90)} = 0.30$, $p = 0.85$	26 – 32, 34, 35
daytime leaf water potential	$F_{(1, 10)} = 2.58$ $p = 0.14$	$F_{(1, 10)} = 0.29$ $p = 0.60$	**$F_{(8, 80)} = 10.08$, $p < 0.001$**	$F_{(1, 10)} = 2.37$, $p = 0.15$	$F_{(8, 80)} = 0.33$, $p = 0.78$	$F_{(8, 80)} = 0.70$, $p = 0.54$	$F_{(8, 80)} = 0.61$, $p = 0.60$	26 – 32, 34, 35

Suppl. Table 2.1 continued	compaction	irrigation	calendar week	compaction: irrigation	compaction: cal. week	irrigation: cal. week	compaction: irrigation: cal. week	weeks used in the analysis
stomatal conductance	$F_{(1, 8)} = 1.48$, $p = 0.26$	$F_{(1, 8)} = 0.51$, $p = 0.49$	$F_{(3, 24)} = 3.50$, $p = 0.07$	$F_{(1, 8)} = 0.01$, $p = 0.94$	$F_{(3, 24)} = 0.80$, $p = 0.44$	$F_{(3, 24)} = 0.18$, $p = 0.77$	$F_{(3, 24)} = 0.38$, $p = 0.63$	29 – 31, 35
photosynthesis rate	$F_{(1, 8)} = 2.61$, $p = 0.15$	$F_{(1, 8)} = 0.54$, $p = 0.48$	**$F_{(3, 24)} = 7.62$, $p = 0.001$**	$F_{(1, 8)} = 0.28$, $p = 0.61$	$F_{(3, 24)} = 0.24$, $p = 0.87$	$F_{(3, 24)} = 0.38$, $p = 0.77$	$F_{(3, 24)} = 1.23$, $p = 0.32$	29 – 31, 35
Leaf ABA concentration	$F_{(1, 6)} = 0.05$, $p = 0.84$	$F_{(1, 6)} = 0.83$, $p = 0.40$	**$F_{(5, 30)} = 3.67$, $p = 0.01$**	$F_{(1, 6)} = 0.36$, $p = 0.57$	$F_{(5, 30)} = 1.04$, $p = 0.41$	**$F_{(5, 30)} = 2.79$, $p = 0.03$**	$F_{(5, 30)} = 1.17$, $p = 0.97$	26 – 28, 32, 35

Supplementary Table 2.2: Supplementary Table 2: outcome of repeated measures ANOVAs for penetrometer resistance measurements. F-values and p-values are reported and printed bold where $p < 0.05$. P-values have been adjusted by Greenhouse-Geisser correction for the main factor 'week' and all interactions with the factor 'week' involved

outcome of repeated measures ANOVA	compaction	irrigation	depth measured	compaction: irrigation	compaction: depth	irrigation: depth	compaction: irrigation: depth
penetrometer resistance in April	**$F_{(1, 11)} = 20.32$, $p = 0.0009$**	$F_{(1, 11)} = 0.14$, $p = 0.72$	**$F_{(10, 110)} = 176.64$, $p < 0.0001$**	$F_{(1, 11)} = 1.81$, $p = 0.21$	**$F_{(10, 110)} = 3.11$, $p = 0.046$**	$F_{(10, 110)} = 0.42$, $p = 0.72$	$F_{(10, 110)} = 0.45$, $p = 0.70$
penetrometer resistance in July	$F_{(1, 11)} = 0.82$, $p = 0.38$	$F_{(1, 11)} = 0.21$, $p = 0.65$	**$F_{(10, 110)} = 0.006$, $p < 0.001$**	$F_{(1, 11)} = 90.51$, $p = 0.94$	$F_{(10, 110)} = 2.02$, $p = 0.11$	$F_{(10, 110)} = 1.04$, $p = 0.40$	$F_{(10, 110)} = 1.18$, $p = 0.33$
penetrometer resistance in Sep (only 0-30 cm depth)	**$F_{(1, 12)} = 5.40$, $p = 0.04$**	$F_{(1, 12)} = 2.32$, $p = 0.15$	**$F_{(5, 60)} = 163.07$, $p < 0.001$**	$F_{(1, 12)} = 0.03$, $p = 0.86$	**$F_{(5, 60)} = 17.96$, $p < 0.001$**	**$F_{(5, 60)} = 3.91$, $p = 0.03$**	$F_{(5, 60)} = 0.59$, $p = 0.58$

8. Supplementary data to Chapter 3

Supplementary Table 3.1: F-values and p-values of repeated measures ANOVAS for all presented parameters.

RM ANOVA outcome	treatment	time point	interaction
Stomatal Conductance			
stomatal Conductance **pre re-watering**	$F_{(1,6)}$ = **10.87** **p = 0.017 ***	$F_{(1,21)}$ = 0.42 p = 0.53	$F_{(1,21)}$ = **44.66** **p < 0.0001 *****
Only 1 time point post-watering			
stomatal Conductance **overall**	$F_{(1,6)}$ = **13.47** **p = 0.011 ***	$F_{(1,37)}$ = 0.65 p = 0.43	$F_{(1,37)}$ = 2.35 p = 0.13
Photosynthesis rate			
photosynthesis rate **pre re-watering**	$F_{(1,6)}$ = **10.79** **p = 0.017 ***	$F_{(1,21)}$ = **14.33** **p = 0.0011 ****	$F_{(1,21)}$ = **65.68** **p < 0.0001 *****
Only 1 time point post-watering			
photosynthesis rate **overall**	$F_{(1,6)}$ = **16.16** **p = 0.007 ****	$F_{(1,37)}$ = 4.04 p = 0.052	$F_{(1,37)}$ = **5.27** **p = 0.028 ***
Leaf water potential			
leaf water potential **Pre-rewatering**	$F_{(1,6)}$ = **13.28** **p = 0.011 ***	$F_{(1,6)}$ = **28.92** **p = 0.0017 ****	$F_{(1,6)}$ = **45.18** **p = 0.0005 *****
leaf water potential **Post-rewatering**	$F_{(1,6)}$ = 5.28 p = 0.061	$F_{(1, 5)}$ = **14.25** **p = 0.013 ***	$F_{(1, 5)}$ = 2.04 p = 0. 21
leaf water potential **overall**	$F_{(1,6)}$ = 1.70 p = 0.24	$F_{(1, 14)}$ = 0.28 p = 0. 60	$F_{(1, 14)}$ = 0.28 p = 0. 60
Leaf tissue ABA			
leaf tissue ABA **pre re-watering**	$F_{(1,6)}$ = 2.19 p = 0.19	$F_{(1,6)}$ = **6.22** **p = 0.047 ***	$F_{(1,6)}$ = **13.15** **p = 0.010 ***
leaf tissue ABA **post re-watering**	$F_{(1,6)}$ = 5.28 p = 0.061	$F_{(1,5)}$ = **14.25** **p = 0.013 ***	$F_{(1,5)}$ = 2.04 p = 0.21
leaf tissue ABA **overall**	$F_{(1,6)}$ = 3.62 p = 0.11	$F_{(1, 21)}$ = 0.67 p = 0. 42	$F_{(1, 21)}$ = 0.07 p = 0. 80
mean soil moisture			
mean soil moisture **pre rewatering**	$F_{(1,6)}$ = **18.90** **p = 0.005 ****	$F_{(1,30)}$ = **75.28** **p < 0.0001 *****	$F_{(1,30)}$ = **48.15** **p < 0.0001 *****
mean soil moisture **post re-watering**	$F_{(1,6)}$ = **34.27** **p = 0.0011 ****	$F_{(1,6)}$ = **22.11** **p = 0.0033 ****	$F_{(1,6)}$ = 4.02 p = 0.09
mean soil moisture **overall**	$F_{(1,6)}$ = 2.32 p = 0.18	$F_{(1,46)}$ = 0.64 p = 0.43	$F_{(1,46)}$ = **5.52** **p = 0.023 ***
Normalised relative tuber water content			
normalised relative tuber water content **pre rewatering**	$F_{(1, 6)}$ = **13.65** **p = 0.010 ***	$F_{(1,78)}$ = 3.13 p = 0.081	$F_{(1,78)}$ = **4.42** **p = 0.039 ***
normalised relative tuber water content **post re-watering**	$F_{(1,6)}$ = 3.64 p = 0.11	$F_{(1, 38)}$ = **23.00** **p < 0.0001 *****	$F_{(1,38)}$ = **6.49** **p = 0.02 ****
normalised relative tuber water content **overall**	$F_{(1,6)}$ = **13.47** **p = 0.010 ***	$F_{(1,126)}$ = **5.15** **p = 0.025***	$F_{(1,126)}$ = 0.03 p = 0.86

| RM ANOVA outcome	treatment	time point	interaction
Normalised tuber volume			
normalised tuber volume **pre re-watering**	$F_{(1,6)} = 27.38$ $p = 0.002$ **	$F_{(1,78)} = 19.18$ $p < 0.0001$ ***	$F_{(1,78)} = 31.98$ $p < 0.0001$ ***
normalised tuber volume **post re-watering**	$F_{(1,6)} = 12.49$ $p = 0.012$ *	$F_{(1,38)} = 36.56$ $p < 0.0001$ ***	$F_{(1,38)} = 6.78$ $p = 0.013$ *
normalised tuber volume **overall**	$F_{(1,6)} = 9.48$ $p = 0.02$ *	$F_{(1,126)} = 39.66$ $p < 0.0001$ ***	$F_{(1,126)} = 2.17$ $p = 0.14$
Normalised total tuber water content			
normalised tuber water content **pre re-watering**	$F_{(1,6)} = 9.45$ $p = 0.021$ *	$F_{(1,78)} = 9.02$ $p = 0.0036$ **	$F_{(1,78)} = 12.12$ $p = 0.0008$ ***
normalised tuber water content **post re-watering**	$F_{(1,6)} = 3.63$ $p = 0.11$	$F_{(1,38)} = 23.0$ $p < 0.0001$ ***	$F_{(1,38)} = 6.49$ $p = 0.015$ *
normalised tuber water content **overall**	$F_{(1,6)} = 3.69$ $p = 0.10$	$F_{(1,126)} = 16.43$ $p = 0.0001$ ***	$F_{(1,126)} = 0.03$ $p = 0.86$
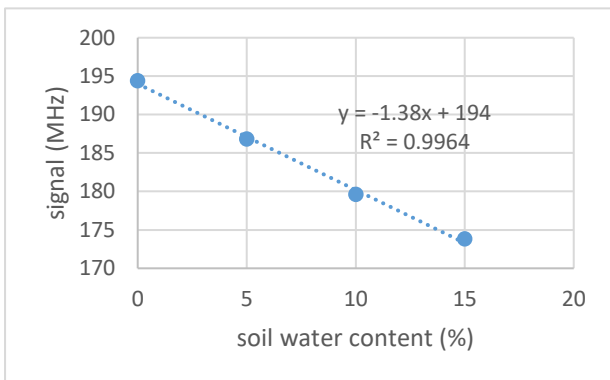
Supplementary Figure 1: Calibration curve of the soil water profiler for different soil water contents of the soil used in this experiment. Each symbol indicates the same soil with different amounts of water added.
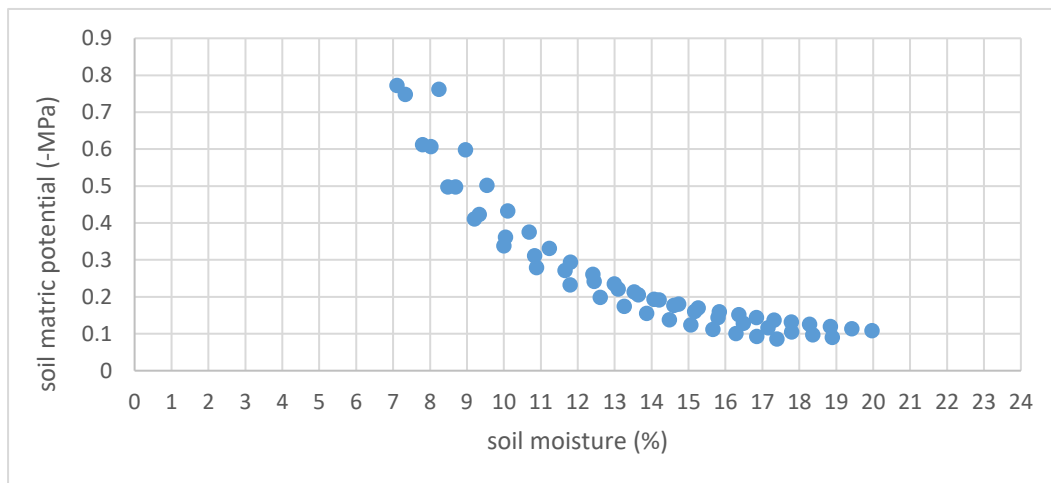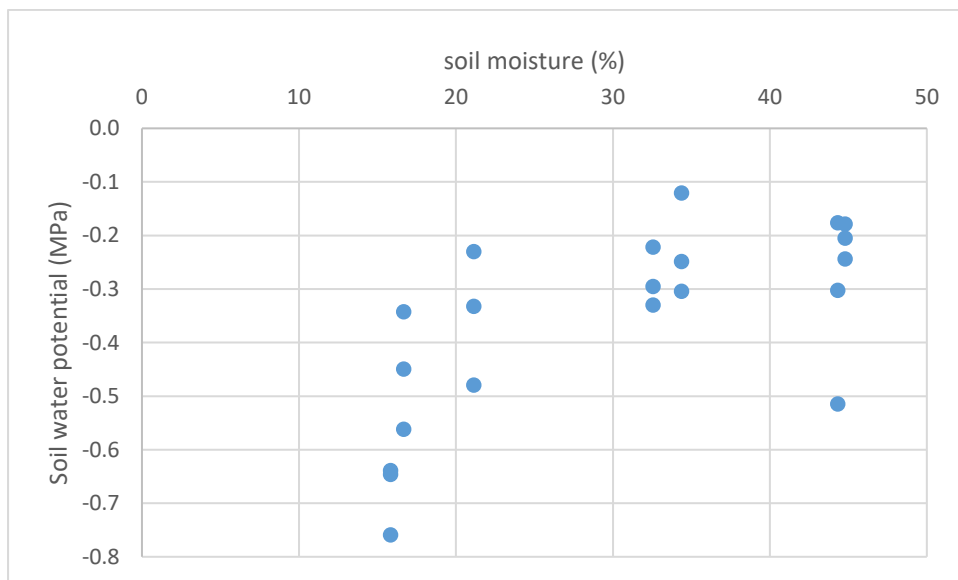

Supplementary Figure 2: Soil water retention curve for the soil ("Kaldenkirchen soil with 10 % sand) used in the experiment. Data from three independent measurement courses.
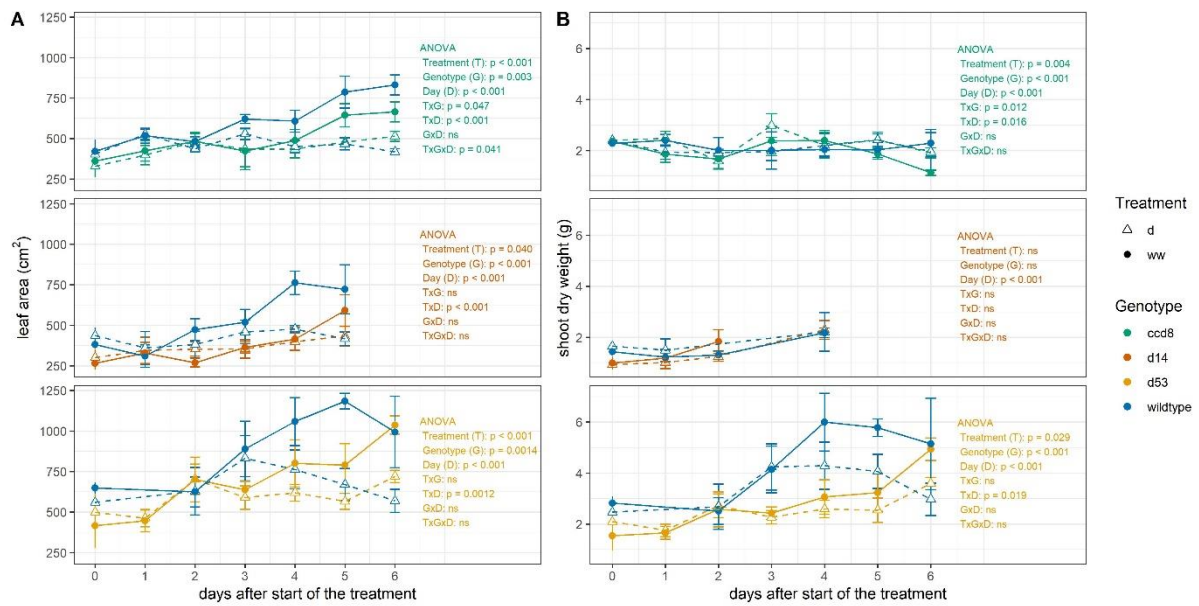
9. Supplementary data to Chapter 4
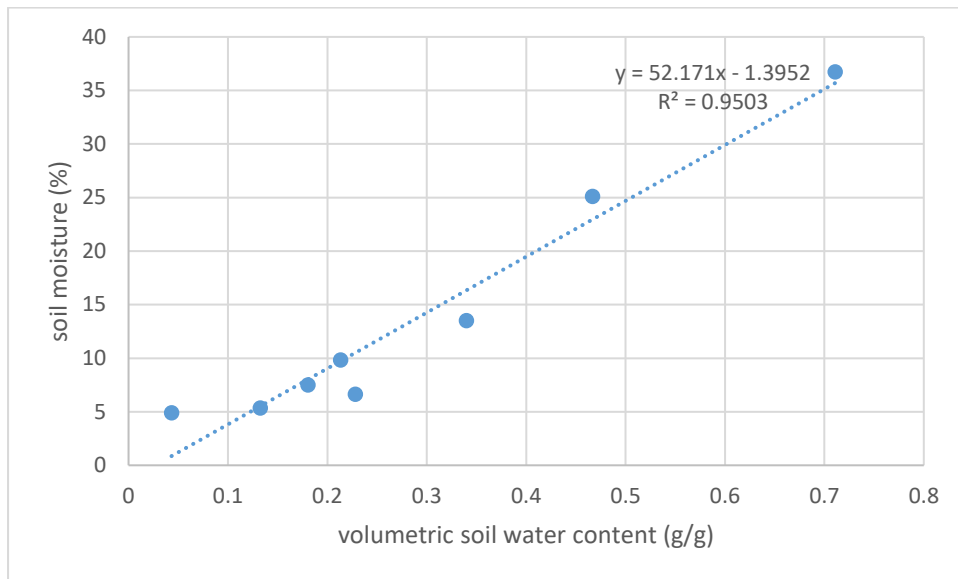
Supplementary Figure 3: Relation between volumetric soil water content (g/g) and soil moisture (%) measured with the WET sensor used in these experiments.

Supplementary Figure 4: Relation between soil moisture (%) measured with the WET sensor and soil water potential (MPa) measured with a psychrometer.

Supplementary Figure 5: Leaf area (A) and shoot dry weight (B) of three different potato genotypes compared to the wildtype in well-watered conditions (closed circles) and drying soil (open triangles). Means ± SE of 4 plants per day and treatment. Data acquired in the conditions described in Chapter 4