# Suberin biosynthesis in barley roots in response to osmotic stress

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

Vorgelegt von

**Tino Kreszies**

aus

Lichtenstein

Bonn, 2018

Angefertigt mit der Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität Bonn

1. Gutachter: Prof. Dr. Lukas Schreiber
2. Gutachter: Prof. Dr. Frank Hochholdinger

Tag der Promotion: 05.10.2018

Erscheinungsjahr: 2018

**Teile dieser Arbeit wurden bereits veröffentlicht:**

**Kreszies, T.**, Schreiber, L., Ranathunge, K., 2018. Suberized transport barriers in Arabidopsis, barley and rice roots: from the model plant to crop species. Journal of Plant Physiology, doi: 10.1016/j.jplph.2018.02.002

**Kreszies, T.**, Shellakkutti, N., Osthoff, A., Peng, Y., Baldauf, J.A., Zeisler-Diehl, V.V., Ranathunge, K., Hochholdinger, F., Schreiber, L., 2018. Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses. New Phytologist, doi: 10.1111/nph.15351

## Acknowledgements

At first I would like to thank my supervisor Prof. Dr. Lukas Schreiber for giving me the chance to work in his lab at the University of Bonn. I am thankful for his scientific advice and his always open door for fruitful discussions. It was a great pleasure for me to work in this project.

Next I would like to thank Dr. Kosala Ranathunge for teaching me the root pressure probe and all the theoretical background about root water transport during my stay in Guelph. Moreover I am thankful to him answering my various questions via email as well as for proofreading and discussion of my manuscripts.

I would like to thank Dr. Viktoria Zeisler-Diehl for teaching me how to perform gas chromatography and mass spectrometry, analyze the data and how to take care on all aspects of these machines. We had a lot of fun together in the lab and so I wish her a great future with little Lotta.

Many thanks go to my second supervisor Prof. Dr. Frank Hochholdinger and his colleagues Alina, Jutta and Peng who helped me within all my transcriptomic experiments.

I am grateful to Prof. Dr. Jens Leon, PD Dr. Ali Naz and Karola Müller for providing the barley seeds and for their help with propagation of the wild barley accessions.

I thank my students Nandhini, Priya, Annika, Jonas, Paul and Stella for the opportunity to pass some knowledge further and also for the new insights and ideas I got during teaching them.

I am thanking my office mates Filip and Charlie for the great time we spend together in discussion about football, beer and further things.

I thank all GRK2064 members for making this project possible and the helpful discussion after various progress reports we spend together.

Lastly I thank my great wife Victoria for convincing me to do a PhD.

# Index of Contents

# 1 Introduction

This dissertation consists of 3 chapters dealing with root suberization in barley and its response to water deficit, which was mimicked by adjusting different osmotic potentials in hydroponic solutions varying between -0.4 to -1.2 MPa.

Chapter 1 “Suberized transport barriers in *Arabidopsis*, barley and rice roots: From the model plant to crop species” is accepted and in press by Journal of Plant Physiology. It represents a general introduction into the topic of this dissertation, summarizing most recent findings in suberin research. Similarities and differences in root anatomy, suberized apoplastic root barrier development and its chemical composition, and finally the influence of these barriers on water and solute transport in roots. The review highlights that transfer of knowledge on root water transport from the model plant *Arabidopsis thaliana* to crop plants, such as barley and rice, may not always be straightforward, because of different complex anatomical structures.

Chapter 2 “Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses” is accepted and in press by New Phytologist. This chapter extends our view on how barley roots respond to low water potentials. A multifaceted approach was chosen including (i) detailed investigations of root anatomy by histochemistry and microscopy, (ii) quantitative and qualitative investigations of changes in suberin composition using gas chromatography and mass spectrometry, (iii) investigations on transcript changes by RNAseq and (iv) the functional measurements of radial water and solute transport in roots in response to osmotic stress. Obtained results indicate that an increased amount of aliphatic suberin can be an effective adaption to water stress by sealing the apoplastic pathway and thus preventing uncontrolled passive water loss to the dry soil/medium when roots are exposed to osmotic stress. But, at the same time water can still be taken up through the highly regulated cell-to-cell pathway thus allowing the plant to maintain its water status even under water stress conditions.

Chapter 3 “Osmotic stress has different effects on suberized transport barriers in roots of cultivated and wild barley” represents an extension of the detailed experimental approaches developed in chapter 2 comparing three wild barley accessions, from different countries, with three different modern cultivated barley accessions. Wild barley has a wider diversity, which results in superior traits helping to survive under abiotic stress better than its cultivated progeny. In accordance with this, wild barley

shows different responses to osmotic stress, such as forming an exodermis or having no significant change in aliphatic suberin amounts when exposed to water stress. In addition, very different from cultivated accessions, wild barley showed no decrease in radial root water uptake in response to osmotic stress conditions.

# 2 Chapter 1: Suberized transport barriers in *Arabidopsis*, barley and rice roots: from the model plant to crop species

Tino Kreszies[1], Lukas Schreiber[1] and Kosala Ranathunge[2]*

[1]Department of Ecophysiology, Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, 53115 Bonn, Germany.

[2]School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley 6009, Perth, Australia.

*Author for correspondence: Kosala Ranathunge
Email: kosala.ranathunge@uwa.edu.au

*In Press* in Journal of Plant Physiology, doi: 10.1016/j.jplph.2018.02.002

**Abstract:**

Water is the most important prerequisite for life and plays a major role during uptake and transport of nutrients. Roots are the plant organs that take up the major part of water, from the surrounding soil. Water uptake is related to the root system architecture, root growth, age and species dependent complex developmental changes in the anatomical structures. The latter is mainly attributed to the deposition of suberized barriers in certain layers of cell walls, such as endo- and exodermis. With respect to water permeability, changes in the suberization of roots are most relevant. Water transport or hydraulic conductivity of roots ($Lp_r$) can be described by the composite transport model and is known to be very variable between plant species and growth conditions and root developmental states. In this review, we summarize how anatomical structures and apoplastic barriers of roots can diversely affect water transport, comparing the model plant *Arabidopsis* with crop plants, such as barley and rice. Results comparing the suberin amounts and water transport properties indicate that the common assumption that suberin amount negatively correlates with water and solute transport through roots may not always be true. The composition, microstructure and

localization of suberin may also have a great impact on the formation of efficient barriers to water and solutes.

**Keywords:**

Apoplast, *Arabidopsis*, barley, composite transport model, hydraulic conductivity, rice, solutes, suberin, water transport

**Abbreviations:**

Hydrostatic hydraulic conductivity, $Lp_{hy}$ ; osmotic hydraulic conductivity, $Lp_{os}$ ; pressure chamber, PC ; radial oxygen loss, ROL ; reflection coefficient $\sigma_{sr}$ ; root hydraulic conductivity, $Lp_r$ ; root pressure probe, rpp ; soil-plant-atmosphere continuum, SPAC ; solute permeability, $P_{sr}$ ; α–ω dicarboxylic acids, diacids ; ω-hydroxyl acids, ω-OH acids

## 2.1 Introduction

Plant roots are designed to take up water and nutrient ions from the surrounding soil and supplying them to shoots and leaves. It is well documented that the water moves through plants by water potential gradients set up by transpiration through the soil-plant-atmosphere continuum (SPAC) (Steudle, 2000a,b; Nobel, 2009; Kramer and Boyer 1995). Resistances in plant water uptake can be described by Ohm's Law using simple force and flow relations (van den Honert, 1948; Landsberg & Fowkes, 1978; Steudle, 2000b). The water and solute transport of roots are known to depend on (1) complex root anatomical features, which are species dependent, (2) different growth conditions, and (3) different growth stages/age of roots (Steudle & Peterson, 1998; Steudle, 2000b). Such factors also lead to a highly variable water and solute movement, which are not only related to permeability of root cell membranes but also to apoplastic barriers such as Casparian bands and the suberin lamellae (Steudle & Peterson, 1998; Hose *et al.*, 2001; Steudle & Ranathunge, 2007). Suberin in cell walls can also be induced by plant exposure to different abiotic (drought, salinity, anoxia/hypoxia, organic acids, high nutrients etc.) and biotic (pathogens) stresses (Hose *et al.*, 2001; Enstone *et al.*, 2002; Krishnamurthy *et al.*, 2009, 2011; Ranathunge *et al.*, 2011c; Shiono *et al.*, 2014a; Barberon *et al.*, 2016; Tylová *et al.*, 2017). Induced suberin in cell walls is known to strengthen the barriers in order to minimize the entry of pathogens,

toxic gases and organic acids into the roots (Lulai *et al.*, 1998; Thomas *et al.*, 2007; Ranathunge *et al.*, 2008; Lanoue *et al.*, 2010). Suberized cell walls also act as strong barriers to prevent radial oxygen loss (ROL) from roots to the substrate under anoxia/hypoxia and uncontrolled back flow of water and solutes from root to the surrounding soil/environment (Kotula *et al.*, 2009a, 2014, 2017; Ranathunge *et al.*, 2011c).

In this review, we compare the similarities and differences in root anatomy (first section), suberized apoplastic barrier development and its composition/biosynthesis (second section) between the model plant of *Arabidopsis* (*Arabidopsis thaliana*) and crop plants, such as barley (*Hordeum vulgare*) and rice (*Oryza sativa*); and subsequently their influence on water and solute transport of roots (third section).

## 2.2 Anatomy/structure and suberized apoplastic barriers in roots

A comprehensive knowledge of root anatomy is essential to understand water and solute transport of roots. Different anatomical features as well as system architectures of roots from various plant species result in complex ways of water movement through roots (Steudle & Peterson, 1998; Steudle, 2000b).

In roots, there are three major radial pathways for transport of water and solutes across the cylinder: (1) the apoplastic path around the protoplast, where water and solutes can move towards the stele through free spaces and cell walls of the rhizodermis and cortex, (2) the symplastic pathway, in which transport occurs through plasmodesmata from one cell (protoplast) to the other using cytoplasmic continuum, and (3) the transmembrane pathway, where water and solutes move through cell walls and aquaporins/transporters localized in the cell membrane (Steudle & Peterson, 1998; Peterson & Cholewa, 1998; Steudle, 2000a,b). To date, there are no simple and straightforward experimental approaches to separate the latter two components. Therefore, these two pathways together are summarized as a ‘cell-to-cell’ or ‘protoplastic’ component (Steudle, 2000b). Water transport across roots should be considered as radial, in which water has to cross series of cell layers such as rhizodermis, cortex (including endodermis and/or exodermis) and stele. Once water entered into the vascular tissue of the root, its direction is longitudinal through the xylem vessels towards the shoot. The apoplast can be interrupted by Casparian bands

and suberin lamellae in endodermal and exodermal cell walls. This blockage can only be bypassed in young root zones close to the tips, where these structures are not yet fully developed and also through lateral roots, which emerge from pericycle cells and directly grow through the endodermis thus disturbing the continuity of endodermal barrier (Krishnamurthy et al., 2011; Steudle, 2000b; Steudle and Jeschke, 1983; Steudle and Peterson, 1998).

Over the length of the root, from the root tip to the base, roots can be divided into developmental stages which exhibit different apoplastic modifications with suberin. At stage I, close to the root apex, Casparian bands are deposited in the transverse and radial cell walls of the endodermis (Ma & Peterson, 2003; Karahara *et al.*, 2004; Krishnamurthy *et al.*, 2009; Chen *et al.*, 2011). They are formed mainly by lignin (Schreiber, 1996; Naseer *et al.*, 2012) and only partly by suberin (Zeier & Schreiber, 1998). It was shown that Casparian bands can block the movement of ions and fluorescents dyes through the apoplastic pathway (Singh & Jacobsen, 1977; Peterson, 1987). At stage II, the suberin lamellae start to lay down interior to the primary cell walls but outside of the plasma membrane of some endodermal cells, which increases the blockage of the apoplast. The transition zone from Casparian bands to a fully developed suberized endodermis is called patchy suberin lamellae. This patchy suberin lamellae still is a permeable barrier, because unsuberized passage cells allow movement of water and solutes through the plasma membrane (Peterson & Enstone, 1996; Enstone *et al.*, 2002; Franke & Schreiber, 2007; Schreiber, 2010). Formation of Casparian bands and suberin lamellae does not only occur in the endodermis but also in the hypodermis, which is the cell layer most adjacent to the outermost rhizodermis. A hypodermis exhibiting Casparian bands is called exodermis (Peterson, 1988; Hose *et al.*, 2001; Meyer & Peterson, 2013). The development of an exodermis is not a common character for all plant species. For example in contrast to rice and corn (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2016), no exodermis is present in *Arabidopsis*, soybean, castor bean and barley roots (Schreiber *et al.*, 2005a; Thomas *et al.*, 2007; Ranathunge *et al.*, 2008, 2017; Ranathunge & Schreiber, 2011). Also its development is highly dependent on the environmental and growth conditions. Another form of suberin occurrence in roots is the deposition of diffuse suberin into intermicrofibrillar spaces of the rhizodermal cell walls in certain plant species such as onion and soybean (Peterson and Cholewa, 1998; Ranathunge et al., 2008; Thomas et al., 2007).

It is necessary to have a detailed map over the length of the root for the different developmental stages under different growth conditions (e.g. stress *vs*. control) to interpret measured water and solute transport data correctly, as well as to decide which root zones should be taken for further analyses, for example tissue specific transcriptomics or cell wall specific chemical analysis. This can be done by staining root cross sections with Sudan red or Fluorol yellow 088 to detect suberin lamellae and berberine aniline blue to detect Casparian bands (Brundrett *et al.*, 1988, 1991).

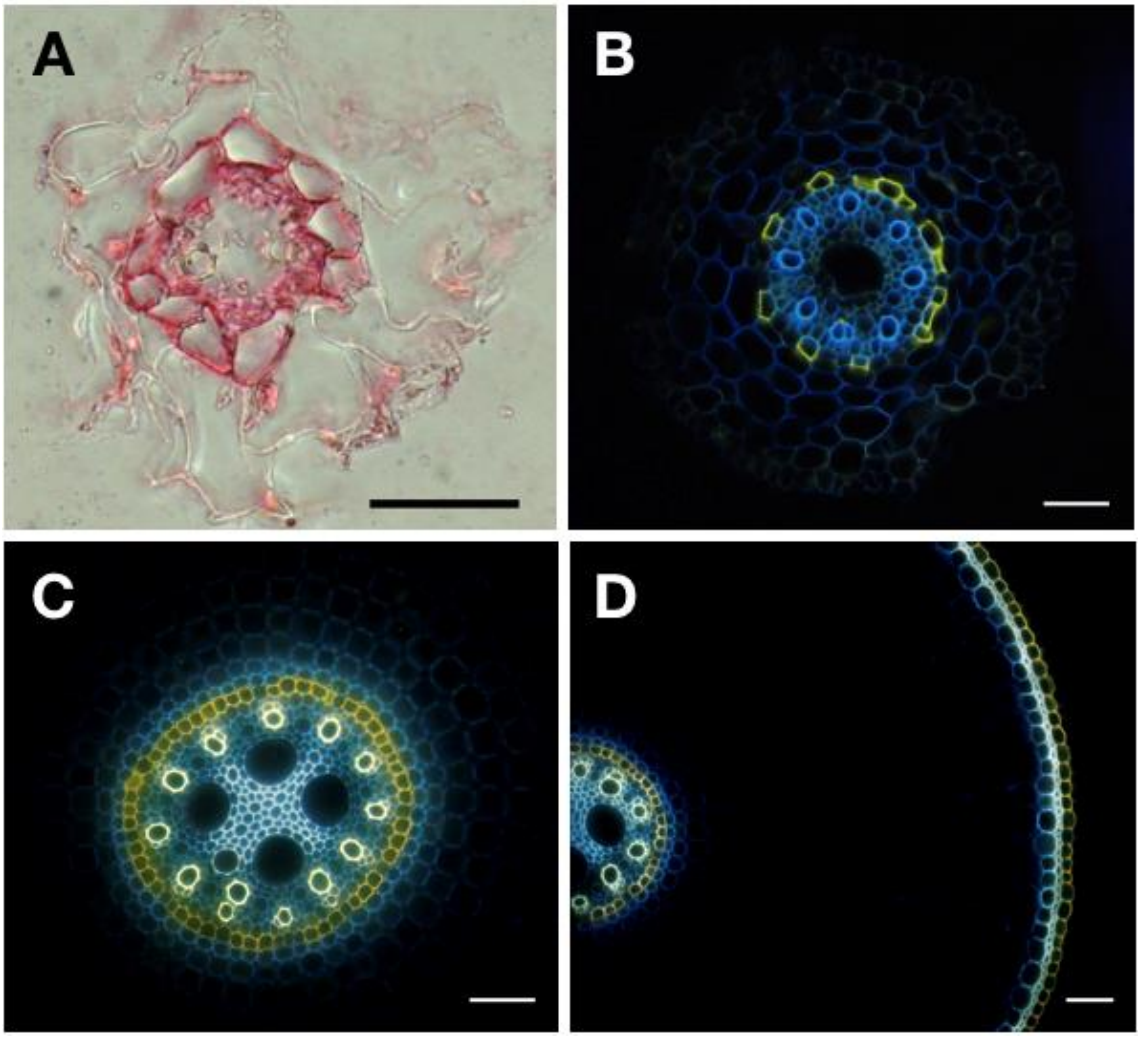


**Figure 1 Comparison of cross sections of *Arabidopsis*, barley and rice roots.** (A) *Arabidopsis* root cross section stained with Sudan red 7B. The endodermis (red colour) shows suberized cells. Bar = 25µm. (B) Barley seminal root cross section stained with fluorol yellow 088. The yellow fluorescence shows the suberized cells in the endodermis, whereas, unsuberized passage cells do not have yellow fluorescence. Bar = 50µm. (C and D) Rice root cross sections stained with Fluorol yellow 088, in which intense yellow fluorescence shows suberized endo- and exodermis. Bars = 50µm.

Besides the root system architecture, there are major differences in the root anatomy between *Arabidopsis*, barley and rice, which affect water and solute transport. *Arabidopsis* roots consist of a rhizodermis, one layer of unmodified cortical cells, endodermis and the stele. Casparian bands and suberin lamellae can only be detected in the endodermis of *Arabidopsis* (Fig. 1) (Ranathunge & Schreiber, 2011). On the other hand, barley develops two types of roots, seminal and adventitious roots. While seminal roots contain one large central late metaxylem together with five to eight early metaxylem vessels (Fig. 1) and four to five cortical cell layers, adventitious roots have five to six late metaxylem vessels and eight cortical cell layers. Besides that, Casparian bands and suberin lamellae can only be found in the endodermis but not in the hypodermis of barley roots (Jackson, 1922; Knipfer & Fricke, 2011; Ranathunge *et al.*, 2017). In contrast, rice roots have three to five late metaxylems together with ten to fourteen early metaxylem vessels and they form a suberized endo- and exodermis (Fig. 1) (Ranathunge *et al.*, 2016), which is different from *Arabidopsis* and barley roots. Rice is often grown in lowland, water-logged soils with anoxic/hypoxic environment. Thus, rice roots need a very different structure compared to non-wetland species. To cope with oxygen deprivation in the medium, rice roots develop an aerenchyma as a result of programmed cell death in the mid cortex (Clark & Harris, 1981; Ranathunge *et al.*, 2011a). Over the length of the root, from tip to the base, the volume of the aerenchyma increases, which facilitates longitudinal oxygen transport/diffusion to the rapidly growing root tips, which needs a steady oxygen supply (Kotula *et al.*, 2009a,b).

## 2.3 Composition and biosynthesis of suberized apoplastic barriers

Suberin is a complex biopolyester that forms an apoplastic transport barrier, which is deposited in the inner layer of the cell wall of the endo- and exodermis as suberin lamellae or within the primary cell walls forming Casparian bands (Nawrath *et al.*, 2013). Suberized cell walls contain poly aliphatic and poly aromatic domains which are cross linked (Kolattukudy *et al.*, 1975; Bernards, 2002). The common aliphatic components are primary alcohols, fatty acids, α–ω dicarboxylic acids (diacids) and ω-hydroxyl acids (ω-OH acids), while the most abundant aromatic components are ferulic- and coumaric acids (Zeier & Schreiber, 1997; Ranathunge *et al.*, 2011c; Graça, 2015). Aliphatic suberin is generally attributed to be the main barrier for water transport

because of its high hydrophobicity (Zimmermann *et al.*, 2000; Hose *et al.*, 2001), whereas, aromatic suberin primarily poses a barrier for solutes and pathogen penetration (Lulai *et al.*, 1998; Enstone *et al.*, 2002).

Not only the total content of suberin but also the composition of suberin varies immensely among *Arabidopsis*, barley and rice roots (Fig. 2). Different cultivars of rice, e.g. lowland *vs*. upland, as well as growth conditions, result in altered total suberin amounts (Schreiber *et al.*, 2005b; Ranathunge & Schreiber, 2011; Ranathunge *et al.*, 2016, 2017). For example, in *Arabidopsis*, the carbon chain length distribution of monomers ranges from $C_{16}$ to $C_{24}$, whereas, in barley and rice, the monomer chain length distribution reaches up to $C_{32}$. Anatomical studies with different suberin staining techniques revealed that *Arabidopsis* and barley roots do not form an exodermis even at the very base of the roots (Ranathunge *et al.*, 2011a, 2017). Hence, the quantified suberin amounts using gas chromatography (GC) represent the suberin from the endodermis. On the other hand, rice roots formed a suberized exodermis in addition to the endodermis, and total suberin represents the amounts from both barriers (Ranathunge *et al.*, 2011a). The total aliphatic suberin amount of *Arabidopsis* is approximately three times lower than the amount of barley (1.5 *vs.* 5 µg $cm^{-2}$) (Ranathunge & Schreiber, 2011; Ranathunge *et al.*, 2017). Measured suberin amounts in rice vary between comparable amounts to barley (Ranathunge *et al.*, 2011a) and up to more than twelve times higher compared with *Arabidopsis*. In upland rice, the total amount of 18 µg $cm^{-2}$ is made of 12 µg $cm^{-2}$ from the endodermis and 6 µg $cm^{-2}$ from the exodermis (Fig. 2) (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2016). There are distinct differences in the aromatic suberin domain among plant species too. In *Arabidopsis*, the aromatic suberin is negligible since there are only traces, whereas, barley has two-fold more aromatics than aliphatics (Ranathunge & Schreiber, 2011; Ranathunge *et al.*, 2017). Among these three species, rice has the greatest amount of aromatic suberin and it is more than five- to eight-fold the amount of the aliphatics (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2016). This high amount of aromatics in barley and rice roots has to be discussed carefully since in graminaceae fairly high amounts of aromatics are bound to all cell walls (Carpita, 1996). However, different suberin monomer compositions and amounts suggest that the ultrastructure of the suberin polyester is likely to be different among the plant species of *Arabidopsis*, barley and rice.

The biosynthesis of suberin in *Arabidopsis* has been studied extensively due to the availability of suberin mutants (Tab. 1) and has been reviewed elsewhere (Franke & Schreiber, 2007; Ranathunge *et al.*, 2011c; Vishwanath *et al.*, 2015). In contrast, only a few suberin mutants are available in rice, whereas, in barley, according to our best knowledge, there are no suberin mutants that have been analyzed so far. This is apparently due to relatively easy genetic manipulation in the model plant of *Arabidopsis* compared to barley and rice, and thus *Arabidopsis* suberin mutants are well established. Even though, predictions of the putative orthologous genes in rice and barley are possible using bioinformatics tools (Tab. 1) it remains unclear whether the biological functions of those genes are the same. However, it is likely that future investigations will unravel these current uncertain predictions.

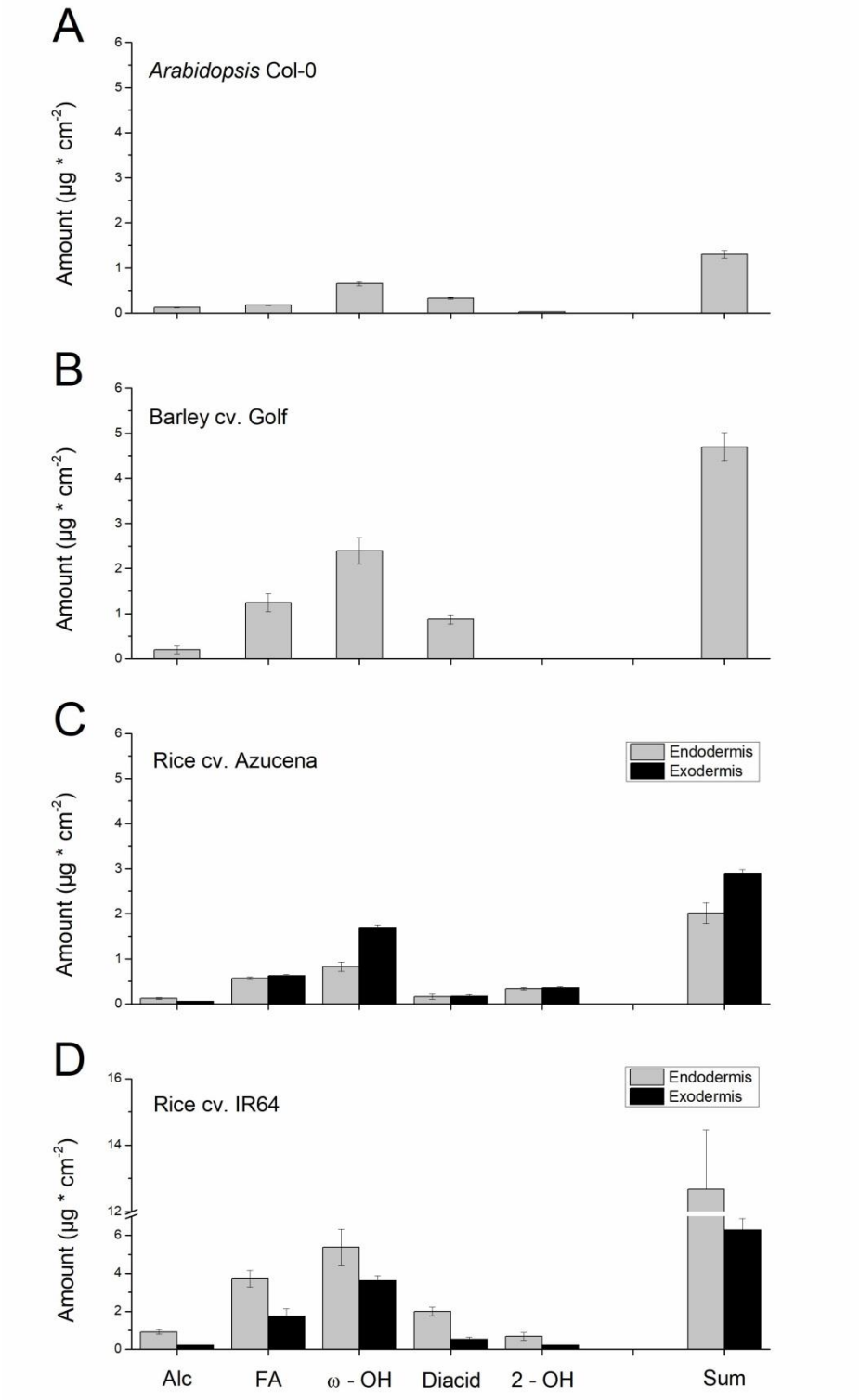


**Figure 2** Comparison of substance class composition of aliphatic suberin released from (A) endodermis of whole roots of *Arabidopsis* Col-0, (B) endodermis of the mature half of barley cv. Golf roots, and (C and D) endo- and exodermis of the mature half of rice cv. Azucena and cv. IR64 roots. Data replotted from (A) Ranathunge and Schreiber, 2011, (B) Ranathunge et al., 2017, (C) Ranathunge et al., 2011a, (D) Schreiber et al., 2005b.

**Table 1: Genes involved in suberin biosynthesis**

Arabidopsis genes involved in suberin biosynthesis, and putative orthologous in rice and barley plants obtained from EnsemblPlants database are listed (Kersey *et al.*, 2016). One-to-many indicates that one gene from the query is orthologous to many genes in the target. Many-to-many indicates that multiple orthologous can be found in the target species due to paralogous genes in the query species.

| **Gene Name** | **Annotated function** | ***Arabidopsis*** | **Rice** (japonica) | **Barley** |
|---|---|---|---|---|
| ***CYP86A1 / HORST*** | Cytochrome P450 monooxygenase | At5g58860 (Höfer *et al.*, 2008) | OS01G0854800 | HORVU3Hr1G085020 |
| ***CYP86B1 / RALPH*** | Cytochrome P450 monooxygenase | At5g23190 (Molina *et al.*, 2009; Compagnon *et al.*, 2009) | OS10G0486100 (Waßmann, 2014) | HORVU1Hr1G042810 |
| ***KCS2 / DAISY KCS20*** | β-Ketoacyl-CoA synthase | At1g04220; At5g43760 (Lee *et al.*, 2009; Franke *et al.*, 2009) | OS11G0591200 (one-to-many) | no orthologous found |
| ***GPAT5 ; GPAT7*** | Acyl-CoA:glycerol-3-phosphate acyltransferase | At3g11430; At5g06090 (Beisson *et al.*, 2007; Yang *et al.*, 2012) | OS05G0457800 (one-to-many) | HORVU1Hr1G072590 (one-to-many) |
| ***FAR1 ; FAR4 ; FAR5*** | Fatty acyl CoA reductase | At5g22500; At3g44540; At3g44550 (Domergue *et al.*, 2010) | OS07G0416600 (one-to-many) | many to many |
| ***ESB1 (Dir10)*** | Dirigent protein | At2g28670 (Baxter *et al.*, 2009; Hosmani *et al.*, 2013) | OS01G0155300 (one-to-many) | no orthologous found |
| ***ABCG2; ABCG20 ; ABCG6*** | ATP-binding cassette (ABC) transporters | At2g37360; At3g53510; At5g13580 (Yadav *et al.*, 2014) | OS03G0281900 OsABCG5/RCN1 (Shiono *et al.*, 2014a) | many to many |
| **ABCG11/WBC11/DSO/COF1** | ATP-binding cassette (ABC) transporters | At1g17840 (Panikashvili *et al.*, 2010) | OS04G0528300 OS10G0494300 | HORVU2Hr1G090960 |
| ***GDSL*** | GDSL-motif esterase/acyltransferase/lipase | At2g23540 (Soler *et al.*, 2007) | no orthologous found | no orthologous found |
| ***MYB41; MYB9; MYP107*** | MYB transcription factor | At4g28110; At5g16770; At3g02940 (Kosma *et al.*, 2014; Lashbrooke *et al.*, 2016; Gou *et al.*, 2017) | Many to many | Many to many |

## 2.4 Water and solute transport in roots

Water uptake in roots, which consist of complex anatomical structures, can be described according to the composite transport model, proposed by Steudle and co-workers in the last decades (Steudle, 1993, 2000a,b; Steudle & Peterson, 1998; Ranathunge *et al.*, 2017). The apoplastic and the cell-to-cell (symplastic and transcellular) pathways are the main components in the composite transport model. Once water and solutes entered into the rhizodermis from the soil solution, they have to move radially into the vascular tissue crossing many cell layers including suberized barriers such as endo- and exodermis, which poses resistance to the radial flows. As mentioned in the previous section of this review, species-dependent root anatomy, including the development of Casparian bands and suberin lamellae, plays an important role in the water uptake, and also preventing water losses (back flow) from root to the dry and/or saline soils (Steudle & Jeschke, 1983; Steudle & Peterson, 1998; Steudle, 2000b).

Water flow in plant roots is usually measured as hydraulic conductivity ($Lp_r$ in m $s^{-1}$ $MPa^{-1}$), which is a measure of conductance per unit surface area per unit driving force. $Lp_r$ depends on the plant species and the root developmental stage or age, but it can also be altered by exposure of plants to different abiotic stresses, such as drought, salinity, anoxia, nutrient stress, heavy metals, temperature stress etc. (Steudle & Peterson, 1998). The apoplastic pathway can be altered, reduced or eventually completely interrupted by deposition of Casparian bands and suberin lamellae, while the cell-to-cell pathway can be affected by suberin lamellae and the parallel activity of aquaporins or water channels (Steudle & Ranathunge, 2007; Maurel *et al.*, 2015; Gambetta *et al.*, 2017). In general, $Lp_r$ can be measured hydrostatically or osmotically using a root pressure probe (Steudle, 1993). The hydrostatic hydraulic conductivity ($Lp_{hy}$) determines the water flow through both the apoplastic and cell-to-cell (symplastic and transcellular) paths (Zhu & Steudle, 1991; Steudle, 2000b). Osmotic pressure gradients, created by adding different osmotic solutions into the medium, can only represent a considerable force for water movement across the semipermeable plasma membranes and not for the porous and non-selective apoplast (Steudle, 1993, 2000b). Hence, the osmotic hydraulic conductivity ($Lp_{os}$) measures the water transport across the cell-to-cell path (Zhu & Steudle, 1991; Steudle, 2000b). The ratios of hydrostatic to osmotic conductivities indicate which pathway contributes most to the overall water transport across the roots (Steudle & Peterson,

1998). Large differences in root $Lp_r$ can be observed either during osmotic (such as during conventional exudation of an excised root) or hydraulic water flow (such as during transpiration) and this depends on the species investigated. According to the composite transport model this can be explained in terms of (1) the variability of root hydraulic properties, i.e. changes in forces which cause a switching between the pathways, (2) the resistance or conductance along the pathways, and (3) cross-sectional areas.

The radial movement of solutes across the root can be described by the solute permeability coefficient ($P_{sr}$ in $m.s^{-1}$) and the passive selectivity of roots for solutes can be explained by the reflection coefficient ($\sigma_{sr}$). For example, the cell-to-cell (protoplastic) path is semipermeable and it exhibits a $\sigma_s^{cc}$ of close to unity ($\sigma_s^{cc} \approx 1$). The porous, non-selective apoplastic path, on the other hand, is having a reflection coefficient of virtually zero ($\sigma_s^{cw} \approx 0$). The two pathways interact with each other, and the interaction results in phenomena such as a circulation flow of water and a low overall reflection coefficient of the root ($\sigma_{sr}$) (Steudle & Frensch, 1996; Steudle, 1997, 2000b). This means that root $\sigma_{sr}$ is smaller than unity. The values of $\sigma_{sr}$ are by definition between zero and one, which would describe a non-perfect barrier against a solute or deviation from the ideal osmometer model (Steudle & Peterson, 1998; Tomos & Leigh, 1999; Steudle & Ranathunge, 2007).

In *Arabidopsis*, water and solute transport was measured exclusively using whole roots so far, while in rice, these measurements for whole roots and individual adventitious roots were carried out using different techniques (Miyamoto *et al.*, 2001; Ranathunge *et al.*, 2003, 2011a). For barley, which contains different types of roots, the measurements were mainly targeted for seminal roots due to their superior contribution to the overall root water uptake compared with adventitious roots (92% vs. 8%; (Knipfer & Fricke, 2010)). In general, the model plant *Arabidopsis* and the crop plant rice have smaller $Lp_r$ values compared with other crop species such as barley (Tab. 2) and corn (Miyamoto *et al.*, 2001; Ranathunge *et al.*, 2003, 2017), but still greater values than woody plants (Rüdinger *et al.*, 1994; Steudle & Meshcheryakov, 1996; Steudle & Heydt, 1997).

**Table 2 Hydraulic conductivities (Lpr) of Arabidopsis, rice and barley roots.**

Lpr of individual roots or whole root systems were measured using a root pressure probe (RPP), a pressure chamber (PC) or a pump perfusion technique, respectively.

| Plant species | Description | $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | | | Reference |
|---|---|---|---|---|---|
| | | Hydrostatic $Lp_r$ ($Lp_{hy}$) | Osmotic $Lp_r$ ($Lp_{os}$) | Ratio of $Lp_{hy}$/ $Lp_{os}$ | |
| ***Arabidopsis* - Col-0** | Whole root systems, measured using a root pressure probe (RPP). | 3.7 ± 0.3 | 3.6 ± 0.4 | 1.04 ± 0.07 | (Ranathunge & Schreiber, 2011) |
| ***Arabidopsis* - Col-8** | | 3.8 ± 0.2 | 3.5 ± 0.1 | 1.12 ± 0.06 | |
| ***Arabidopsis* - *horst*** | | 9.5 ± 0.2 | 5.7 ± 1.0 | 1.71 ± 0.15 | |
| ***Arabidopsis* – *esb1-1*** | | 3.3 ± 0.2 | 3.2 ± 0.2 | 1.07 ± 0.04 | |
| ***Arabidopsis* – *esb1-2*** | | 3.1 ± 0.3 | 2.8 ± 0.3 | 1.09 ± 0.03 | |
| **Rice cv. IR64** | Individual adventitious roots, measured using a RPP. | 3.8 ± 0.6 | 1.1 ± 0.5 | | (Ranathunge *et al.*, 2003) |
| **Rice cv. Azucena** | | 4.0 ± 1.2 | 1.1 ± 0.4 | | |
| **Rice cv. IR64** | Whole root systems, measured using a pressure chamber (PC). | 4.0 ± 1.7 | 3.1 ± 0.9 | | |
| **Rice cv. Azucena** | | 2.8 ± 1.3 | 2.4 ± 1.1 | | |
| **Rice cv. IR64** | Outer part of the roots, measured using a pump perfusion technique. | 150 ± 50 | | | |
| **Rice cv. Azucena** | | 130 ± 50 | | | |
| **Rice cv. IR64** | Individual adventitious roots, measured using a RPP. | 5.0 ± 2.5 | 9.2 ± 3.0 | 0.7 ± 0.2 | (Miyamoto *et al.*, 2001) |
| **Rice cv. Azucena** | | 4.7 ± 1.0 | 4.0 ± 2.4 | 1.9 ± 1.6 | |
| **Rice cv. IR64** | Whole root systems, measured using a PC. | 5.6 ± 2.7 | 4.2 ± 2.5 | 1.8 ± 1.3 | |
| **Rice cv. Azucena** | | 6.3 ±3.1 | 5.5 ± 3.7 | 1.4 ± 0.7 | |
| **Rice cv. IR64** | Individual adventitious roots, measured using a RPP. | 3.7 ± 0.6 | 1.1 ± 0.5 | 3.6 ± 0.9 | (Schreiber *et al.*, 2005b) |
| **Rice cv. Azucena** | Root systems from stagnant growth, measured using PC. | 6.7 ± 2.9 | 2.0 ± 1.4 | 4.3 ± 1.6 | (Ranathunge *et al.*, 2011a) |
| **Rice cv. Azucena** | Root systems from aerated growth, measured with PC. | 7.1 ± 2.8 | 3.0 ± 1.3 | 2.9 ± 2.0 | |
| **Rice cv. Azucena** | Individual roots from stagnant growth, | 5.1 ± 1.9 | 4.3 ± 1.6 | 1.5 ± 0.5 | |

| Plant species | Description | $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | | | Reference |
|---|---|---|---|---|---|
| | | Hydrostatic $Lp_r$ ($Lp_{hv}$) | Osmotic $Lp_r$ ($Lp_{os}$) | Ratio of $Lp_{hv}$/ $Lp_{os}$ | |
| | measured using RPP. | | | | |
| **Rice cv. Azucena** | Individual roots from aerated growth, measured using a RPP. | 5.9 ± 1.8 | 3.4 ± 1.2 | 2.0 ± 0.5 | |
| **Barley cv. Golf** | Individual seminal roots, measured using a RPP | 13 ± 2.6 | 5.4 ± 2.0 | | (Knipfer & Fricke, 2011) |
| **Barley cv. Golf** | Individual adventitious roots, , measured using a RPP | 10 ± 5.1 | 6.3 ± 3.4 | | |
| **Barley cv. Golf** | Individual seminal roots in circulating medium, measured using RPP | 12.2 ± 3.7 | 5.1 ± 1.6 | 2.5 ± 0.8 | (Knipfer & Fricke, 2010) |
| **Barley cv. Golf** | Individual seminal roots in stagnant medium, measured using a RPP | 3.2 ± 0.5 | 0.4 ± 0.1 | 8.4 ± 1.9 | |
| **Barley cv. Golf** | Individual end segment of seminal roots from aerated growth, well stirred, measured using a RPP | 9.4 ± 3.1 | 9.5 ± 3.7 | 1.1 ± 0.3 | (Ranathunge *et al.*, 2017) |
| **Barley cv. Golf** | Individual end segment of seminal roots from aerated growth, unstirred, measured using a RPP | 9.7 ± 4.2 | 4.2 ± 2.6 | 2.6 ± 0.8 | |
| **Barley cv. Golf** | Whole root, from aerated growth, well stirred, measured using a RPP | 1.5 ± 0.4 | | | |

Measurement of $Lp_r$ and $P_{sr}$ on hydroponically-grown wild type and suberin mutants of *Arabidopsis* plants allowed studying of water and solute transport in comparison to altered suberin amount and composition. The *atcyp86a1* (*horst*) mutant has in total more than 60% reduced suberin amount compared to wild type *Arabidopsis*, because of a major reduction in $C_{16}$ and $C_{18}$ ω-OH acids and diacids (Li *et al.*, 2007; Höfer *et al.*, 2008). This effect resulted in a 1.6-fold increase of $Lp_r$ and $P_{sr}$ compared to the wild type (Ranathunge & Schreiber, 2011). The enhanced suberin mutant, *esb1*, which exhibited defects in Casparian band formation, accumulated two-fold more total suberin amounts compared to the wild type (Baxter *et al.*, 2009). However, measurements of $Lp_r$ and $P_{sr}$ for NaCl revealed that there were no differences between *esb1* and wild type. When grown in soil, *esb* mutants deposited twice the amount of suberin of wild type plants, as observed for hydroponics, but failed to decrease $Na^+$ accumulation in the shoot (Baxter *et al.*, 2009). In contrast, *esb* mutants had significantly lower levels of $Ca^{2+}$, $Mn^{2+}$, and $Zn^{2+}$ compared to the wild type. This suggested that different nutrient ions use different pathways, i.e. apoplastic or cell-to-cell paths, to radially move into the vascular tissue of roots from the soil solution. For water, the ratio of $Lp_{hy}$ (water transport through the apoplast) and $Lp_{os}$ (water transport through plasma membrane) is close to 1, which reveals that in *Arabidopsis* water is mainly transported via the cell-to-cell pathway (Ranathunge & Schreiber, 2011).

Rice roots of both lowland (cv. IR64) and upland (cv. Azucena) cultivars have lower $Lp_r$ and $P_{sr}$ values compared to *Arabidopsis* and other cereal crops (Tab. 3). In contrast to *Arabidopsis* and barley, which form only an endodermis, rice roots form both the endodermis and exodermis (Ranathunge *et al.*, 2011a). The apoplastic biopolymer, suberin is deposited into both layers, which contribute to the overall resistance for water and solute flows. High-yielding rice is mostly lowland cultivars which often grow in water-logging, anaerobic soils (Shin-ichiro & Ishihara, 1959; Kawata *et al.*, 1964). To cope with anoxia, rice roots develop an internal air channel system (aerenchyma) with a very low resistance to diffuse oxygen from the leaves to the roots. In order to successfully transport oxygen to rapidly-growing root tips without radially losing to anaerobic soils, these roots build up a strong suberized exodermis or a barrier against radial oxygen loss (Colmer et al., 1998; Kotula et al., 2009; Miyamoto et al., 2001; Ranathunge et al., 2011a, 2003). However, increased suberization of the exodermis by stagnant growth did not necessarily reduce the water transport further (Ranathunge *et al.*, 2011a), confirming the earlier finding that the endodermis is the

major barrier for water and nutrient uptake in rice roots (Ranathunge *et al.*, 2003). This is likely due to the differences in the microstructure between suberized endodermis and exodermis (Schreiber *et al.*, 2005b). Rather low $Lp_r$ of rice roots is mainly due to the higher resistance at the endodermis, while the exodermis has a markedly greater permeability which is approximately 30-fold larger than the endodermis (Ranathunge *et al.*, 2003). The higher Lpr of the exodermis is attributed to greater apoplastic bypass flow at the root periphery (Ranathunge *et al.*, 2003, 2004). When grown in oxygen-deprived medium, the exodermis of rice roots is optimized for reduction of oxygen efflux from the root to anaerobic soil substrate, while keeping higher water uptake rates. However, as a trade-off, the suberized and strengthened exodermis, due to anoxia/hypoxia, negatively affected the solute transport, in which the root $P_{sr}$ for NaCl was reduced by 60%, whereas, the root selectivity for NaCl or $\sigma_{sr}$ was increased by 55% (Ranathunge et al., 2011). In contrast to rice, development of an exodermis in corn by mist culture reduced the $Lp_r$ by 3-fold (Zimmermann & Steudle, 1998).

Barley roots form no exodermis, thus only the suberized endodermis acts as a sole barrier to block water and solvent movement into the vascular tissue of the root. When comparing different root zones of barley, the basal or older part of the root, where the endodermis is fully suberized, the $Lp_r$ is markedly lower compared with the apical zone, which is less suberized or remains as a patchy structure (Ranathunge *et al.*, 2017). Comparison of measured $Lp_r$ and calculated Lpr from the Lp of individual cortical cells revealed that at least one-quarter of water moves across the root via apoplast (Ranathunge *et al.*, 2017). Different studies of $Lp_r$ measurements in barley roots revealed that the values are in the same range and comparable (Knipfer & Fricke, 2010, 2011; Suku *et al.*, 2013; Ranathunge *et al.*, 2017). Some cautious should be required, when considering roots as semi-permeable membranes for solutes and their behavior as perfect osmometers. A perfect osmometer should have a reflection coefficient ($\sigma_{sr}$) of one (unity) and a solute permeability ($P_{sr}$) of zero. Since the measured $\sigma_{sr}$ values are smaller than unity and Psr is greater than zero (Tab. 3) for the roots of barley and other plant species, it clearly shows that roots deviate substantially from the predicted ideal osmometer model (Steudle & Peterson, 1998; Steudle, 2000a,b; Ranathunge *et al.*, 2017).

**Table 3: Solute permeability (Psr) and reflection coefficients (σsr) for NaCl of Arabidopsis, rice and barley**

Psr and σsr of individual roots or whole root systems were measured using a root pressure probe (RPP) or a pump perfusion technique, respectively.

| Plant species | Description | Solute permeability $P_{sr}$ ($10^{-9}$m $s^{-1}$ ) | Reflection coefficient ($\sigma_{sr}$) | Reference |
|---|---|---|---|---|
| ***Arabidopsis* - Col-0** | Whole root systems, measured using a root pressure probe (RPP). | 3.0 ± 0.2 | 0.34 ± 0.03 | (Ranathunge & Schreiber, 2011) |
| ***Arabidopsis* - Col-8** | | 2.9 ± 0.2 | 0.35 ± 0.04 | |
| ***Arabidopsis* - *horst*** | | 5.7 ± 0.6 | 0.30 ± 0.02 | |
| ***Arabidopsis* – *esb1-1*** | | 2.8 ± 0.1 | 0.40 ± 0.01 | |
| ***Arabidopsis* – *esb1-2*** | | 2.9 ± 0.2 | 0.41 ± 0.02 | |
| **Rice cv. IR64** | Individual adventitious roots, measured using a RPP. | 1.7 ± 1.0 | 0.28 ± 0.11 | (Miyamoto *et al.*, 2001) |
| **Rice cv. Azucena** | | 0.73 ± 0.32 | 0.28 ± 0.17 | |
| **Rice cv. IR64** | Individual adventitious roots, measured using a RPP. | | 0.18 ± 0.06 | (Ranathunge *et al.*, 2003) |
| **Rice cv. Azucena** | | | 0.16 ± 0.11 | |
| **Rice cv. IR64** | Outer part of the roots, measured using a pump perfusion technique | | 0.09 ± 0.02 | |
| **Rice cv. Azucena** | | | 0.08 ± 0.02 | |
| **Rice cv. Azucena** | Individual roots from stagnant growth, measured using RPP. | 1.2 ± 0.3 | 0.56 ± 0.10 | (Ranathunge *et al.*, 2011a) |
| **Rice cv. Azucena** | Individual roots from aerated growth, measured using a RPP. | 2.5 ± 0.4 | 0.38 ± 0.08 | |
| **Barley cv. Golf** | Individual seminal roots in circulating medium, measured using RPP | | 0.7 ± 0.1 | (Knipfer & Fricke, 2010) |
| **Barley cv. Golf** | Individual seminal roots in stagnant medium, measured using a RPP | | 0.4 ± 0.1 | |
| **Barley cv. Golf** | Individual end segment of seminal roots from aerated growth, well stirred, measured using a RPP | 2.8 ± 0.5 | 0.51 ± 0.09 | (Ranathunge *et al.*, 2017) |

## 2.5 Conclusions

Suberin, a heterogeneous secondary cell wall biopolymer, can build an effective apoplastic barrier against water and solute movement as well as pathogen penetration into plant roots. Increased root suberin by abiotic stresses such as drought, salinity, anoxia, organic acids and higher nutrient levels or decreased root suberin by low nutrients or even genetic manipulations (*horst* mutant) often coincided with decreased or increased root water ($Lp_r$) and solute permeabilities ($P_{sr}$). In contrast, *Arabidopsis* mutants such as *esb1* with increased suberin amounts failed to reduce $Lp_r$ of roots. These results indicate that the predicted assumption of increased amount of root suberin negatively correlates with water and solute transport of roots, which was often found in studies where plants were exposed to abiotic stresses (Steudle & Peterson, 1998; Zimmermann *et al.*, 2000; Schreiber *et al.*, 2005b), may not always be correct. Suberin composition, microstructure of suberized barriers (e.g. how suberin clogs the intermicrofibrillar spaces of cell walls), as well as the specific location of the barrier in roots also play an important role to make an efficient functional barrier for water and solute transport (Schreiber *et al.*, 2005b; Ranathunge & Schreiber, 2011). Thus it is of great importance to know the root anatomy, suberin composition and suberin amounts, and the location of the suberin barrier in roots to better understand and predict the connection between suberized barriers and root water transport in plants. Since root anatomy and suberization significantly differ among plant species such as *Arabidopsis*, barley and rice, a simple and straightforward transfer of knowledge on root water transport will not always be justified.

In the future, accessibility of new mutants preferentially in crops, such as barley and rice may help to identify and verify suberin genes and their function/s. More detailed studies of altered suberin compositions, amounts and their effect on water and solute transport will improve our knowledge and might help to develop breeding strategies making these crops more stress (e.g. drought and salt) tolerant.

# 3 Chapter 2: Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses

Tino Kreszies[1,*], Nandhini Shellakkutti[1], Alina Osthoff[2], Peng Yu[2], Jutta A. Baldauf[2], Viktoria V. Zeisler-Diehl[1], Kosala Ranathunge[3], Frank Hochholdinger[2], and Lukas Schreiber[1]

[1]Department of Ecophysiology, Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, 53115 Bonn, Germany.
[2]Crop Functional Genomics, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, 53113 Bonn, Germany.
[3]School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley 6009, Perth, Australia.

*Author for correspondence: Tino Kreszies
Email: kreszies@uni-bonn.de
Tel.: +49 228 73 4996

*In Press* in New Phytologist, doi: 10.1111/nph.15351

**Summary**

- Barley (*Hordeum vulgare* L.) is more drought tolerant than other cereals, thus making it an excellent model for studying the chemical, transcriptomic and physiological effects of water deficit. Roots are the first organ to sense soil water deficit. Therefore, we studied the response of barley seminal roots to different water potentials induced by PEG 8000.
- We investigated changes in (1) anatomical parameters by histochemistry and microscopy, (2) quantitative and qualitative changes in suberin composition by analytical chemistry, (3) transcript changes by RNA-Seq and (4) the radial water and solute movement of roots using a root pressure probe.
- In response to osmotic stress, genes in the suberin biosynthesis pathway were up-regulated that correlated with increased suberin amounts in the endodermis and overall reduction of hydraulic conductivity ($Lp_r$). In parallel, transcriptomic data indicated no or only weak effects of osmotic stress on aquaporin expression.
- These results indicate that osmotic stress enhanced cell wall suberisation and markedly reduced $Lp_r$ of the apoplastic pathway, whereas $Lp_r$ of the cell-to-cell pathway was not altered. Thus, the sealed apoplast markedly reduced the uncontrolled back flow of water from the root to the medium while keeping constant water flow through the highly-regulated cell-to-cell path.

**Keywords:**

Apoplast; barley; osmotic stress; transcriptomics; root; suberin; water deficit; water transport

## 3.1 Introduction

Climate changes and extreme weather conditions, such as drought, will become more intensive in the future (Melillo *et al.*, 2014). This will have a major impact on agricultural productivity. Compared with other abiotic stresses, drought accounts for the highest crop losses (Boyer, 1982). Barley (*Hordeum vulgare* L.) is more drought tolerant than other crop plants, and represents the fourth most abundant cereal after wheat, maize and rice (http://faostat.fao.org). Other than drought, barley is also fairly resistant to other abiotic stresses such as salinity, alkalinity and cold and can survive better under non-optimal environmental conditions (Colmer *et al.*, 2006; Kosová *et al.*, 2014). These unique properties make barley a model crop for studying the effect of abiotic stresses in general. Drought starts with decreasing of the soil water potential. Consequently, plant roots are the first organs which sense drought and have to cope with water deficiency (Zingaretti *et al.*, 2013).

The main function of roots is water and nutrient uptake, which is highly dependent on anatomical structures, growth conditions and plant age. Water and solute uptake of plant roots is best described by the composite transport model. According to the model, there are three major pathways for water and solute transport in roots: (i) the apoplastic (cell walls), (ii) the symplastic and the (iii) transcellular pathway. The latter two are also referred to as cell-to-cell pathway. The apoplastic pathway can be blocked by Casparian bands and suberin lamellae in endodermal and exodermal cell walls. The cell-to-cell pathway can additionally be regulated by aquaporins (Steudle & Peterson, 1998; Peterson & Cholewa, 1998; Steudle, 2000a,b).

The formation of the biopolyester suberin was shown to be enhanced by abiotic (Hose *et al.*, 2001; Enstone *et al.*, 2002; Krishnamurthy *et al.*, 2009; Ranathunge *et al.*, 2011a; Barberon *et al.*, 2016; Kotula *et al.*, 2017) and biotic stresses (Lulai *et al.*, 1998; Thomas *et al.*, 2007; Ranathunge *et al.*, 2008; Lanoue *et al.*, 2010). The suberin lamellae contains a polyaliphatic and a polyaromatic domain, which are poylmerized (Kolattukudy *et al.*, 1975; Bernards, 2002). The aliphatic monomers are primary alcohols, fatty acids, α–ω dicarboxylic acids (diacids) and ω-hydroxy acids (ω-OH acids), while the aromatic components are ferulic- and coumaric acids (Schreiber *et al.*, 1999; Graça, 2015). Casparian bands are mainly constituted of lignin and partly of suberin (Schreiber, 1996; Zeier & Schreiber, 1998; Schreiber *et al.*, 1999; Naseer *et al.*, 2012). Lignin consists of syringyl, guaiacyl and p-hydroxypenol monomers which form a complex aromatic biopolymer (Fraser & Chapple, 2011; Lupoi *et al.*, 2015).

Here, the effect of water deficit induced by osmotic stress through PEG 8000 on suberized barrier development in barley roots and its physiological effects are reported. Apoplastic barrier development along the root using microscopy and histochemical studies of barley roots grown under different low water potentials were investigated. Subsequently, changes in root suberization and global gene expression patterns during the different root developmental stages in response to osmotic stress were quantified. Finally, the effect of osmotic stress on water and solute transport in roots using a root pressure probe was studied. These findings indicate that an increased amount of suberin could be an effective adaptation to water deficit due to sealing of roots and preventing uncontrolled passive water loss from the root to the dry soil by back flow via the non-selective apoplastic pathway. At the same time, roots maintain uptake of water through the cell-to-cell pathway.

## 3.2 Material and methods

### 3.2.1 Plant material and growth conditions

Seeds of barley (*Hordeum vulgare spp. vulgare* cv. Scarlett) were stratified for one week at 4°C. Then they were germinated in the dark at 25 °C covered with wet filter paper. After three days, seedlings were transferred into an aerated hydroponic system containing half–strength Hoagland solution in a climatic chamber under long day conditions (16 h : 8 h, light : dark), an air temperature of 23 : 20 °C (day : night) and a relative humidity of 50–65%. When the plants were six days old, stress treatment was applied for another six days in all experiments described thus plants were grown for 12 days (Fig. 1a) and at this stage they had two leaves and five to six seminal roots.

### 3.2.2 Water deficit application induced by osmotic stress through PEG 8000

Low water potentials were applied when the plants were six days old (Fig. 1a). Plants were moved from the half–strength Hoagland solution (20 mOsmol/kg or -0.04 MPa of osmotic pressure) to half–strength Hoagland solution adjusted to a defined water potential with PEG8000 (Roth, Karlsruhe, Germany) simulating water deficit induced by osmotic stress. The water potential of the medium was reduced up to -0.4

MPa, -0.8 MPa and -1.2 MPa by adding 17.5%, 25.4% and 31.6% (w/w) PEG8000 (Michel, 1983). The water potential of the nutrient solutions with different levels of PEG8000 were measured using a WP4C Water Potential Meter (METER Group, USA).

Simulating water deficit by PEG8000 treatment represents a widely accepted experimental approach offering various important advantages. An exactly defined and homogeneous osmotic potential acting on the roots can be adjusted. Since in nature water stress during drought mostly occurs in a combination with heat and highlight, PEG treatment allows to look at water deficit separately (Kramer and Boyer, 1995; Verslues *et al.*, 2006; Frolov *et al.*, 2017). In addition for our experiments hydroponic culture was essential because only with this approach root transport properties using the pressure probe technique could be measured.

### 3.2.3 Histochemical detection of Casparian bands and suberin lamellae in roots

Cross-sections were made at 1 cm increments along the whole seminal root using a cryostat microtome (Microm HM 500M, Microm International, Walldorf, Germany). To detect the development of Casparian bands over the root length, cross-sections were stained with 0.1% (w/v) berberine hemisulfate for 1 h and with 0.5% (w/v) aniline blue for 30 min (Brundrett *et al.*, 1988). Suberin lamellae were stained with 0.01% (w/v) lipophilic fluorol yellow 088 for 1 h (Brundrett *et al.*, 1991). Cross-sections were analyzed by epifluorescence microscopy using an ultraviolet (UV) filter set (excitation filter BP 365, dichroic mirror FT 395, barrier filter LP 397; Zeiss, Oberkochen, Germany). Pictures were taken with a Canon EOS 600D camera at ISO 200 or 400 for 1 s to 2 s.

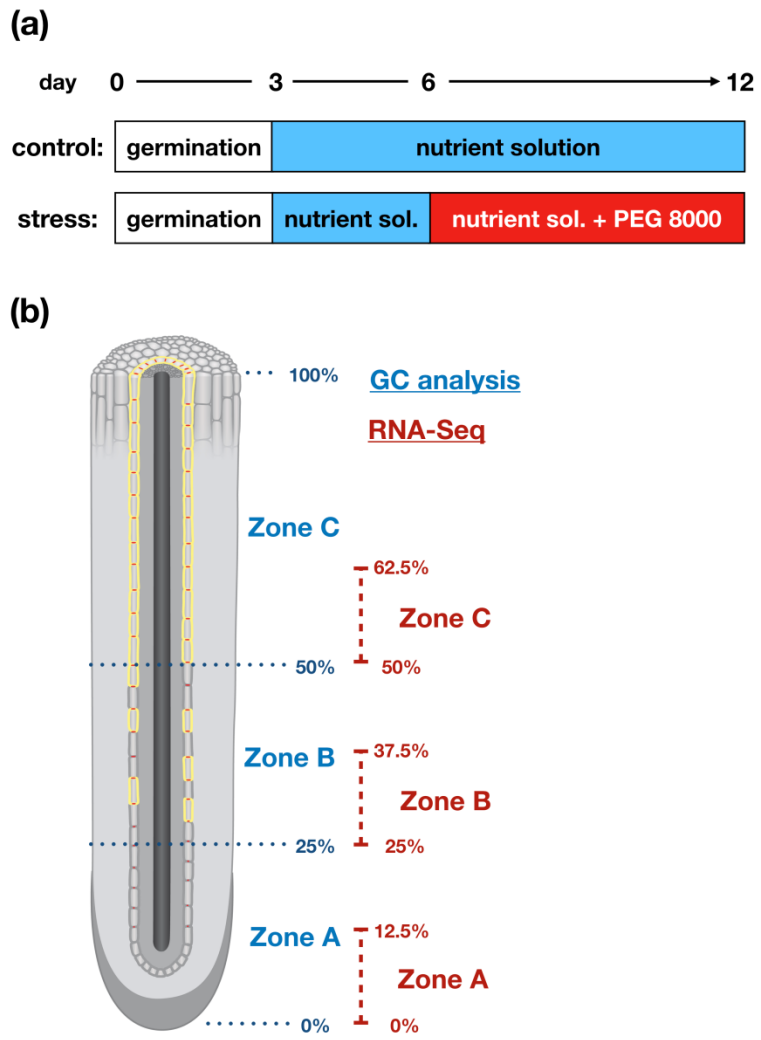


**Fig. 1:** Experimental Setup of long-term osmotic stress. (a) Schematic diagram of growth conditions and low water potential application with PEG8000. After three days of germination seedlings were transferred to hydroponic nutrient solution. For stress treatment the nutrient solution was exchanged with nutrient solution adjusted to a defined water potential with PEG8000 at day six. When the plants were twelve days old they were harvested for experiments. (b) Schematic diagram showing the different root zones, which were harvested for the GC analysis (blue) and RNA-Seq analysis (red). The seminal roots were divided into three zones based on the developmental of apoplastic barriers such as Casparian bands and suberin lamellae. For suberin analysis by gas chromatography, three zones were selected: (1) zone A - from 0% to 25%, (2) zone B - from 25% to 50% and (3) zone C from 50% to 100% of the total seminal root length. For RNA-Seq analysis, the length of the zones were reduced to avoid an overload of material and to get more specific information. Here, zone A corresponds to 0% to 12.5%, zone B from 25% to 37.5% and zone C from 50% to 62.5% of the total seminal root length.

### 3.2.4 Chemical analysis of barley root suberin

The seminal roots were divided into three zones - A, B and C based on the previous microscopic investigations (Fig. 1b). Zone A (0-25% of total root length) was the youngest part of the root, which included the root apex. In this zone, only Casparian bands were present in the endodermis but no suberin lamellae were deposited. Zone B (25-50%) was the transition zone, in which all endodermal cells had Casparian bands, but only a limited number of cells had suberin lamellae depositions. Zone C (50-100%) was the mature part of the root close to the root base, in which all endodermal cells characterized by the presence of Casparian bands and suberin lamellae (Fig. 1b).

For each replicate, ten segments of seminal roots from each of the three zones were pooled together. The root segments were enzymatically digested for three weeks with 0.5% (w/v) cellulase and 0.5% (w/v) pectinase at room temperature under continuous shaking (Zeier & Schreiber, 1997). The enzyme solution was replaced four times within the three weeks and roots were vacuum infiltrated with the solution. Subsequently, isolated cell walls were washed in borate buffer and then transferred to 1:1 (v/v) chloroform:methanol for soluble lipid extraction at room temperature under continuous shaking for two weeks. The chloroform:methanol solution was replaced four times. Finally, samples were dried on PTFE in a desiccator, containing activated silica gel. The dried samples were subjected to transesterification with $BF_3$-methanol to release suberin monomers (Kolattukudy & Agrawal, 1974). Gas chromatographic analysis and mass spectrometric identification were performed as described earlier (Zeier & Schreiber, 1997, 1998). Suberin amounts were referred to the endodermal surface area. Endodermal area was calculated for each root zone: $A = 2\pi \cdot r \cdot L$ (r = endodermis radius; L = length of the individual root zone). Three biological replicates were used for each experiment.

### 3.2.5 RNA isolation

For RNA isolation, five seminal roots from five 12 day old barley plants grown under control or -0.8 MPa osmotic stress conditions were pooled. Samples of each of the three root zones were taken for specific transcriptome analysis. Different from samples taken for chemical analysis only half of each zone was collected (Fig. 1b). The samples were collected in 2 mL reaction tubes with sterile steel beads inside. The

sample was frozen in liquid nitrogen and ground with a mixer mill (Retsch MM400) at a frequency of 30 rounds per second for one minute. RNA was isolated with the RNeasyPlus Universal Mini Kit (Qiagen, Venlo, Netherlands). RNA quality was analyzed via NanoDrop and Agilent Bioanalyzer. For all samples, a RNA integrity number ≥ 9.1 was detected. Four biological replicates were used for each experiment.

### 3.2.6 Processing of raw reads and analysis of differentially expressed genes

Raw sequencing data of 100 bp paired-end reads, obtained with an IlluminaHiSeq 4000 sequencer (BGI TECH SOLUTIONS, Hong Kong, China), was processed with CLC Genomics Workbench Version 10.0.1 (https://www.qiagenbioinformatics.com/) for further analyses. After quality trimming for low quality scores and ambiguous nucleotides, only reads with a length of more than 40 bp were retained for mapping. These reads were mapped to the barley reference genome, Ensembl plants: Hv_IBSC_PGSB_v2, v2.36 (Mascher *et al.*, 2017, ftp://ftp.ensemblgenomes.org/pub/plants/release-36/fasta/hordeum_vulgare/dna/) allowing large gaps of up to 50 kb to span introns. Only reads that matched uniquely with ≥80% of their length and an identity of ≥90% to the reference genome were considered as mapped. Stacked reads, i. e. read pairs that have identical start and end coordinates and orientation, were merged into one. Subsequently, the remaining reads were mapped to the high-confidence annotation of the genome sequence (Mascher *et al.*, 2017, ftp://ftp.ensemblgenomes.org/pub/plants/release-36/gff3/hordeum_vulgare/; v2.36). Sequences had to match with ≥90% of their length and ≥90% similarity to the set of high confidence gene models. Reads with more than one hit were excluded from subsequent read counting. Prior to differential expression analysis, read counts were normalized by sequencing depth and $\log_2$-transformed to meet the assumptions of a linear model. Furthermore, the mean-variance relationships were estimated and used to assign precision weights to each observation to adjust for heteroscedasticity (Law *et al.*, 2014). To test the quality of the data, samples were clustered in a multidimensional scaling plot (MDS plot) using the plotMDS function implemented in the Bioconductor package limma (Smyth, 2005) in R (R Version 3.4.0, limma_3.32.2). Distances between sample pairs were displayed as the leading $\log_2$-fold changes ($\log_2$FC), which are defined as the estimated root-mean-square deviation for the top 500 genes with the

largest standard deviation among all samples. This analysis provided a visual representation of sample relationships by spatial arrangement. To assess differences in gene expression between osmotic stress treatment and control in each root tissue, a linear model including a fixed effect for treatment and tissue and an interaction effect was applied. An empirical Bayes approach was used to estimate the variability over all genes in the fitted model and to shrink the variances towards a common value (Smyth, 2004). The contrast.fit function of the R package limma was used to compute pair-wise comparisons between osmotic stress treatment and control for each tissue. To correct calculated p-values of the performed pairwise t-tests for multiplicity, the false discovery rate (FDR) was adjusted to ≤5% according to Benjamini & Hochberg, 1995. Transcripts per million (TPM) for each gene (Table S1) was calculated according to Wagner *et al.*, 2012. The raw sequencing data have been deposited at the NCBI sequence read archive (SRA accession: SRP136092).

### 3.2.7 Functional annotation and Gene Ontology (GO) analysis

Annotations were retrieved from *EnsemblPlants* (Kersey *et al.*, 2016http://plants.ensembl.org/index.html) and the IPK Barley BLAST server (Deng *et al.*, 2007; http://webblast.ipk-gatersleben.de/barley_ibsc/downloads/). AgriGOv2.0 (Tian *et al.*, 2017) was used for Singular Enrichment Analysis (SEA) by comparing the list of differentially expressed genes with the customized annotated reference from the IPK Barley BLAST server. The cross comparison of SEA (SEACOMPARE) tool was used to combine the SEA results.

Putative barley orthologous of suberin, lignin, fatty acid elongation and aquaporin genes are based on known mutants described in *Arabidopsis* and rice (Fraser & Chapple, 2011; Ranathunge *et al.*, 2011c; Li-Beisson *et al.*, 2013; Vishwanath *et al.*, 2015; Kreszies *et al.*, 2018a). The barley genes were retrieved via the IPK Barley BLAST server (Deng *et al.*, 2007) and the orthologous search from *EnsemblPlants* (Kersey *et al.*, 2016).

### 3.2.8 Root pressure probe experiments

Root pressure probe experiments were conducted with the end segments/apical part of the seminal roots lacking lateral roots (zone A and zone B together) as described earlier (Steudle *et al.*, 1987; Ranathunge *et al.*, 2017). The measurements were only performed for plants grown in control and -0.8 MPa treatment conditions. Plants grown in -0.8 MPa PEG8000 solution were transferred back to half–strength Hoagland nutrient solution at least 1 h before root pressure probe measurements. Between 2 and 4 h after fixing to the pressure probe, roots reached the steady-state root pressure. In the hydrostatic experiments, water flow was induced by moving the micrometer screw forward and backward, and thus inducing radial water flow out of or into the root. The subsequent pressure changes were used to calculate hydraulic conductivity ($Lp_r$) of the roots from the half times of water exchange ($t_{1/2}^{w}$):

$$k_{wr} = \frac{\ln(2)}{t_{1/2}^{w}} = Lp_r * A_r * \beta$$

β (MPa · $m^{-3}$) is the elastic coefficient of the measuring system. It was measured by inducing step changes in the volume, which results in changes in the root pressure. $A_r$ is the surface area of the root segment mounted on the pressure probe. The hydraulically isolated non-conductive part of the root was approximately 15 mm from the root apex.

For the osmotic experiments, the nutrient solution was rapidly exchanged with nutrient solution containing 30 mM NaCl (60 mOsmol · $kg^{-1}$). To minimize the effect of unstirred layers, the medium was stirred with aeration during all experiments. The changes in root pressure in response to the osmotic change in the medium were biphasic. A rapid water phase was followed by a slower solute phase. The water phase was used to calculate the osmotic hydraulic conductivity of the root. The solute phase was used to calculate solute permeabilities ($P_{sr}$) of NaCl:

$$k_{sr} = \frac{\ln(2)}{t_{1/2}^{s}} = \frac{A_r * P_{sr}}{V_x}$$

$k_{sr}$ is the rate constant of permeation of solutes. Here $t_{1/2}^{s}$ is the half-time of solute exchange and $V_x$ is the volume of functional xylem in the root. It was 1.5 % measured in the cross-sections of seminal roots. Total root volume was calculated with the conductive root length and the root diameter. Reflection coefficients ($\sigma_{sr}$) of NaCl were calculated with:

$$\sigma_{sr} = \frac{\Delta P_r}{\Delta \pi^{\circ}_{s}} \exp(k_{sr} * t_{min})$$

$\Delta P_r$ is the maximum change in root pressure and $t_{min}$ is the time which is required to reach the minimum root pressure. $\Delta\pi^0_s$ is the change in the osmotic pressure of the medium, which is calculated as $\Delta\pi^0_s = R \cdot T \cdot C_s$, with R = universal gas constant, T = absolute temperature, $C_s$ = osmolarity of the solute (60 mOsmol · $kg^{-1}$).

At the end of each measurement roots were cut close to the seal to check the proper fixation of the root: if the root pressure did not drop rapidly down to zero and if there was no drastic decrease in $t^w_{1/2}$ to approx. one order of magnitude faster than during hydrostatic pressure relaxations, the roots were discarded. This usually happens due to overtight of roots at the fixing point of the pressure probe that blocked the xylem vessels.

### 3.2.9 Statistical analysis of chemical and physiological data

Data analysis and statistical tests were performed with Origin Pro 9. Normal distribution of the data was tested with Shapiro-Wilk test. Since all data was normal distributed we tested for statistical significance of differences between means of plants grown under different water potentials at a significance level of 0.05. Two sample t-test for root pressure probe experiments or analysis of variance (Fisher LSD) for chemical analyses.

## 3.3 Results

### 3.3.1 Root morphology and anatomy

The average length of 12 day old barley seminal roots decreased in increasing osmotic stress treatments (-0.4, -0.8 and -1.2 MPa) (Fig. 2). Reduction in root length at -0.4 MPa (21.5 ± 4.0 cm) was not statistically different from control conditions (22.9 ± 5.5 cm), whereas root length was significantly reduced at -0.8 MPa (19.2 ± 6.9 cm) and -1.2 MPa (19.3 ± 3.6 cm). Seminal root length was not significantly different for two lowest water potential treatments of -0.8 MPa and -1.2 MPa (Fig. 2).

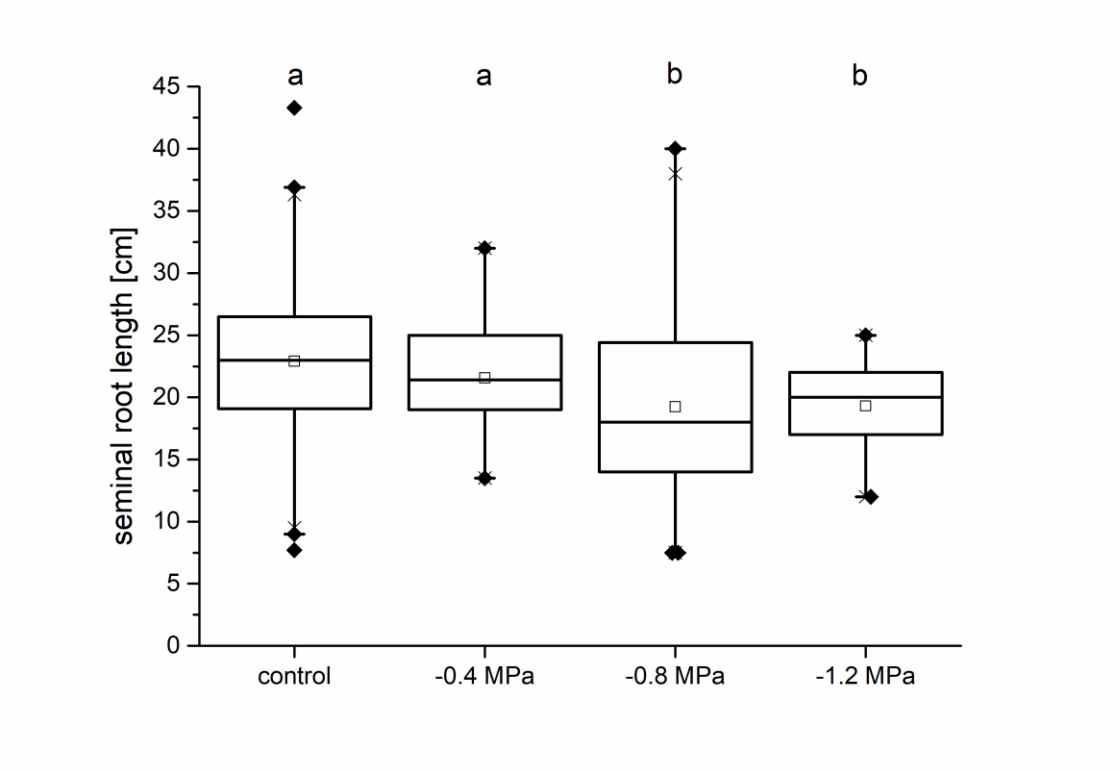


**Fig. 2:** Root lengths of 12-day-old barley plants, grown under control conditions or at a water potential of -0.4 MPa, -0.8 MPa or -1.2 MPa. The boxes range from 25 to 75 percentiles. The square in the box represents the mean value. The whiskers range to the outliers. Each box represents more than 150 individual seminal roots. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way ANOVA (Fishers LSD).

Endodermal Casparian bands were visible even near the root apex as small dot-like structures (Fig. 3a and e). Starting at 12.5% of the root length, they develop to continuous bands in the radial cell wall (Fig. 3). There were no obvious differences between the control (Fig. 3a to d) and water stressed plants (-0.8 MPa) in the development of Casparian bands (Fig. 3e to h). Casparian bands were not detected in the rhizodermis of control and water-deficit plants, even in the older root zones, where Casparian bands were completely developed in the endodermis. Thus, barley seminal roots fail to develop an exodermis, even under osmotic stress conditions.

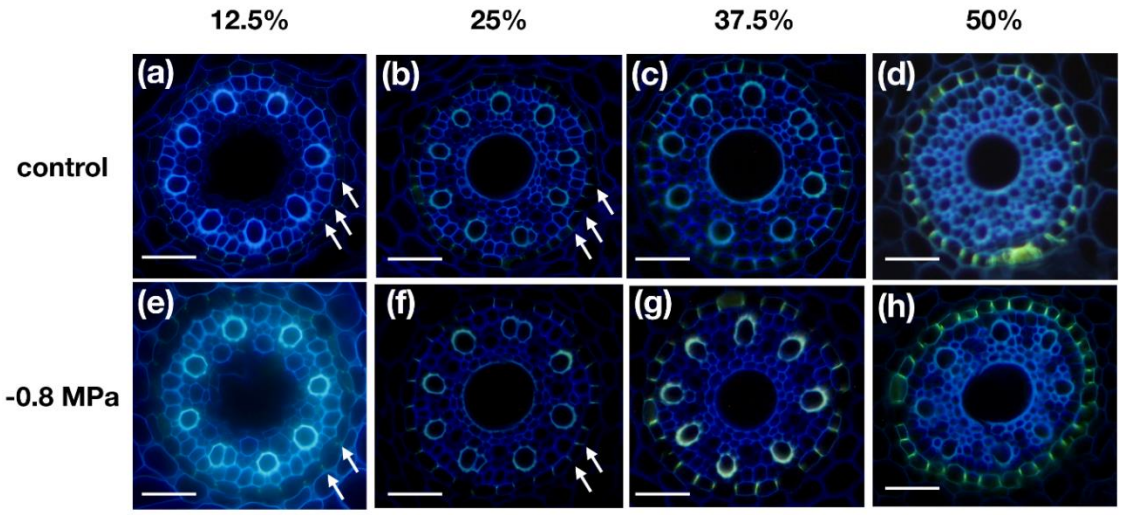


**Fig. 3:** Development of Casparian bands in the endodermis of barley seminal roots in different root zones (Fig. 1b). Casparian bands of roots grown under control (a - d) and in presence of -0.8 MPa (e - h) were stained with berberine aniniline sulfat. The presence of Casparian bands is indicated by yellow fluorescence. At a distance of 12.5% thin Casparian bands are visible (arrows), which increase in length and fluorescence intensity going from 25%, via 37.5% to 50% relative root length. Bars = 50 µm.

The suberin lamellae in the endodermis started to deposit further back from the root tip than the Casparian bands and were not detectable at 12.5% of the total root length (Fig. 4a, e, i and m). In control and all osmotic stress treatments, first appearance of single suberized cells was observed at 25% of the root length (Fig. 4b, f, j and n). At 37.5% of the total root length, there was a patchy development of suberization detected in the endodermis of both control and osmotic stress treatment (Fig. 4c, g, k and o). At higher osmotic stress levels of -0.8 MPa and -1.2 MPa, the number of suberized cells in the endodermis was higher compared with the control (Fig. 4k and o). At 50% of the root length, the endodermal cells were fully suberized (complete ring of suberized cells) in control and all osmotic stress treatments (Fig. 4d, h, i and p).

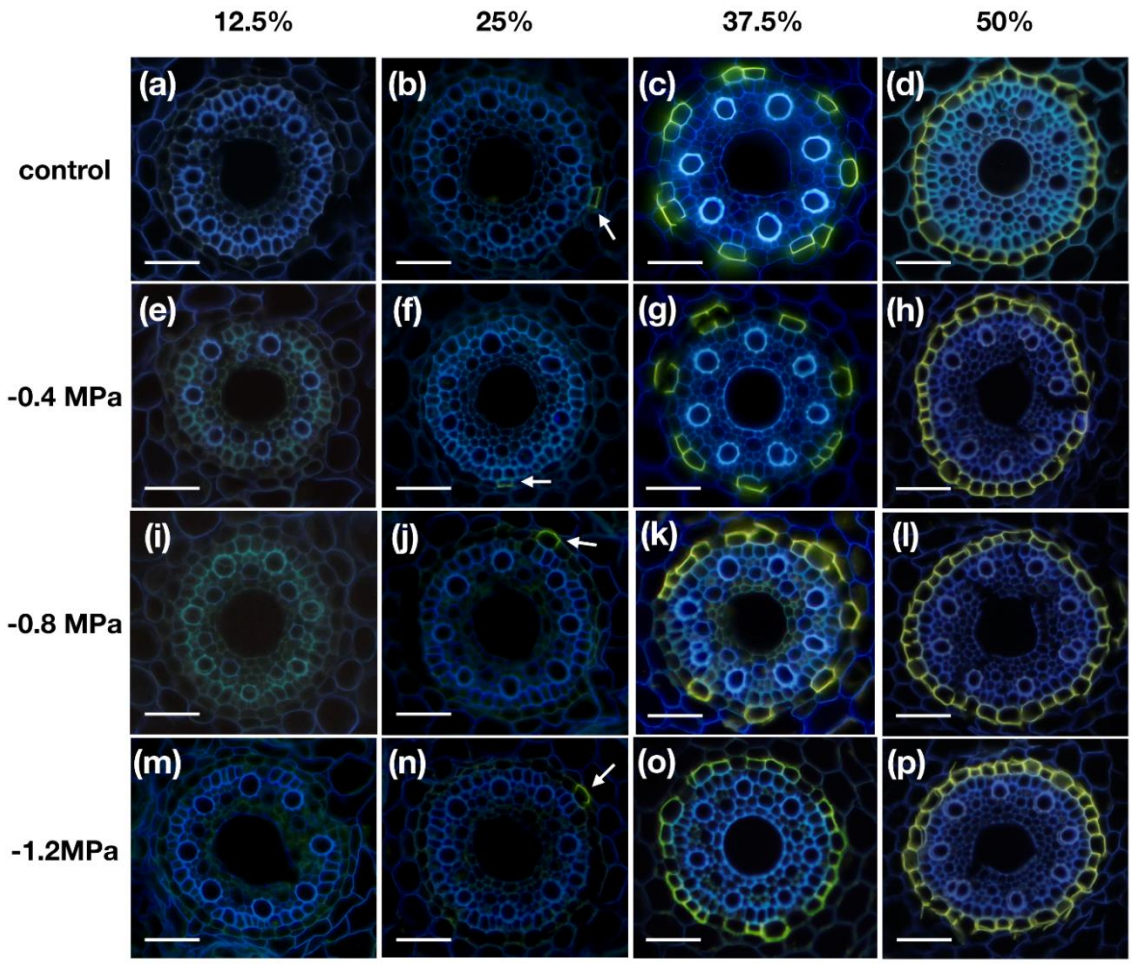


**Fig. 4:** Development of suberin lamellae in the endodermis of barley seminal roots. Suberin lamellae in different root zones (Fig. 1b) of roots grown under different water potentials were stained with fluorol yellow 088. The presence of suberin lamellae is indicated by a bright yellow fluorescence. At a distance of 12.5% no suberin lamellae is visible (a, e, i and m). At 25% of relative root length first single only partially suberized cells (arrows) are visible (b, f, j and n). At 37.5 % of relative root length a patchy suberization is visible, which is stronger with in roots grown in the presence of -0.8 MPa and -1.2 MPa (h, k) compared to control (c) and - 0.4 MPa (g). At the distance of 50% the endodermis is complete suberized in all growth conditions (c, f, i and l). Bars = 50 µm.

### 3.3.2 Chemical analysis of suberin of barley seminal roots in response to different osmotic stress levels

For chemical suberin analysis, barley seminal roots were divided into the three zones A, B and C (Fig. 1b), based on the endodermal suberization (Fig. 4). Aliphatic suberin in barley seminal roots was composed of the four monomers classes: alcohols (alc), fatty acids (fa), α,ω-dicarboxylic acids (diacids) and ω-hydroxy acids (ω-OH acids) (Fig. 5). The most abundant aliphatic suberin monomers were the $C_{18:1}$ diacid and

ω-OH acids ($C_{18:1}$ and $C_{24}$ ω-OH acids) (Fig 5 and 6). Chain length of the different suberin monomers varied from $C_{16}$ to $C_{26}$ (Fig. 6). Aromatic suberin component were composed of coumaric and ferulic acids (Fig. S1). There were no significant differences in substance classes (Fig. 5) or single monomer composition (Fig. 6) between control and osmotic stress conditions.

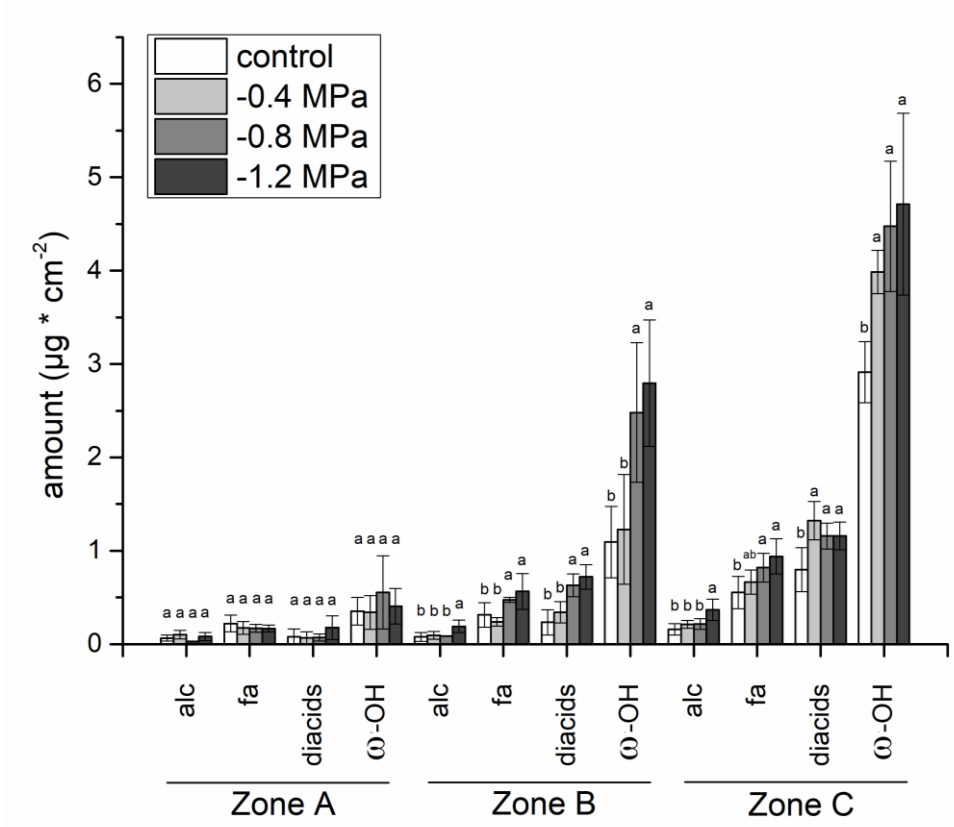


**Fig. 5:** Amounts of substance classes of aliphatic suberin detected in barley seminal roots grown under control conditions or at water potentials of -0.4 MPa, -0.8 MPa or -1.2 MPa. The roots were divided into three root zones from the apical root tip zone A over zone B to the basal part zone C. The substance classes are primary alcohols (alc), fatty acids (fa), α–ω dicarboxylic acids (diacids) and ω-hydroxy acids (ω-OH). The bars represent mean values with standard deviation of three biological replicates. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way ANOVA (Fishers LSD).

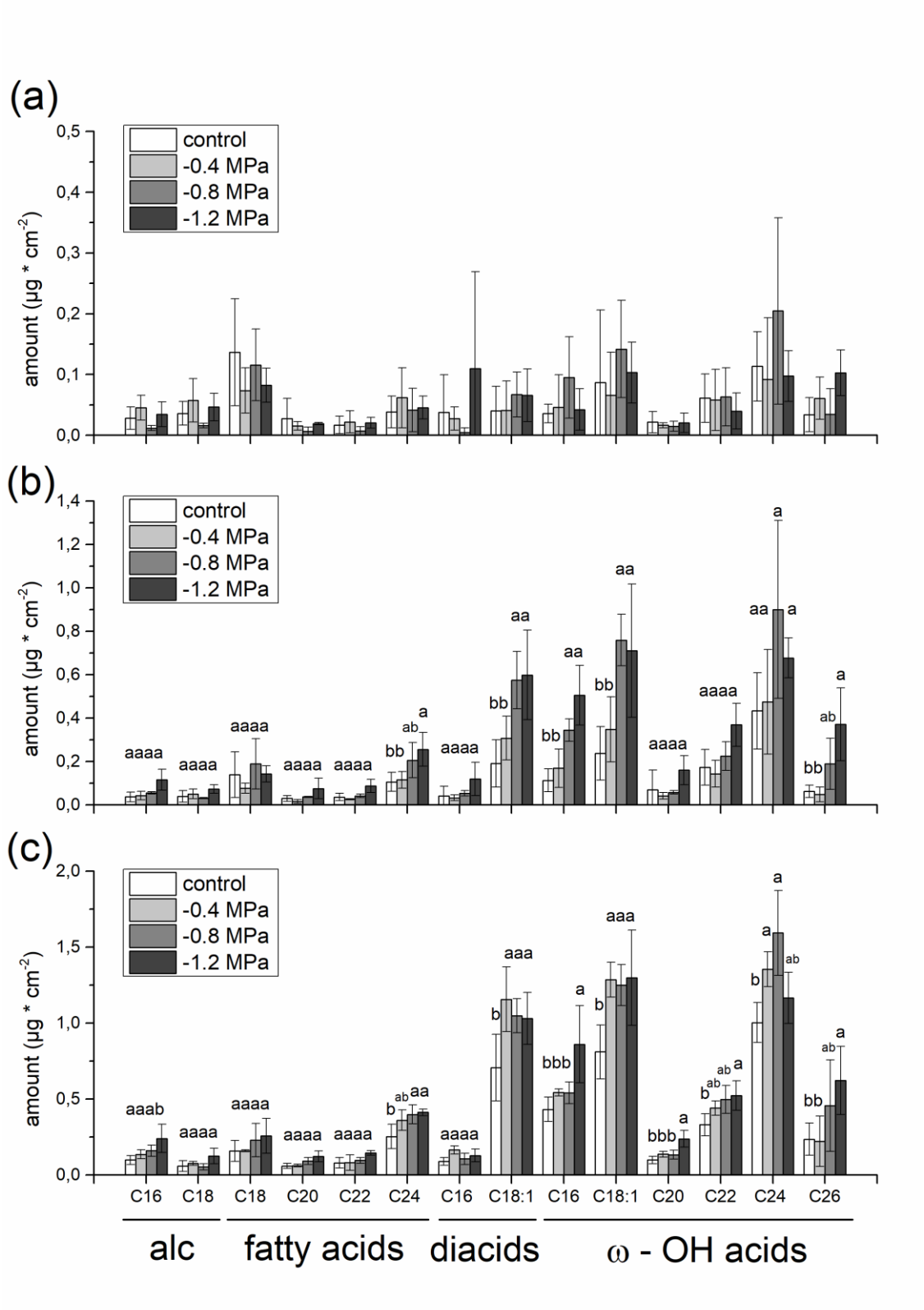


**Fig. 6:** Amounts of monomers of aliphatic suberin detected in barley seminal roots grown under control conditions or at water potential of -0.4 MPa, -0.8 MPa or -1.2 MPa. The roots were divided into three root zones from the apical root tip (a) zone A over (b) zone B to the basal part (c) zone C. The bars represent mean values with standard deviation of three biological replicates. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way ANOVA (Fishers LSD). In (a) zone A no significant difference were detected.

However, the absolute (Fig. 5 and 6) and the relative amounts (Fig. S2) of substance classes changed over the length of the root from zone A to zone C in all treatments (control and osmotic stress conditions). This change was pronounced in particular for the total amounts of aliphatic (Fig. 7a) and aromatic suberin (Fig. 7b). Barley seminal roots showed a significant increase in total aliphatic and aromatic suberin (Fig 7a, b) from zone A to C (Fig 5), which correlated well with the suberin histochemical observations (Fig. 3). Comparing the severity of osmotic stress treatments on the degree of aliphatic suberization, there was no significant difference between treatments in zone A (Fig. 7a). In zone B, mild osmotic stress (-0.4 MPa) did not significantly enhance suberization in comparison to the control. However, stronger osmotic stress treatments of -0.8 MPa and -1.2 MPa increased the aliphatic suberin amounts by two-fold compared to the control and -0.4 MPa (Fig. 7a). In zone C, all water stress treatments significantly increased the aliphatic suberin amounts compared to the control, but to there was no significant difference between the treatments (Fig. 7a). In contrast to the total aliphatic suberin (Fig. 7a), the total aromatic suberin content significantly increased from zone A to C but there were no significant differences between control and osmotic stress treatments (Fig. 7b). In control, the total aromatic suberin amount was two-fold higher than aliphatic suberin, but this ratio decreased under water stress, because of the increase of aliphatic suberin (Fig. 7).

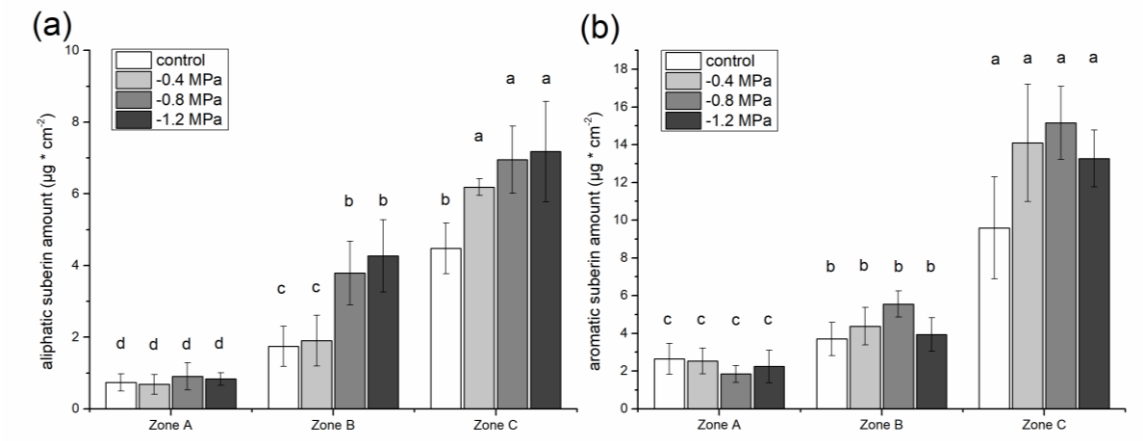


**Fig. 7:** Total amounts of (a) aliphatic and (b) aromatic suberin in barley seminal roots grown under control conditions or at water potentials of -0.4 MPa, -0.8 MPa or -1.2 MPa. The roots were divided into three root zones from the apical root tip zone A over zone B to the basal part zone C. The bars represent mean values with standard deviation of three biological replicates. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way ANOVA (Fishers LSD).

The increase in aliphatic suberin between the three zones was mainly due to increases in the amounts of diacids and ω-OH acids (Fig. 5 and 6). For example, the amount of alcohols and fatty acids in zone C was twice the amount of zone A, but this was a ten-fold increase for diacids and ω-OH acids (Fig. 5). In osmotic stress treatments, this increment was even more pronounced with a twelve-fold increase in diacids and ω-OH acids in zone C compared with zone A (Fig. 6). The relative amounts of fatty acids and alcohols decreased from 33% and 9% in zone A to 12% and 4% in zone C, respectively; whereas, the diacids and ω-OH acids increased from 9% and 49% in zone A to 18% and 66% in zone C, respectively (Fig. S2).

### 3.3.3 Transcriptome analysis of barley seminal roots using RNA-Seq

To identify global gene expression changes in barley seminal roots with respect to suberin development, total RNA was extracted from the three root zones (A, B and C) from control and -0.8 MPa conditions (Fig. 1b) and subjected to RNA-Sequencing (RNA-Seq). We chose a water potential of -0.8 MPa for the stress treatment, because the responses of roots for growth and suberization were more pronounced compared with -0.4 MPa but not different form the treatment with -1.2 MPa (Fig. 2, 4 and 7).

RNA-Seq yielded on average 35 million reads for each of the four biological replicates per zone by treatment combination. In a multidimensional scaling plot, the replicate samples of the three root zones and the control versus stress conditions clustered separately, and were thus clearly distinguishable (Fig. 8a). Analysis of differentially-expressed genes with FDR ≤5% showed that in total 5531 unique genes were up-regulated and 5146 unique genes were down-regulated. However, the response to osmotic stress was also root zone specific with 1101, 1139 and 1204 unique up-regulated genes and 750, 2980 and 227 unique down-regulated genes in the zones A, B and C, respectively (Fig. 8b, Table S2). Functional categorization was performed by using preliminary annotated barley gene ontology (GO) terms from the IPK barley server (Deng *et al.*, 2007), and identification of significantly enriched GO terms by single enrichment analysis with AgriGOv2 (Tian *et al.*, 2017). The analysis showed 95 unique enriched GO terms when comparing the differentially expressed genes between the three root zones under control and stress conditions (Table S3). Significantly enriched biological processes in response to osmotic stress shared by three root zones

were (1) organic acid metabolic process, (2) carboxylic acid metabolic process and (3) oxoacid metabolic process (Table S3).

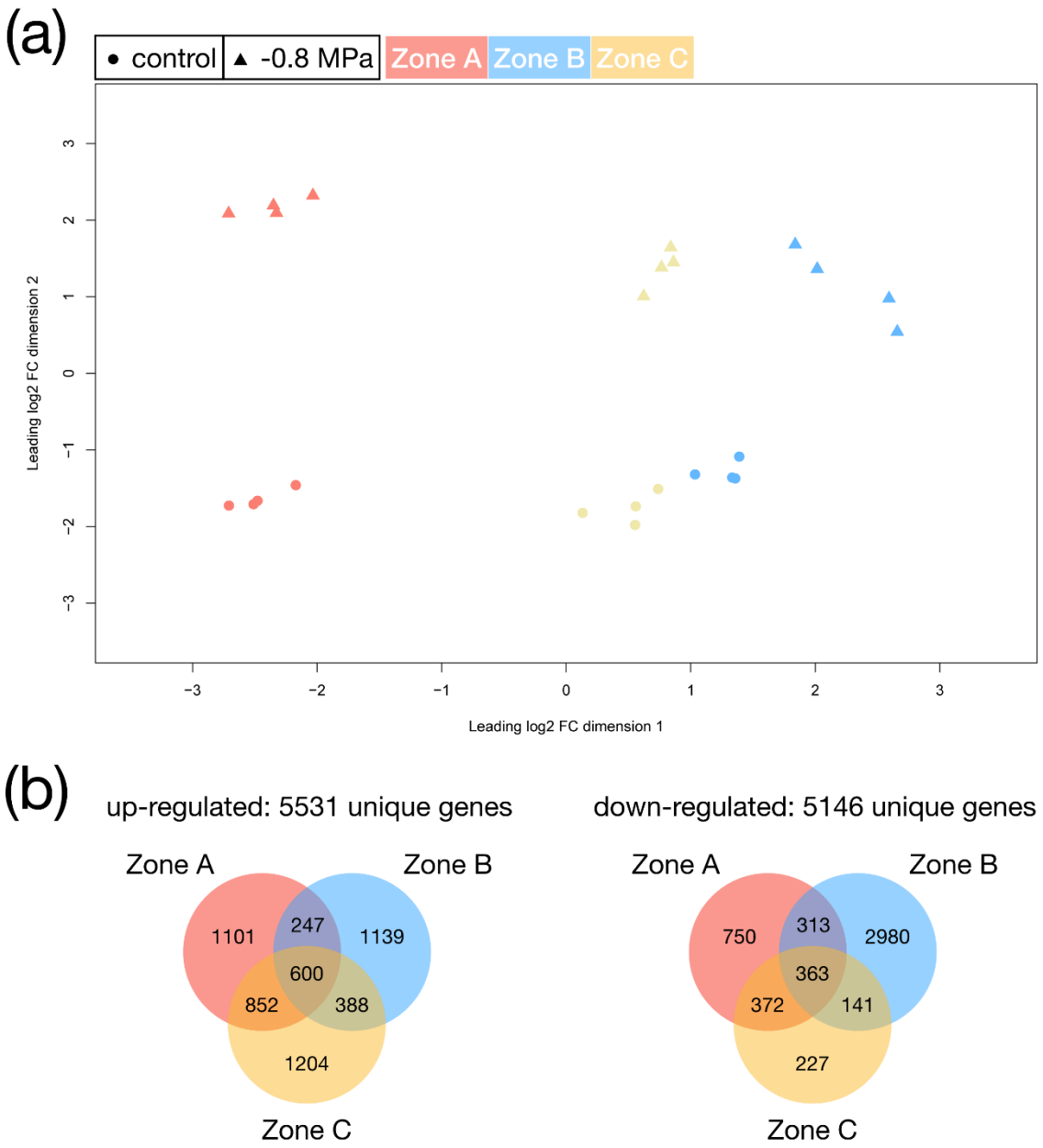


**Fig. 8:** (a) Multidimensional scaling plot of barley seminal root zones, grown under control or water potential of -0.8 MPa. The roots were divided into three root zones from the apical root tip zone A over zone B to the basal part zone C. Dots: control, triangles: -0.8MPa, Zone A: red, Zone B blue and Zone C: yellow. (b) Numbers of differentially expressed genes in barley root zones in response to osmotic stress. Overlap of the 5531 up-regulated genes. Overlap of the 5146 down-regulated genes.

A significant up-regulation of barley suberin genes in control as well as in -0.8 MPa treatments was detected in all three root zones (Fig. 9). In most cases, the highest expression was in zone B (Fig. 9). In total, more suberin genes were up-regulated in zone B and C with higher $log_2FC$ values compared with zone A (Fig. 9). On average, the expression of aquaporin genes was 50-times higher than barley suberin associated genes in barley roots. In addition, different from suberin genes, expression of the majority of barley aquaporin genes was not significantly different in response to osmotic stress, in which few genes were up- and few genes were down-regulated. Only HORVU1Hr1G047100, a putative NIP5;1 ortholog (portable aquaporin for boric acid and water), was highly up-regulated in all three root zones (Table S4). Genes from the phenylpropanoid pathway, which are involved in the biosynthesis of lignin that is part of the composition of Casparian bands and which is heavily deposited in the central cylinder of roots, were also found to be up-regulated (Table S4).

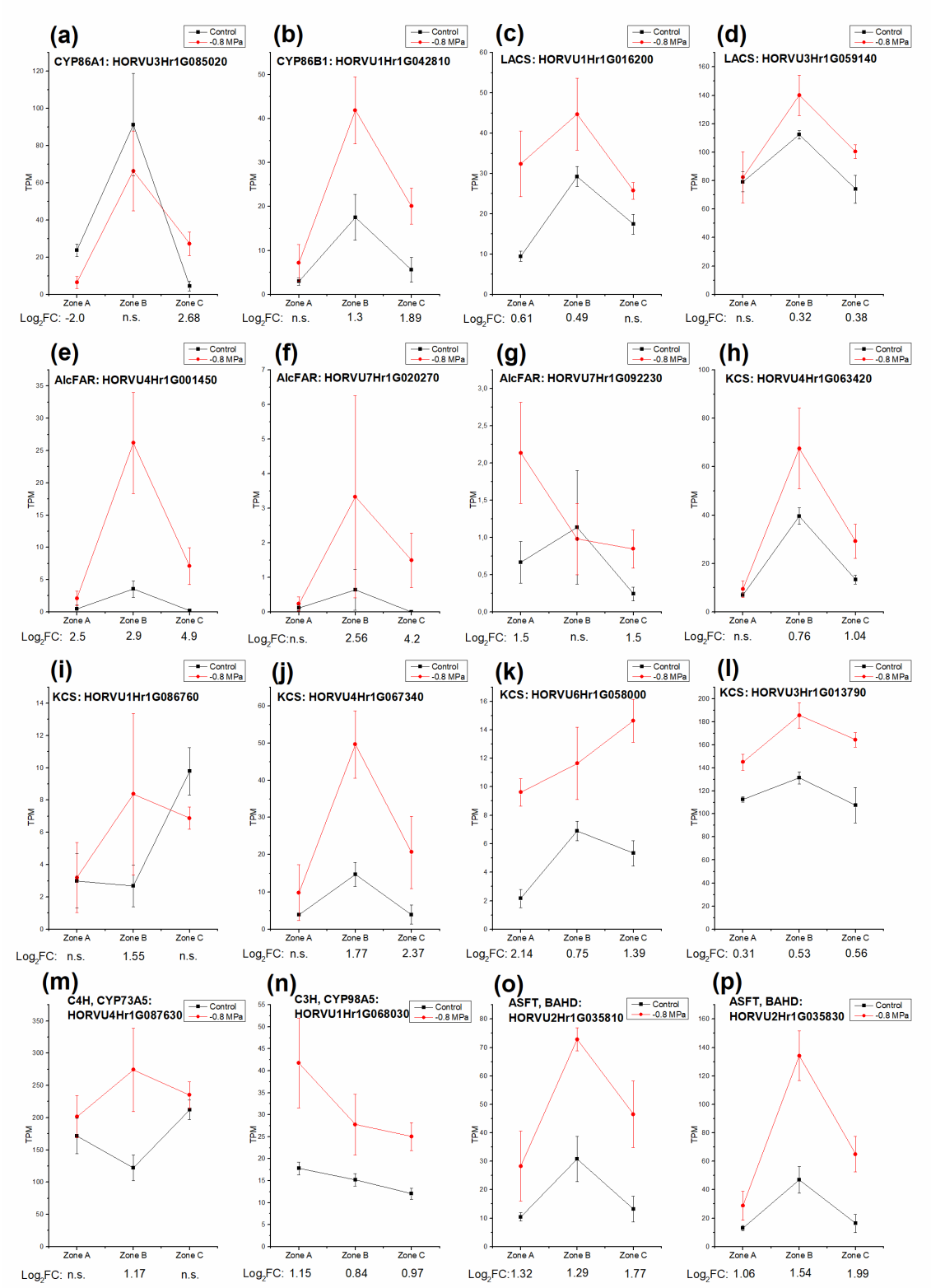


**Fig. 9:** Expression patterns of most highly up-regulated suberin biosynthesis genes in barley roots obtained by RNA-seq. The roots were divided into three root zones from the apical root tip zone A over zone B to the basal part zone C. Transcripts per millions (TPM) for the root zones A, B and C of selected genes and their $log_2FC$ in response to osmotic stress is given. $Log_2FC$ are given when control and PEG8000 treated roots display significantly different expression levels at a significance level of 0.05 in pairwise t-tests. n.s. represents not significant changes. (a and b) Cytochromes P450 converting fatty

acids into ω-hydroxy acids and α-ω dicarboxylic acids. (c and d) LACS: Long-Chain Acyl-CoA Synthetases. (e, f and g) AlcFAR: Alcohol-forming Fatty Acyl-CoA Reductase. (I, j, k and l) KCS: Ketoacyl-CoA Synthase from the fatty acid elongation complex. (m and n) Cytochromes P450 synthesize coumaric and ferulic acids. (o and p) ASFT/BAHD: Aliphatic Suberin Feruloyl Transferase link aliphatic and aromatic suberin monomers to suberin building units.

### 3.3.4 Hydraulic conductivity, solute permeability and reflection coefficient of barley seminal roots in response to osmotic stress

Similar to the RNA-Seq analysis we have chosen a water potential of -0.8 MPa to compare the hydraulic conductivity ($Lp_r$) and solute permeability of barley seminal roots between control and osmotic stress conditions (Table 1). The hydrostatic $Lp_r$ was significantly reduced by 2.5-fold (from 8.11 to $3.19 \cdot 10^{-8}$ $m \cdot s^{-1} \cdot MPa^{-1}$) in response to osmotic stress. In contrast, the osmotic $Lp_r$ did not change in response to osmotic stress (Table 1). Thus, the ratios of hydrostatic/osmotic $Lp_r$ declined in the osmotic stress treatment and showed that there is a shift of water flow from the apoplastic pathway to the cell-to-cell pathway in the treatment of osmotic stress (-0.8 MPa).

Solute permeability ($P_{sr}$) of roots for NaCl was also reduced by the osmotic stress treatment compared with the control, but was not statistically significant because of the high variability among the water stressed roots (Table 1). There was no change in the reflection coefficient ($\sigma_{sr}$) for NaCl in response to osmotic stress treatment compared with the control (Table 1).

**Table 1:** Hydrostatic and osmotic hydraulic conductivity ($Lp_r$), solute permeability ($P_{sr}$) and reflection coefficient ($\sigma_{sr}$) for NaCl of individual barley seminal roots grown under control or osmotic stress conditions (water potential of -0.8 MPa). Values are given as means ± SD of eight independent replicates (n=8). Different letters indicate significant differences at significance level of 0.05, analyzed using a two-sample t-test.

| **Parameters** | Control | -0.8MPa (osmotic stress) |
|---|---|---|
| Hydrostatic $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | 8.11 ±2.37 a | 3.19 ± 1.45 b |
| Osmotic $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | 3.15 ± 3.0 a | 3.59 ± 1.91 a |
| Hydrostatic/Osmotic | 4.27 ± 2.58 a | 1.11 ± 0.36 b |
| Solute permeability $P_{sr}$ ($10^{-9}$m $s^{-1}$ ) | 2.24 ± 1.54 a | 0.61 ± 0.61 a |
| Reflection coefficient ($\sigma_{sr}$) | 0.38 ± 0.06 a | 0.38 ± 0.17 a |

### 3.4 Discussion

Plant roots are the first organs sensing water deficit in dehydrating soil and thus play a crucial role in plant drought responses. In this approach, multifaceted techniques were used to test the hypothesis that an increased suberization of barley roots could represent an efficient response to water deficit by limiting uncontrolled, passive water loss from roots to the dry soil. By adding different concentrations of PEG8000 to the nutrient solutions of hydroponically growing barley plants, specific water potentials from mild (-0.4 MPa) to more severe water deficit (-0.8 and -1.2 MPa) were adjusted.

One of the most important parameters in seedling root system architecture in response to osmotic stress is seminal root length, because barley seminal roots contribute to overall root water uptake during early development (Knipfer & Fricke, 2010). At more negative water potentials of -0.8 MPa and -1.2 MPa, barley roots developed 10% significantly shorter seminal roots compared to control and mild osmotic stress treatment (-0.4MPa) (Fig. 2). This phenotypic alteration of seminal roots is likely due to osmotically-driven reduced cell elongation and organ development in declining water potentials (Yamaguchi & Sharp, 2010) resulting in reduced root length.

Detailed knowledge of the anatomy of the developmental stages along the root was important for our further analyses including chemical, transcriptomic and water transport measurements and their interpretations (Steudle & Peterson, 1998; Steudle, 2000b; Kreszies *et al.*, 2018a). The suberin lamellae were only visible in the endodermis and we detected no exodermis not even under the most severe osmotic stress conditions (-1.2 MPa) applied. This is very different compared to other crop plants such as rice and maize which develop a strong exodermis in response to stress (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2011a, 2016). Our results on barley seminal root anatomy are consistent with previous studies (Knipfer & Fricke, 2011; Ranathunge *et al.*, 2017).

In the youngest root zone (0% and 12.5% from the root tip), suberized cells were never detected (Fig. 4a, e, i and m) and only Casparian bands were visible in some instances. First single suberized cells appeared at the border of zone A to zone B at 25% (Fig. 4b, f, j and n). At the beginning of 50% of the root length more than 90% of the endodermal cells were suberized (Fig. 4d, h, l and p). The histochemical observations show that barley roots undergo strong suberization in response to osmotic stress (Fig. 4), which was observed previously in plant roots as general response towards abiotic stresses (Hose *et al.*, 2001; Enstone *et al.*, 2002; Krishnamurthy *et al.*, 2009, 2011;

Ranathunge *et al.*, 2011c; Shiono *et al.*, 2014a; Barberon *et al.*, 2016; Tylová *et al.*, 2017). Nevertheless, histochemical studies on suberization only provide a qualitative picture of root developmental status, whereas direct analytical methods such as gas chromatography and mass spectrometry can be used for quantification of suberin amounts (Schreiber *et al.*, 2005b).

Suberin monomers obtained after transesterification belonged to fatty acids, alcohols, ω-OH acids and diacids (Fig. 5). Aromatic monomers consisted of coumaric and ferulic acid (Fig. S1). This is in accordance with typical suberin compositions described in the literature (Kolattukudy & Agrawal, 1974; Bernards, 2002; Ranathunge *et al.*, 2011c; Graça, 2015). Different from aliphatic suberin monomers, results of a much higher amounts of aromatic monomers (coumaric and ferulic acid) should be interpreted cautiously, because they can be also bound to all other cell walls in *Graminaceae* species (Carpita, 1996). The suberin monomer composition under control conditions of this study (Fig. 5 and 6) is comparable to a previously described suberin composition in the barley cultivar Golf (Ranathunge *et al.*, 2017), suggesting that suberin monomer composition is well conserved in barley roots even under osmotic stress conditions.

Our chemical analysis confirmed the increase of root suberization along the root and in response to osmotic stress (Fig. 7), also observed by microscopy (Fig. 4). A very low suberization was already observed in zone A (0-25%). This is consistent with the observation of first single suberized cells appearing at the border of zone A to zone B at 25%. However, in the distal half of zone A (0-12.5%), only Casparian bands were detectable in some instances (Fig. 3) and suberin lamellae have never been found with fluorol yellow 088 staining in this root zone (Fig. 4). Interestingly, our transcriptomic data clearly showed that suberin biosynthesis genes were already expressed in this youngest root zone (Fig. 9). Either fluorol yellow 088 staining may not be specific enough to detect very thin suberin lamellae in that zone or the measured suberin monomers were derived from Casparian bands. A third possibility, which cannot be excluded at the moment, could be that histochemically undetectable suberin lamellae are synthesized and deposited somewhere else to the cell walls in this youngest root zone (0-12.5%) which might explain why suberin biosynthesis genes are up-regulated in this zone.

Nevertheless, this observation is of major interest, since there is an ongoing debate, whether the chemical composition of Casparian bands is exclusively pure lignin or a

mixture of lignin as the major component and of suberin also occurring in minor amounts. In isolated Casparian bands of *Clivia miniata, Monstera deliciosa,* soybean, pea and maize mainly lignin but also suberin was detected by GC-MS analyses (Karahara & Shibaoka, 1992; Schreiber *et al.*, 1994, 1999; Schreiber, 1996; Zeier & Schreiber, 1997, 1998; Zeier *et al.*, 1999; Thomas *et al.*, 2007). In fact, just recently direct Raman Scattering Microscopic investigations of Casparian bands in maize roots reported that they are composed of both polymers lignin and suberin (Man *et al.*, 2018). On the other hand, it was concluded from promoter GUS assays of suberin genes with specific endodermal expression in *Arabidopsis* roots that Casparian bands are exclusively made of lignin but not suberin (Naseer *et al.*, 2012).

A final conclusion regarding the presence or absence of suberin as additional polymer in Casparian bands cannot be drawn at the moment for barley roots, since different results were obtained from different species and different experimental approaches. Caution should be exercised when transferring results obtained from *Arabidopsis* to other plant species, including crop plants. Such simple and direct one to one correlations may not always be valid (Kreszies *et al.*, 2018a). On the other hand, future experimental approaches with higher resolution allowing for example the direct analysis of the chemical composition of Casparian bands of *Arabidopsis* roots might help answering this question. Alternatively, the best option would be an endodermis specific transcriptomic analysis by RNA-Seq in combination with chemical analyses of isolated and purified endodermal cell walls, which will provide a higher sensitivity and accuracy than qualitative histochemical staining techniques.

Results of our RNA-Seq analysis in barley roots displayed root zone-specific differential gene expression in response to osmotic stress. This is in agreement with the recently published data for maize and rice roots (Shiono *et al.*, 2014b; Opitz *et al.*, 2016). It was obvious that the transition zone B (25-37.5%) had the highest expression of suberin biosynthesis genes in barley roots for both control and osmotic stress conditions (Fig. 9). This confirms microscopic observations (Fig. 4) and chemical analyses (Fig. 7) that in zone B there was a rapid and pronounced increase in endodermal suberization. In response and adaptation to water stress (-0.8 MPa), suberin genes were often significantly up-regulated in zone B compared with the control (Fig. 9), leading to a faster and greater root suberization. This can be interpreted as a strategy of the root to efficiently block the apoplastic pathway preventing uncontrolled water losses from the root to the surrounding medium/soil.

During the developmental transition of the root from zone A to B, there was a pronounced shift in suberin monomer composition from mono-functional fatty acids to ω-OH and diacids (Fig. S2). This can also be explained by the higher expression of suberin biosynthesis genes such as HORVU3Hr1G085020 and HORVU1Hr1G042910, which are directly located after the fatty acid synthesis in the suberin biosynthesis pathway (Fig. 9 and Fig. S3). In zone C, where the highest amount of suberin (Fig. 7) and a completely suberized endodermis was detected (Fig. 4), the expression of suberin biosynthesis genes became lower compared with zone B, but it was not completely turned off (Fig. 9). Our data shows that there is a maximum amount of about 7 µg $cm^{-2}$ of aliphatic suberin in barley seminal roots in response to osmotic stress (Fig. 7). Since roots failed to develop an induced exodermis in barley under osmotic stress, the endodermal suberin is attributed to the total root suberin. This amount is more than double the amount of *Arabidopsis* suberin (1.5 to 3 µg $cm^{-2}$) (Ranathunge & Schreiber, 2011) but still lower than endodermal suberin measured in rice under different abiotic stress conditions (8 to 12.5 µg $cm^{-2}$) (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2011a, 2016).

In drying soils, it is a major advantage for plants to increase suberization in the older basal part of roots to prevent the backflow of water (Steudle & Jeschke, 1983; Steudle & Peterson, 1998; Steudle, 2000b). At the same time the root tip continuously grows into deeper wet soil layers searching for water. It has been described that the maximum radial water uptake in barley roots occurs through this weakly-suberized younger zone that included the root tip, whereas water uptake is significantly decreased in the strongly-suberized basal part of the root (Sanderson, 1983; Ranathunge *et al.*, 2017). Our measured water and solute permeability values under control conditions with the root pressure probe (Table 1) are perfectly in line with earlier measured values of barely roots in different studies (Knipfer & Fricke, 2010, 2011; Ranathunge *et al.*, 2017).

In response to osmotic stress, there was a 2.5-fold decrease in overall hydrostatic hydraulic conductivity ($Lp_r$) of barley roots (Table. 1), which correlated well with a significant increase in aliphatic suberin amounts. This stress-induced aliphatic suberin, which is composed of hydrophobic monomers, markedly reduced the water flow through the apoplast. However, surprisingly, the measured osmotic $Lp_r$ through the cell-to-cell path, which is mainly facilitated by the plasma membrane bound aquaporins (Steudle & Peterson, 1998; Peterson & Cholewa, 1998; Steudle, 2000a,b; Steudle &

Ranathunge, 2007; Maurel *et al.*, 2015; Gambetta *et al.*, 2017) was not curtailed by the rapid development of suberin lamellae and increased suberization of the endodermis under osmotic stress condition (Table 1). This effect was until now only reported in roots of aeroponic grown maize (Zimmermann *et al.*, 2000). Although, in controls, the expression of barley aquaporin genes in roots was much higher compared to suberin biosynthesis genes (Table S4), especially the PIP and TIP aquaporin family members, which are associated with water transport (Maurel *et al.*, 2015), the majority of barley aquaporin genes were not differentially regulated in response to osmotic stress. Some of the aquaporin genes were slightly up-regulated and other genes were slightly down-regulated (Table S4). This supports our results of root osmotic water permeability that the cell-to-cell pathway was not affected by osmotic stress. In previous studies, it has been shown that the effect of aquaporins on osmotic stress varied and was highly depended on plant species and experimental conditions. The gene expression of some aquaporins was up-regulated, but some down-regulated and others were not affected at all (Aroca *et al.*, 2012; Gambetta *et al.*, 2017). It was previously reported that post transcriptional mechanisms such as phosphorylation/dephosphorylation and membrane internalization of aquaporins play a role in the short term response (within hours) of barley roots to salinity/osmotic stress (Kaneko *et al.*, 2015). In contrast our data shows adaption of barley within six days of osmotic stress. This suggests that quick short term reaction and a long term adaption may be different to each other. In the long term changes of root morphology including enhanced suberin in the endodermis have an effect on $Lp_r$ in barley roots.

To get further insights into understanding drought response in general highly and successfully drought adapted plants are of interest. In roots of *Agave deserti*, which experience prolonged drought of several months or even years, it has been described that the endodermis matured much faster with an accelerated suberization, in which suberin lamellae deposited close to the root apex (North & Nobel, 1998, 2000). In addition, root growth stopped and $Lp_r$ decreased by 62%. Following re-watering of these plants, roots started to elongate again and new lateral roots emerged, which were hardly suberized and thus these new roots preferentially enhanced water uptake. These strategies of a highly drought adapted cactus could partially also be applicable for the recovery of drought exposed barley seminal roots.

In conclusion, this multifaceted study showed that water deficit, mimicked by different osmotic potentials through PEG 8000 treatment markedly up-regulated the

suberin biosynthesis genes in barley seminal roots. In contrast, there was no or low effect on the expression of aquaporin genes, which are the regulatory components of water transport through the plasma membrane. The upregulation of suberin biosynthesis genes resulted in an increased endodermal suberization, thus reducing water movements through the apoplastic cell walls to prevent uncontrolled water losses from the root to the dry soil/medium. In contrast, water transport through the cell-to-cell path remained unaffected and thus maintained further efficient water uptake from the soil into the central cylinder of the root. In the future, barley mutants might help to identify further suberin genes and verify their functions. This could help us to better understand how altered suberin compositions and amounts in roots affect/regulate water and solute transport and will help improving future breeding programs to develop drought tolerant barley cultivars.

## 3.5 Supporting Information for Chapter 2

Additional supporting information may be found in the online version of this article.

**Fig. S1** Amounts of aromatic monomers in barley seminal roots grown under control conditions or at water potential of -0.4 MPa, -0.8 MPa or -1.2 MPa.

For chemical analysis the roots were divided into three root zones from the apical root tip Zone A over Zone B to the basal part Zone C (Fig. 1). The bars represent mean values with standard deviation of three biological replicates.

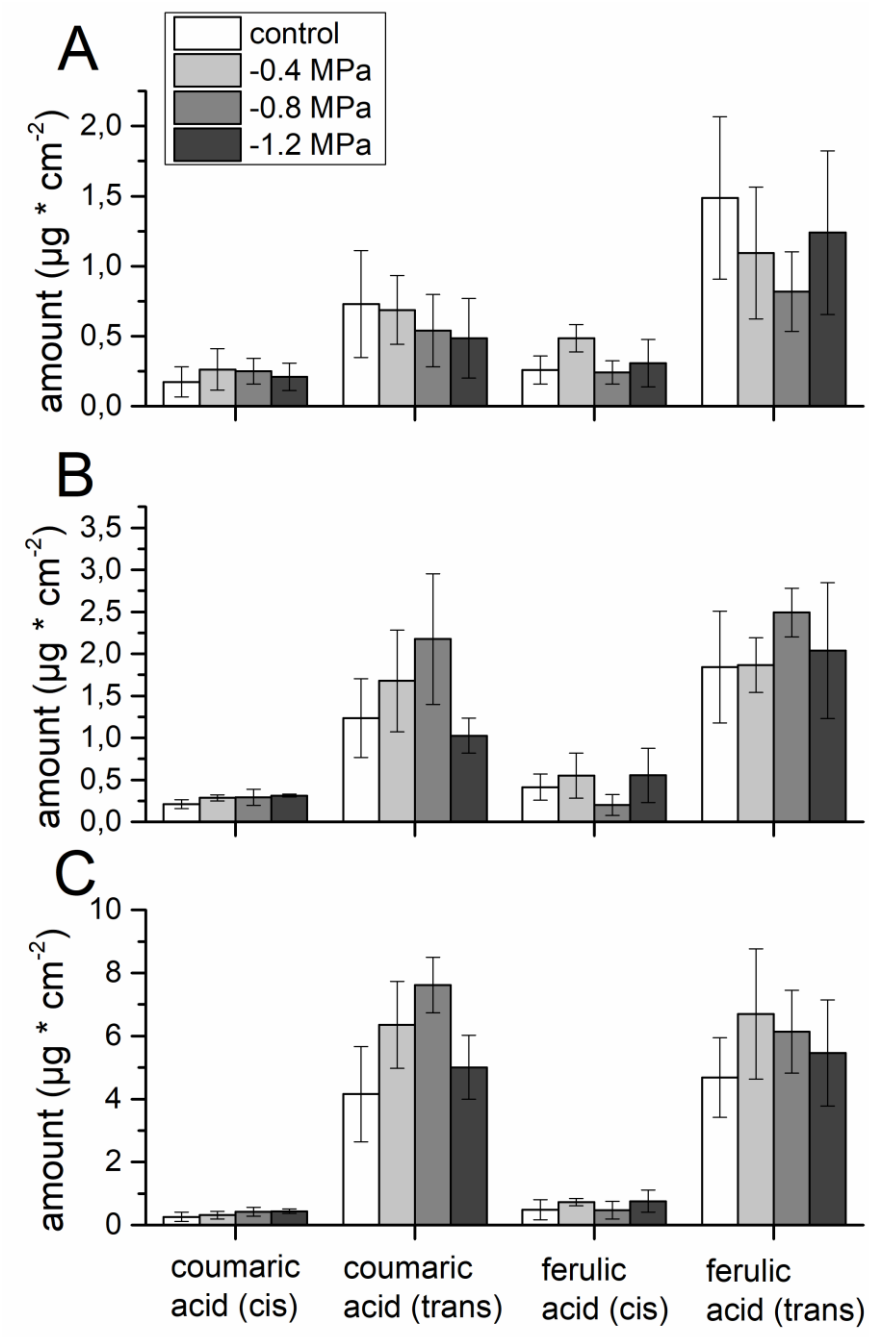

**Fig. S2** Relative amount of aliphatic suberin monomers in barley seminal roots divided in 3 root zones (Fig. 1) grown under control conditions or at water potentials of -0.4 MPa, -0.8 MPa and -1.2 MPa. Alc = alcohol; FA = fatty acids; diacids = α–ω dicarboxylic acids; w-OH = ω-hydroxyl acids

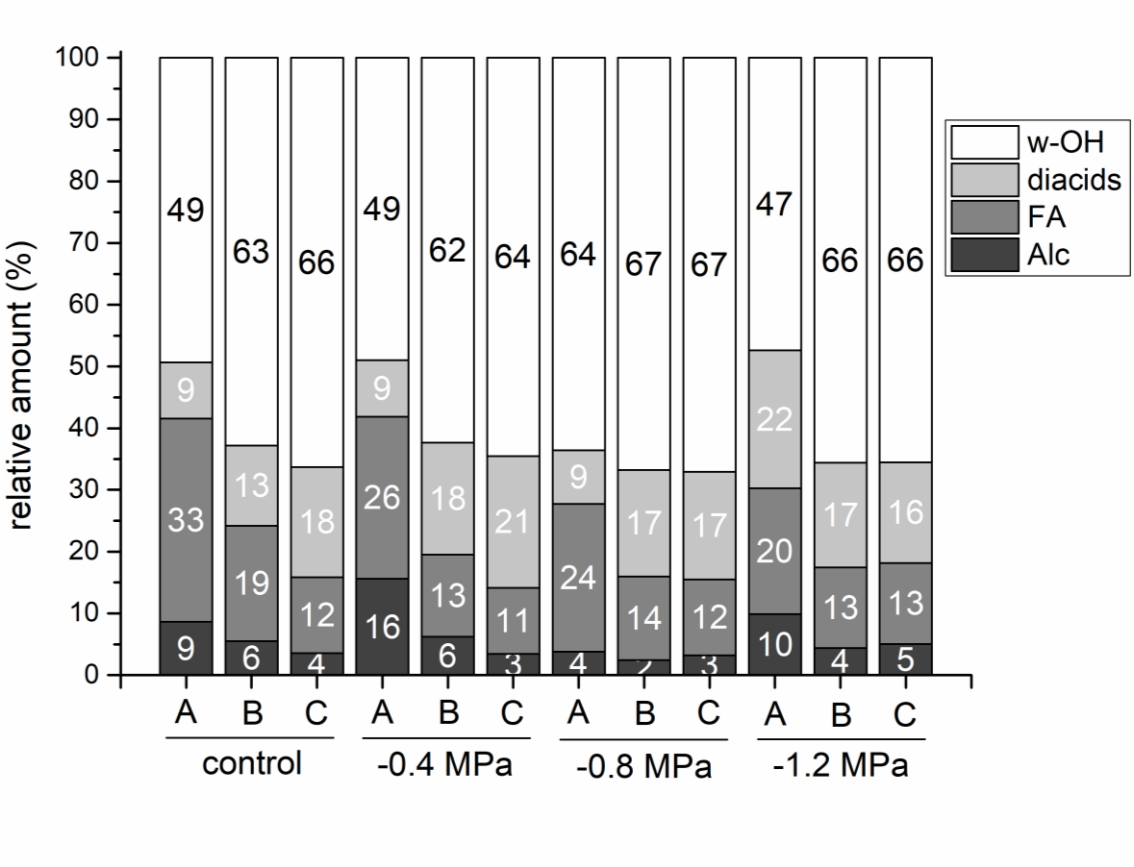

**Fig. S3:** Hypothetical pathway for suberin biosynthesis in barley roots in response to osmotic stress. Genes in red are up-regulated in barley seminal roots in response to drought stress (Fig. 9).
Cytochromes P450 converting fatty acids into ω-hydroxy acids and α-ω dicarboxylic acids. LACS: Long-Chain Acyl-CoA Synthetases. AlcFAR: Alcohol-forming Fatty Acyl-CoA Reductase. KCS: Ketoacyl-CoA Synthase from the fatty acid elongation complex. Cytochromes P450 synthesize coumaric and ferulic acids. ASFT/BAHD: Aliphatic Suberin Feruloyl Transferase link aliphatic and aromatic suberin monomers to suberin building units.

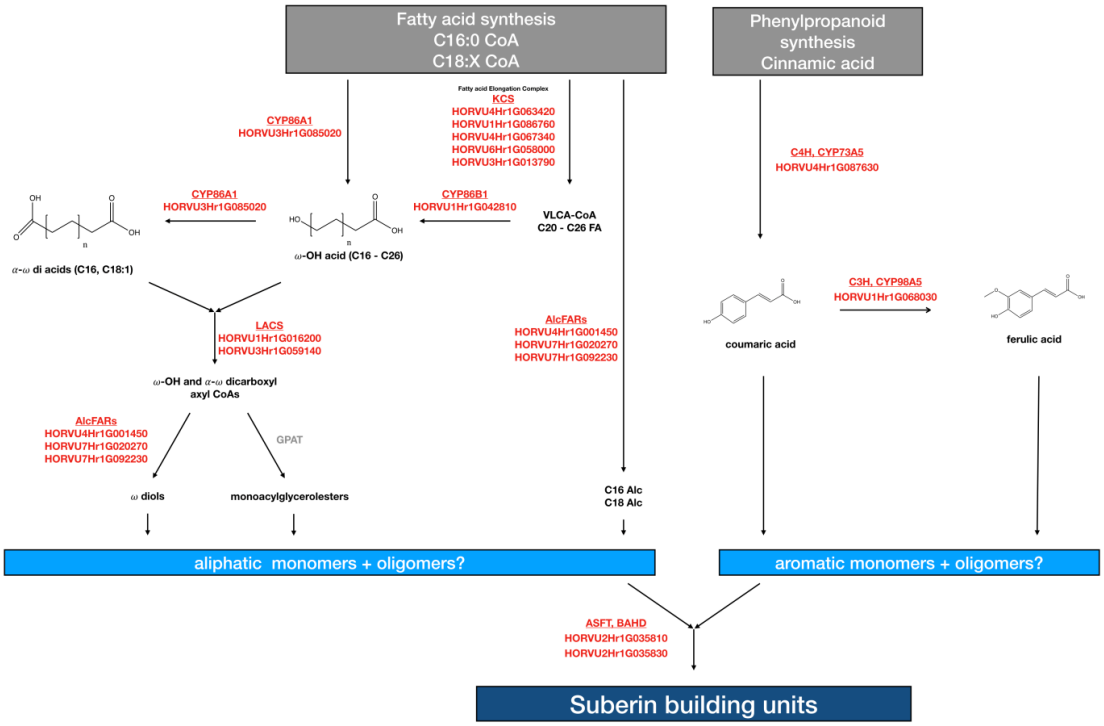

**Table S1:** Complete list of transcript per million values (TPM).
The Table includes the mean values and standard deviation for the three root zones under control and stress treatment of all barley genes IDs

**Table S2:** Complete list of differentially expressed genes.
The Table consists of several sheets which include the barley gene IDs with their log2FC and t, P, adjusted P and B value.
Sheets: A_SvsK; B_SvsK; C_SvsK represent differentially expressed genes in the three root Zones A, B and C each stress versus control
Sheets: K_AvsB; K_BvsC; K_AvsC represent differentially expressed genes in control conditions over the length of the root
Sheets: S_AvsB; S_BvsC; S_AvsC represent differentially expressed genes in control conditions over the length of the root

**Table S3:** Cross comparison of enriched GO terms among differentially expressed genes in the barley seminal root zones A, B and C in response to osmotic stress.
The Table represents the results of the cross comparison of SEA (SEACOMPARE) tool by AgriGOv2.0 as described in material and methods. The sheet “All GO DEG” shows the results of all differently enriched GO while the sheet “GO up and down” separates further between up- and down-regulated enriched GO terms.
The Colour model (CM) shows how small the term's adjusted p-value is. The more significant statistically, the colour is darker and redder. Grey is not significant.

**Table S4:** DEG and TPM values of barley suberin, aquaporin, lignin and fatty acid elongation genes.
This Table includes DEG and TPM values of putative barley homologues to their respective Arabidopsis gene of suberin, aquaporin, lignin and fatty acid elongation genes.

# 4 Chapter 3: Osmotic stress has different effects on suberized transport barriers in roots of cultivated and wild barley

**Summary**

- Wild barley *Hordeum vulgare* spp. *spontaneum* has a wider diversity than its cultivated progenies *Hordeum vulgare* spp. *vulgare*. Both, the wild and the cultivated subspecies, are fairly resistant to abiotic stresses. However wild barley has even better traits to survive under non-optimal conditions, where especially roots play a crucial role for surviving.
- Therefore, the effect of osmotic stress on seminal root development of wild and cultivated barley plants was compared. We investigated changes in (1) basic physiological and anatomical parameters using microscopy, (2) quantitative and qualitative suberin composition using gas chromatography and mass spectrometry, and (3) the radial water and solute flow using the root pressure probe.
- Wild barley plants showed a wide diversity of responses to osmotic stress including a the formation of an exodermis, which is missing in modern cultivars. The hydraulic conductivity was not affected in wild barley as a response to water stress as it was with cultivated barley plants .
- These results show that wild barley is better adapted to osmotic stress through a significantly higher hydraulic conductivity of roots when facing water deficit.

## 4.1 Introduction

Abiotic stresses such as water deficit or osmotic stress limit plant growth and reduce crop yields. Climate change will lead to longer and more frequent drought periods as well as extreme weather conditions in the future. Because of that there will also be a significant increase in yield losses (Kang *et al.*, 2009; Challinor *et al.*, 2014). *Hordeum vulgare* L. is known to be one of the most tolerant crop plants towards abiotic stresses such as extreme weather conditions, drought and salinity (Colmer *et al.*, 2006; Kosová *et al.*, 2014). Today barley as a cereal is almost as cultivated and important as wheat, maize and rice (Mayer *et al.*, 2012; Mascher *et al.*, 2016). However through its early domestication 10000 years ago and due to modern breeding programs to achieve higher yields much of its allelic variation has been lost. A declined genetic diversity is often linked to a higher susceptibility towards stress (Tanksley & McCouch, 1997). Cultivated barley *Hordeum vulgare* ssp. *vulgare* is derived from its wild progenitor *Hordeum vulgare* ssp. *spontaneum*, which originates from the Fertile Crescent (Harlan & Zohary, 1966; Badr *et al.*, 2000). Since wild barley is originally grown in the Middle East and encounters often harsh ecological environments it is adapted to a range of arid to semiarid habitats and exhibits a wider diversity than cultivated barley. Until today wild and cultivated barley plants still can be crossed and progenies are fully fertile which allows to transfer positive traits between wild and cultivated barley (Gunasekera *et al.*, 1994).

The plant root is the organ which takes up water from the surrounding soil and sense and transduces any signal of water deficit. Thus, they play a central role in the plants viability to deal with water deficit (Zingaretti *et al.*, 2013). The hydrophobic biopolyester suberin plays a significant role as an apoplastic barrier for water and nutrient flow at plant surfaces and at the interface between plant tissues (Franke & Schreiber, 2007; Ranathunge *et al.*, 2011b). Suberin can be found in primary roots in the endo- and exodermis. The composite transport model describes water uptake in plant roots via the apoplastic, symplastic and trans-cellular pathway. The apoplastic pathway can be blocked by Casparian bands and suberin lamellae. The symplastic and trans-cellular pathway are often referred together as cell-to-cell pathway which can be regulated by suberin deposition and aquaporin regulation (Steudle & Peterson, 1998; Steudle, 2000a,b; Kim *et al.*, 2018; Kreszies *et al.*, 2018a). The increase of suberin formation in response to abiotic stresses such as water deficit, salinity, hypoxia etc. has been extensively shown in the past (Hose *et al.*, 2001; Enstone *et al.*, 2002;

Krishnamurthy *et al.*, 2009; Ranathunge *et al.*, 2011a; Barberon *et al.*, 2016; Kotula *et al.*, 2017; Kreszies *et al.*, 2018b). The biopolymer suberin contains an aliphatic and an aromatic domain which are cross linked via ester bounds. The aliphatic domain confers mainly in the barrier properties against water transport, whereas it is believed that the aromatic domain connects the polyester to the cell wall (Kolattukudy *et al.*, 1975; Zimmermann *et al.*, 2000; Graça, 2015). The aliphatic domain contains mainly long chain fatty acid derivates with ω-hydroxy acids and α,ω-dicarboxylic acids, primary fatty acids and alcohols as predominant substance classes. The aromatic components are mostly coumaric and ferulic acid (Bernards, 2002; Ranathunge *et al.*, 2011b).

Recently the effect of osmotic stress on the development of root suberization in cultivated barley roots was reported (Kreszies *et al.*, 2018b). In the work presented here three modern barley cultivars and their response towards osmotic stress are compared with three wild barley accessions. The apoplastic barrier development in roots was investigated with microscopy and histochemistry. Further root suberization was quantified using analytical approaches and the effect of solute and water transport was measured with a root pressure probe. The results indicate that wild barley uses different strategies to cope with osmotic stress compared to cultivated barley plants. Cultivated barley has a conserved reaction of increasing the amount of suberin as an adaption to prevent uncontrolled passive water loss from the root into the surrounding environment. Interestingly the wild barley accession has diverse strategies to deal with osmotic stress, ranging from suberization in specific tissues or only specific zones or no effect on suberization at all. In cultivated barley water transport was reduced under osmotic stress, while wild barley was able to keep up the overall water uptake under water deficit conditions.

## 4.2 Material and methods

### 4.2.1 Plant material and growth conditions

Seeds of cultivated barley (*Hordeum vulgare spp. vulgare)* Barke, Morex, Golden Promise and wild barley accessions (*Hordeum vulgare spp. sponataneum)* ICB181160 (Iran), ICB181243 (Pakistan) and ICB181466 (Jordan) were stratified for one week at 4°C. They were germinated in the dark at 25 °C covered with wet filter paper. For simplicity the wild barley accessions are referred to their country of origin. Plant growh

was done as described earlier (Kreszies *et al.*, 2018b). In brief: after three days, seedlings were transferred into an aerated hydroponic system containing half-strength Hoagland solution (Hoagland & Arnon, 1950) in a climatic chamber under long day conditions (16 h : 8 h, light : dark), an air temperature of 23 : 20 °C (day : night) and a relative humidity of 50–65%. Plants were grown for 12 days. At this stage they had two leaves and five to six seminal roots.

### 4.2.2 Osmotic stress application

Osmotic stress was applied when the plants were six days old. Plants were moved from the half–strength Hoagland solution (20 mOsmol/kg or -0.04 MPa of osmotic pressure) to half–strength Hoagland solution adjusted to a defined water potential of -0.8 MPa with PEG8000 (Roth, Karlsruhe, Germany). The water potential of the medium was reduced to -0.8 MPa by adding 25.4% (w/w) PEG8000 (Michel, 1983). The water potential of the nutrient solution as well as the nutrient solution containing PEG8000 was measured using a WP4C Water Potential Meter (METER Group, USA).

### 4.2.3 Histochemical detection of Casparian bands and suberin lamellae in roots

Cross-sections were made at 1 cm increments along the whole seminal root using a cryostat microtome (Microm HM 500M, Microm International, Walldorf, Germany). To detect the development of Casparian bands over the root length, cross-sections were stained with 0.1% (w/v) berberine hemisulfate for 1 h and with 0.5% (w/v) aniline blue for 30 min (Brundrett *et al.*, 1988). Suberin lamellae were stained with 0.01% (w/v) lipophilic fluorol yellow 088 for 1 h (Brundrett *et al.*, 1991). Cross-sections were analyzed by epifluorescence microscopy using an ultraviolet (UV) filter set (excitation filter BP 365, dichroic mirror FT 395, barrier filter LP 397; Zeiss, Oberkochen, Germany). Pictures were taken with a Canon EOS 600D camera at ISO 200 or 400 for 1 s to 2 s.

### 4.2.4 Chemical analysis of barley root suberin

The seminal roots were divided into three (cultivars) or four (wild barley) zones based on the previous microscopic investigations (Fig. 1). In the cultivars Zone A (0-25% of total root length) was the youngest part of the root, which included the root apex. In this zone, only Casparian bands were present in the endodermis but no suberin lamellae were deposited. Zone B (25-50%) was the transition zone, in which all endodermal cells showed Casparian bands, but only a limited number of cells had suberin lamellae depositions. Zone C (50-100%) was the mature part of the root close to the root base, in which all endodermal cells were characterized by the presence of Casparian bands and suberin lamellae. In the wild barley accessions we divide the basal root part further because under control conditions a delayed suberization was observed in the Microscope, thus Zone C was 50-75% and Zone D was 75 to 100% of the seminal root length (Fig 1).

For each replicate, ten segments of each of the zones of the seminal roots were analyzed together. Lateral roots were removed with a razor blade. For gas chromatography root segments were enzymatically digested, soluble lipids extracted and the samples transesterified using $BF_3$-methanol as described earlier (Kolattukudy & Agrawal, 1974; Zeier & Schreiber, 1997, 1998; Kreszies *et al.*, 2018b). Three independent biological replicates were used for each experiment.

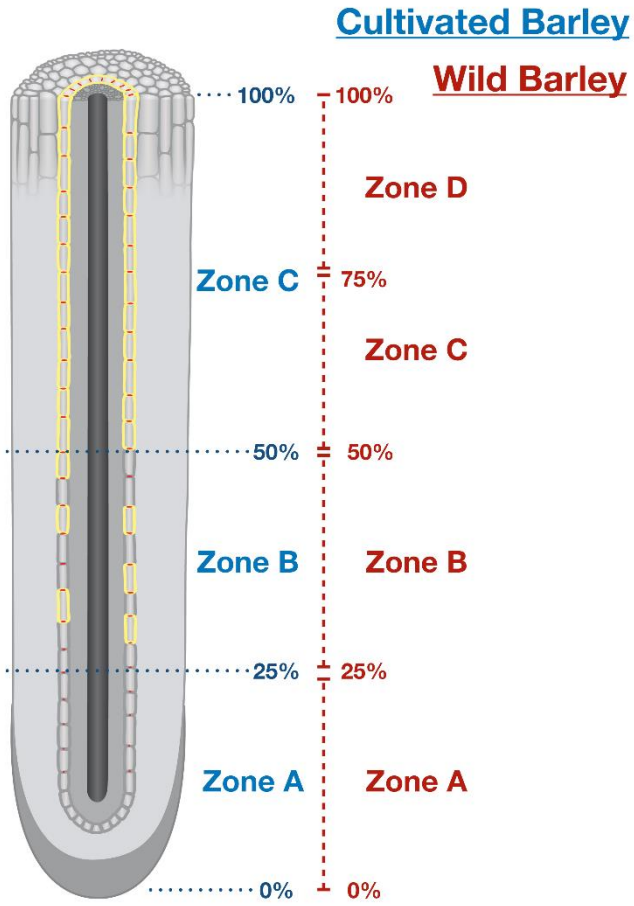


**Fig. 1:** Schematic drawing showing the relative root zones which were used for suberin analysis using gas chromatography. Three zones were selected for cultivated barley (blue): (1) zone A - from 0% to 25%, (2) zone B - from 25% to 50% and (3) zone C from 50% to 100% of the total seminal root length. For wild barley four zones were selected (red): 1) zone A - from 0% to 25%, (2) zone B - from 25% to 50% (3), zone C from 50% to 75% and (4) zone D from 75% to 100% of the total seminal root length. The selected zones were based on the development of the suberin lamellae.

### 4.2.5 Root pressure probe experiments

Root pressure probe experiments were conducted with the end segments/apical part of the seminal roots lacking lateral roots (zone A and zone B together) as described earlier (Steudle *et al.*, 1987; Ranathunge *et al.*, 2017; Kreszies *et al.*, 2018b). Plants grown in -0.8 MPa PEG8000 solution were transferred back to half–strength Hoagland nutrient solution at least 1 h before the measurements. Hydrostatic experiments were done by moving the micrometer screw forward and backward, while osmotic

experiments where induced by exchanging the nutrient solution with nutrient solution containing 30 mM NaCl (60 mOsmol · $kg^{-1}$) (Kreszies *et al.*, 2018b).

### 4.2.6 Statistical analysis

Data analysis and statistical tests were performed with Origin Pro 9. A normal distribution of the data was tested with Shapiro-Wilk test. Significant differences between means of the data were tested with two sample t-test, one-way analysis of variance (Fisher LSD) or two-way (grow conditions vs. barley line) analysis of variance (Fisher LSD). All tests were performed with a significance level of 0.05.

## 4.3 Results

### 4.3.1 Root morphology and anatomy

Seminal roots of the wild barley accessions were always longer than the roots of the cultivated barley plants (Fig. 2). The average seminal root length decreased under osmotic stress in all accessions investigated. The cultivars Golden Promise (17.2 ± 5.8 cm) and Barke (15.3 ± 4.0 cm) had the shortest roots of all accessions. The cultivar with the longest root length, Morex (21.2 ± 4.0 cm), is comparable to the wild barley accession Jordan, having the shortest root length (21.6 ± 10.9 cm). The longest root lengths could be found with the wild barley accessions from Iran (24.6 ± 6.0 cm) and Pakistan (26.8 ± 6.0 cm). Roots from barley plants grown under osmotic stress conditions were always significant shorter than under control conditions (Fig. 2). Here, Barke (9.1 ± 2.4 cm) had the shortest roots, followed by Golden Promise (12.9 ± 5.6 cm). There was no significant difference in the root lengths of Morex (16 ± 2.6 cm), Iran (15.4 ± 3.8 cm) and Jordan (17.5 ± 8.2 cm). Pakistan (21.8 ± 5.9 cm) showed the longest roots under osmotic stress conditions (Fig.: 2).

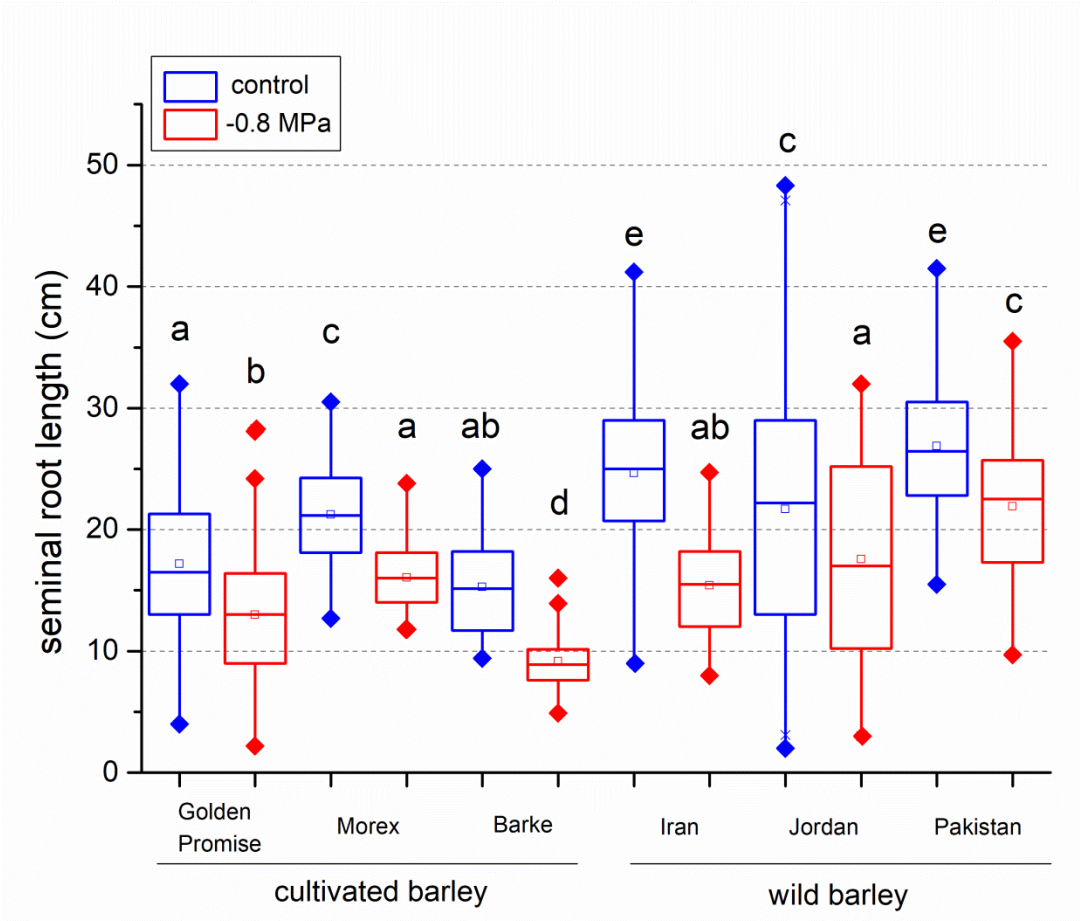


**Fig.2:** Root lengths of 12-day-old cultivated and wild barley plants, either grown under control conditions or at a water potential of -0.8 MPa resulting in osmotic stress. The boxes range from 25 to 75 percentiles. The square in the box represents the mean value. The whiskers range to the outliers. Each box represents more than 150 individual seminal roots. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way and Two-Way ANOVA (Fishers LSD).

Seminal roots of all barley cultivars and wild types showed one large central late metaxylem vessel together with seven to eight early metaxylem vessels (Fig. 3 and 4). The cortex has four to five cell layers. Endodermal Casparian bands were visible in all barley seminal roots even near the root apex developing continuous bands in the radial cell wall over the length of the root. In the hypodermis of 20% of the Jordan plants grown under osmotic stress conditions an exodermis with Casparian bands (Fig. 3a and b) and suberin lamellae (Fig. 3c and d) was detected in root zone D. In all other barley wild types and cultivars no exodermis was detected in the hypodermis of control or osmotic stressed plants.

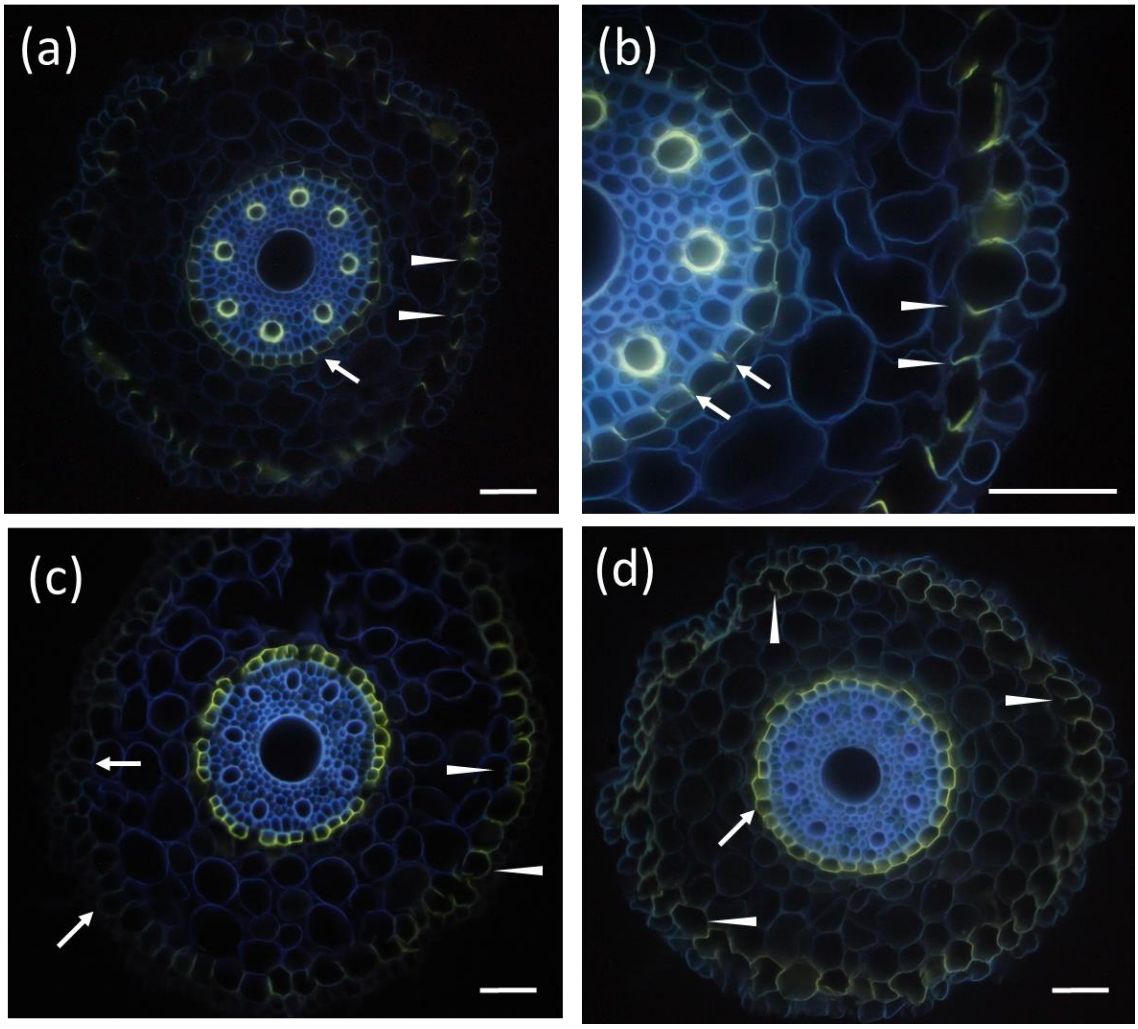


**Fig. 3:** Inducible exodermis in Jordan occurs in approx. 20% of the seminal roots in response to osmotic stress. Cross sections of Jordan seminal roots at a distance of 75% were stained with berberine aniniline sulfat or fluorol yellow 088. The presence of Casparian bands is indicated by a yellowish fluorescence (a and b, arrows endodermis, arrowheads exodermis). The presence of suberin lamellae is indicated by a bright yellowish fluorescence. Some of the roots show an incomplete suberized exodermis (c, arrows no suberin lamellae, arrowheads suberin lamellae) while others have a complete suberized exodermis (d, arrow endodermis, arrowheads exodermis). Bars = 50µm.

The suberin lamellae in the endodermis started to deposit in single cells at 25% (Fig. 4b, g, l and q) of the total root length. Before that no suberization was visible (Fig. 4a, f, k and p). At 37.5% of the total root length a patchy development of suberization in the endodermis of the control and water deficit plants could be found (Fig. 4c, h, m and r). Under osmotic stress conditions the number of suberized cells increased (Fig. 4h and r), but this effect was more pronounced in cultivated compared to wild barley plants. At 50% of the relative root length, the endodermis was fully suberized in cultivated barley

under control and osmotic stress conditions (Fig. 4d, i), while wild barley had still a patchy suberin lamellae in control conditions compared to a complete suberized endodermis under osmotic stress (Fig. 4n and s). At 75% of the root length, the endodermis shows a complete ring of suberized cells in all barley accession in control and stress conditions (Fig. 4e, j, o and t).

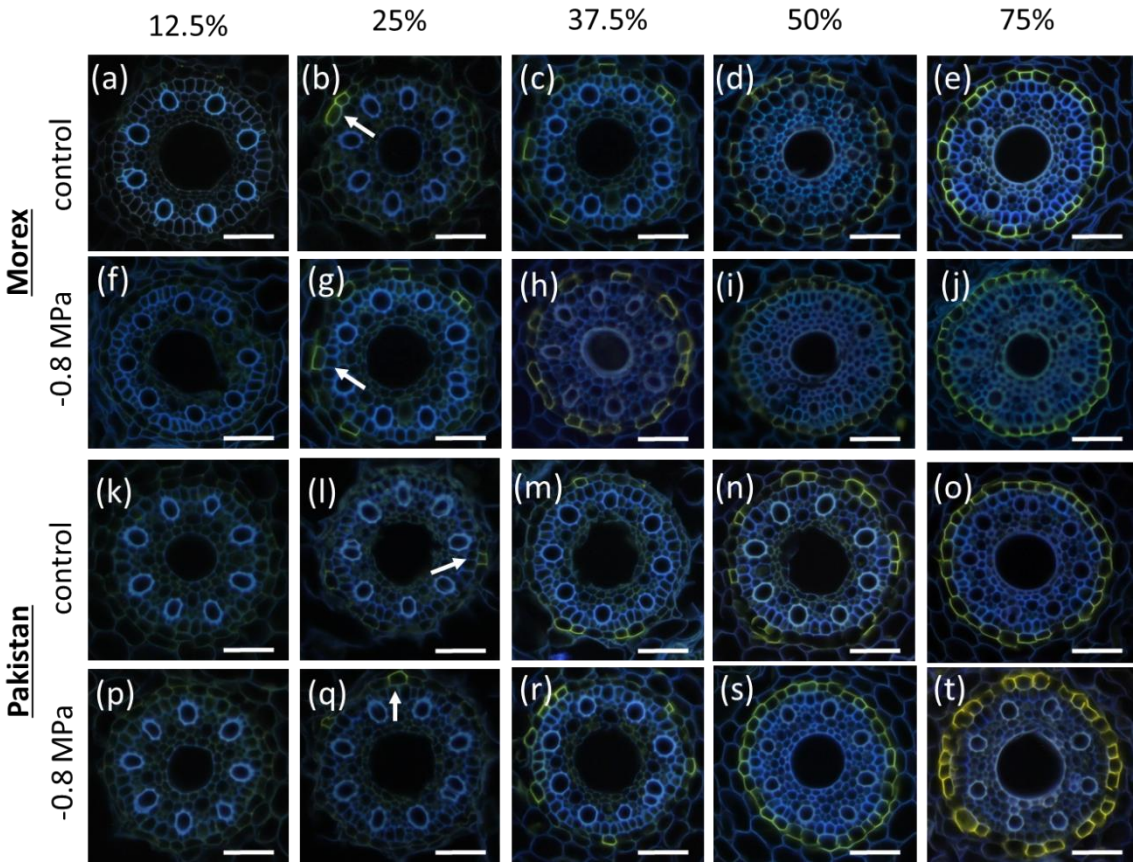


**Fig. 4:** Development of suberin lamellae in the endodermis of barley seminal roots. Cultivated barley is represented by Morex (a-j) and wild barley by Pakistan (k-t). Suberin lamellae in different root zones (Fig. 1) of roots grown under different water potentials were stained with fluorol yellow 088. The presence of suberin lamellae is indicated by a bright yellowish fluorescence. At a distance of 12.5% no suberin lamellae is visible (a, f, k and p). At 25% of the relative root length first single only partially suberized cells (arrows) are visible (b, g, l and q). At 37.5 % of the relative root length a patchy suberization is visible, which is stronger in roots grown under stress conditions (h and r) than under control conditions (c and m). At 50% Morex plants show a complete suberized endodermis (d and j) while with Pakistan the endodermis is still patchy under control conditions (n) but suberized at -0.8 MPa (s). At a distance of 75% the endodermis is completely suberized under all growth conditions (e, j, o and t). Bars = 50 µm.

### 4.3.2 Chemical analysis of suberin of barley seminal roots in response to osmotic stress

For chemical suberin analysis, barley seminal roots were divided into zones (Fig. 1) based on the endodermal suberization (Fig 4). As single monomer classes of aliphatic suberin alcohols (alc), fatty acids (fa), α,ω-dicarboxylic acids (diacids) and ω-hydroxy acids (ω-OH acids) could be found (Fig. 5). The $C_{18:1}$ diacid and ω-OH acids ($C_{18:1}$ and $C_{24}$ ω-OH acids) were the most abundant aliphatic suberin constituents in barley seminal roots. The chain length varied between $C_{16}$ to $C_{26}$ . Aromatic suberin components were composed of coumaric and ferulic acids. There were no significant differences in substance classes (Fig. 5) or single monomer composition between control and osmotic stress conditions or between wild and cultivated barley plants detectable.

The absolute amount (Fig. 5) of substance classes changed over the length of the root from zone A to zone C/D in all treatments (control and stress conditions). This change was pronounced in particular for the total amounts of aliphatic (Fig. 6a) and aromatic suberin (Fig. 6b). Barley seminal roots showed a significant increase in total aliphatic and aromatic suberin (Fig 6a, b) from zone A to C/D, which correlated well with the histochemical observations (Fig. 4). Comparing the effect of osmotic stress on the degree of aliphatic suberization between the cultivars and wild barley accessions no significant differences were observed in zone A (Fig. 6a). In zone B osmotic stress conditions increased aliphatic suberization by two-fold in the cultivars compared to control, while there was no effect in wild barley. In zone C all cultivated and wild barley plants except of Jordan showed a significant increase in aliphatic suberin. In zone D only Jordan had no significant increase in the aliphatic suberin amount compared to osmotic stress, while Iran and Pakistan showed a difference (Fig. 6a). In contrast to the total aliphatic suberin (Fig. 6a), the total aromatic suberin increased from zone A to C/D but there were only significant differences between control and stressed plants in zone D of Iran and Pakistan (Fig. 6b). The total aromatic suberin amount was two-fold higher than the aliphatic suberin amount under control conditions. This ratio decreased under osmotic stress, because of the increase in aliphatic suberin. Wild barley showed a higher amount of aromatic suberin than cultivated barley (Fig. 6).

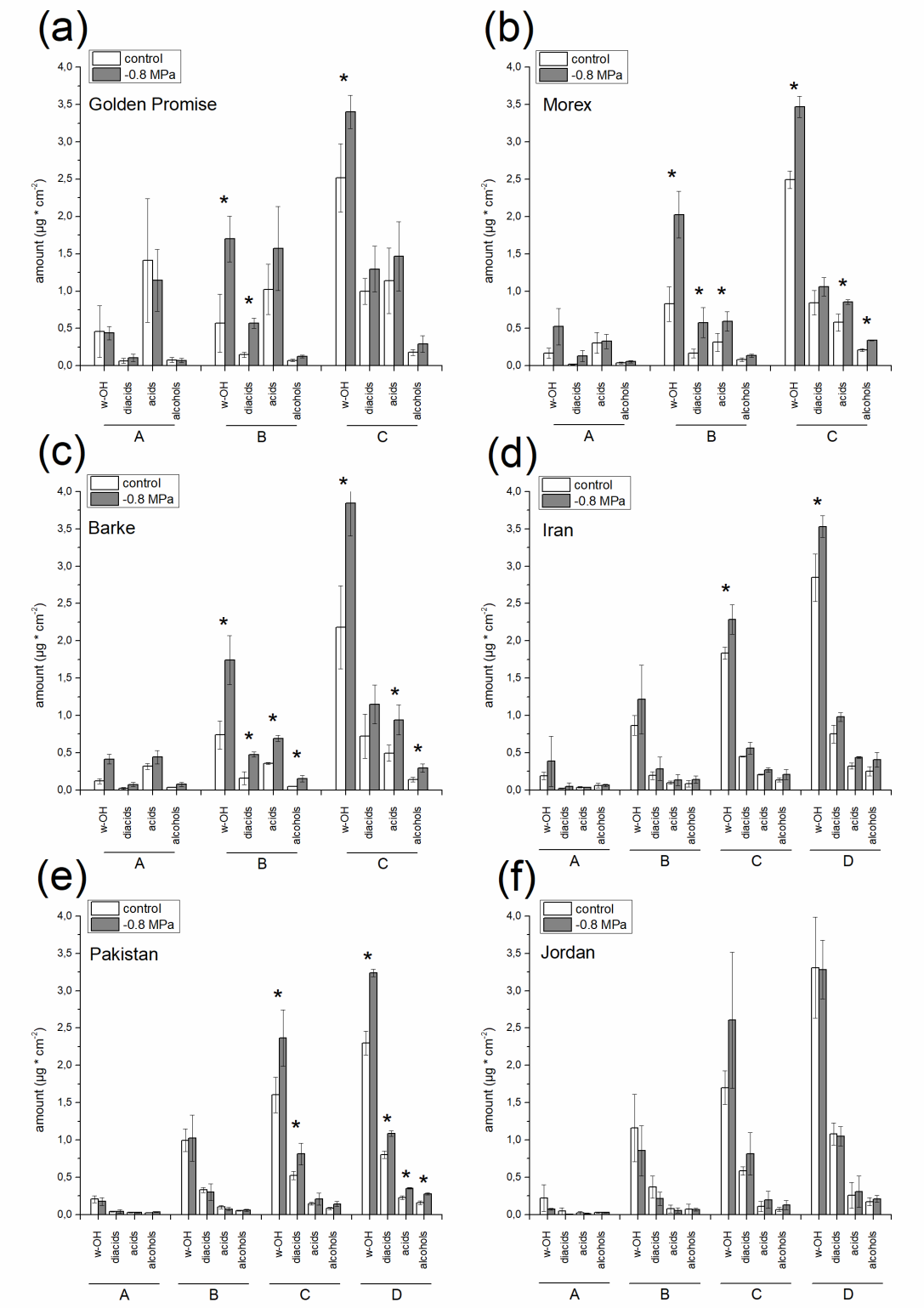


**Fig. 5:** Amounts of substance classes of aliphatic suberin detected in barley seminal roots grown under control conditions or at water potentials of -0.8 MPa performing gas chromatography. The roots of cultivated barley Golden Promise (a), Morex (b) and Barke (c) plants were divided into three root zones starting from the apical root tip zone A followed by zone B to the basal part zone C, while roots of wild barley Iran (d), Pakistan (e) and Jordan (f) were dived into four zones (Fig. 1) . The bars represent mean values with standard deviation of three biological replicates. Asterisks denote significant difference in two-sample t- test with a significance level of 0.05.

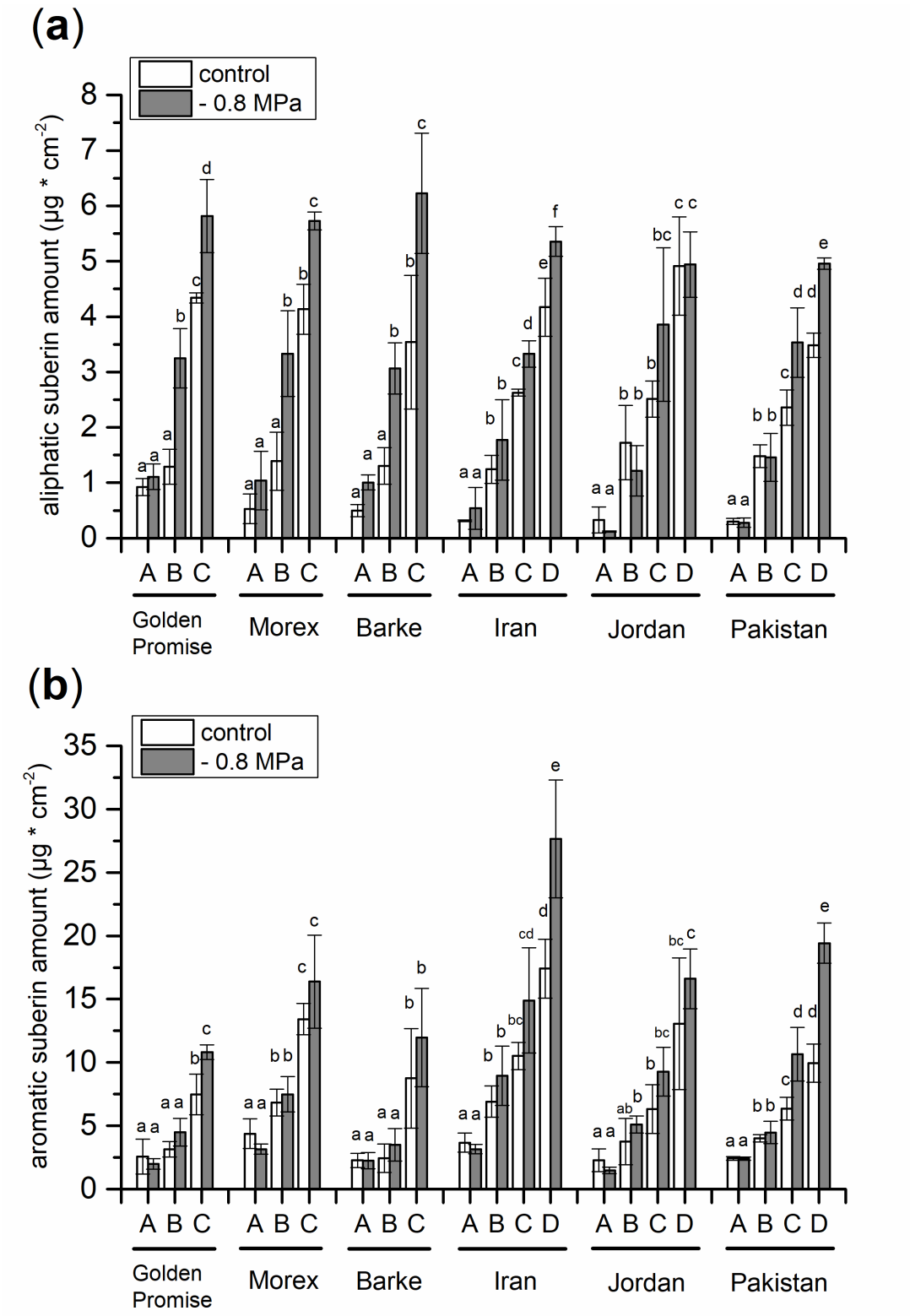


**Fig. 6:** Total amounts of (a) aliphatic and (b) aromatic suberin in barley seminal roots grown under control conditions or at water potential of -0.8 MPa. The roots of cultivated barley (Golden Promise, Morex and Barke) were divided into three root zones from the apical root tip zone A over zone B to the basal part zone C, while roots of wild barley (Iran, Jordan and Pakistan) were divided into four zones. The bars represent mean values with standard deviation of three biological replicates. Different letters indicate significant differences between means at a significance level of 0.05 in One-Way ANOVA (Fishers LSD).

### 4.3.3 Hydraulic conductivity, solute permeability and reflection coefficient of barley seminal roots in response to osmotic stress

Hydraulic conductivity ($Lp_r$) and solute permeability ($P_{sr}$) was measured in Morex (cultivated barley) and Pakistan (wild barley). For both, control plants and plants exposed to -0.8 MPa were investigated (Table. 1). The hydrostatic $Lp_r$ was significantly reduced by three-fold (from 10.0 to 3.39 · $10^{-8}$ $m \cdot s^{-1} \cdot MPa^{-1}$) in Morex in response to water deficit. In contrast Pakistan had a 1.5-fold higher (from 15.46 to 17.3 · $10^{-8}$ $m \cdot s^{-1} \cdot MPa^{-1}$) hydrostatic $Lp_r$ in control conditions and no significant changes in response to osmotic stress (Table 1). There was no difference in the osmotic $Lp_r$ under control conditions between Morex and Pakistan. However, in response to osmotic stress the osmotic $Lp_r$ in Pakistan increased by two-fold whereas in Morex there was no change. Thus the ratios of hydrostatic/osmotic $Lp_r$ declined in the presence of osmotic stress in Morex and Pakistan.

Solute permeability ($P_{sr}$) measured by treating roots with NaCl was also reduced in Morex compared to the control. An opposite effect could be observed for Pakistan, where $P_{sr}$ increased in the presence of osmotic stress. However these changes were not statically significant because of the high variability of roots (Table 1). The reflection coefficient ($\sigma_{sr}$) for NaCl was three-fold higher in Morex compared to Pakistan, but there were no changes in $\sigma_{sr}$ in response to osmotic stress (Table 1).

**Table 1:** Hydrostatic and osmotic hydraulic conductivity ($Lp_r$), solute permeability ($P_{sr}$) and reflection coefficient ($\sigma_{sr}$) for NaCl of individual barley seminal roots grown under control or osmotic stress conditions (water potential of -0.8 MPa). Values are given as means ± SD of five independent replicates (n=5). Different letters indicate significant differences at a significance level of 0.05 in One-Way ANOVA (Fishers LSD).

| | **Morex** | | **Pakistan** | |
|---|---|---|---|---|
| **Parameters** | Control | -0.8MPa | Control | -0.8MPa |
| Hydrostatic $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | 10.0 ± 2.34 a | 3.39 ± 1.95 b | 15.46 ± 2.6 c | 17.3 ± 2.6 c |
| Osmotic $Lp_r$ ($10^{-8}$ m $s^{-1}$ $MPa^{-1}$) | 3.24 ± 1.94 a | 3.05 ± 1.91 a | 2.68 ± 0.93 a | 6.55 ± 0.91 b |
| Hydrostatic/Osmotic | 4.62 ± 3.35 a | 1.11 ± 0.36 b | 6.12 ± 1.48 a | 2.68 ± 0.72 ab |
| Solute permeability $P_{sr}$ ($10^{-9}$m $s^{-1}$ ) | 3.41 ± 5.35 a | 0.41 ± 0.51 a | 0.45 ± 0.62 a | 1.57 ± 0.39 a |
| Reflection coefficient ($\sigma_{sr}$) | 0.60 ± 0.08 a | 0.61 ± 0.17 a | 0.19 ± 0.12 b | 0.19 ± 0.13 b |

## 4.4 Discussion

In dehydrating soil plant roots are the first organs sensing the developing water deficit and thus play an important role in stress response to drought. Here we compared the effect of water deficit, induced by osmotic stress, on suberin formation in three modern barley cultivars and three wild barley accessions. By adding PEG8000 to the nutrient solution of hydroponically grown plants a water potential of -0.8 MPa was specifically adjusted.

In barley seedlings seminal roots contribute to the overall water uptake (Knipfer & Fricke, 2010) thus their development and root length play an important role in response to osmotic stress. By trend roots of wild barley accessions were always longer than those of barley cultivars. A more vigorous root system, including longer roots, was reported earlier for several other wild barley accessions from the Middle East, together with a higher variation of root traits in wild barley (Naz *et al.*, 2012, 2014, Arifuzzaman *et al.*, 2014, 2016). During breeding programs in the last decades the main focus was largely focused on increasing above ground traits such as yield rather than root growth (Koevoets *et al.*, 2016), which explains that roots tend to be shorter in most cultivars. However, in recent years, breeding programs started to include roots as a breeding target (Lynch, 2011). In response to osmotic stress all cultivars and wild barley accessions have significant shorter roots compared to control. This is likely caused by osmotically-driven reduced cell elongation and organ development due to water deficit (Yamaguchi & Sharp, 2010). The longer root system of wild barley plants is of big advantage for the plants performance under well-watered and water stress conditions (Naz *et al.*, 2012). This is because longer roots enable the plants to acquire water in deeper soil layers, which is especially crucial under water deficit, and also are usually linked to a larger surface area for water and nutrient uptake.

To conduct further investigations and their interpretations including chemical and water transport measurements a detailed knowledge of root anatomy is important (Steudle & Peterson, 1998; Steudle, 2000b; Kreszies *et al.*, 2018a). In the barley cultivars the suberin lamellae were only visible in the endodermis and no exodermis was detected even under water stress conditions. This is in accordance with previous descriptions of cultivated barley seminal anatomy (Jackson, 1922; Lehmann *et al.*, 2000; Knipfer & Fricke, 2011; Ranathunge *et al.*, 2017). Suberized cells were never detected in the root tips (Fig. 4a, f, k and p), they appeared first at 25% relative root length which is the crossing from zone A to zone B (Fig. 4b, g, l and q). The majority of

endodermal cells were suberized in cultivated barley at the beginning of 50% of the root length (Fig. 4d and i). In contrast the wild accessions showed in the endodermis of control grown plants a delayed suberization with still patchy suberin lamellae at 50% of the root length (Fig. 4n) and a complete suberization at 75% of the total root length (Fig. 4o). The histochemical observations clearly show that cultivated barley plants undergoes strong suberization in response to osmotic stress, which is not that strong or even delayed with wild barley (Fig. 4). A unique response to osmotic stress could be seen with the wild accession from Jordan. Here the formation of an exodermis with Casparian bands and suberin lamellae was detected in about 20% of the seminal roots in zone D, (Fig. 3). Until now we cannot make clear statements how and why the Jordan accession developed an exodermis in some roots in the presence of osmotic stress, while all other barley accessions as well as modern cultivars did not. Other crop plants such as rice and maize are known to develop a strong exodermis in response to stress (Schreiber *et al.*, 2005b; Ranathunge *et al.*, 2011a, 2016). *Hordeum marinum* a wetland species of the same genus like barley, commonly forms an exodermal barrier to radial oxygen, when it is grown in stagnant or waterlogged conditions (Kotula *et al.*, 2017). An exodermis in response to oxygen deficiency is also very common in other species such as rice (Ranathunge *et al.*, 2011b). But since the nutrient solution was aerated, enough oxygen should have been available for the plants in our experiments. Thus, oxygen deficiency can be excluded as a cause for the partial induction of an exodermis.

Suberin monomers obtained after transesterification belonged to fatty acids, alcohols, ω-OH acids and diacids (Fig. 5). Aromatic monomers consisted of coumaric and ferulic acid. This is in accordance with typical suberin compositions described in the literature (Kolattukudy & Agrawal, 1974; Bernards, 2002; Ranathunge *et al.*, 2011c; Graça, 2015). Different from aliphatic suberin monomers, results of a much higher amounts of aromatic monomers (coumaric and ferulic acid) should be interpreted cautiously, because they can be also bound to all other cell walls in *Graminaceae* species (Carpita, 1996). The suberin monomer composition was the same between cultivated and wild barley and fits to earlier published data from the barley cultivars Golf (Ranathunge *et al.*, 2017) and Scarlett (Kreszies 2018), which suggests that suberin monomer composition is well conserved in barley roots even under stressed conditions.

The chemical analysis confirmed the increase of root suberization over the root length as observed by microscopy (Fig. 6). All three barley cultivars showed the same response to osmotic stress with a significant increase in suberization. This fits to the

earlier described barley cultivar Scarlett (Kreszies *et al.*, 2018b). Most interestingly, compared to cultivated barley, wild barley accessions showed a different response to osmostic stress. Different from modern cultivars, none of the wild barley accessions showed a significant increase in aliphatic suberin in zone B in response to osmotic stress (Fig. 6a). Iran and Pakistan showed only significant increases of suberin in zone C and D while Jordan had no significant increase in any of the zones analyzed comparing control and stress treatments (Fig. 6a). This indicates that Jordan does not increase aliphatic suberin amounts in response to stress (Fig. 6), but it follows other strategies to cope with water stress. This includes the formation of an exodermis in some of the seminal roots (Fig. 3).

Barley cultivars seem to have the strategy of responding to osmotic stress with a greater root suberization to block efficiently the apoplastic pathway preventing uncontrolled water losses from the root to the surrounding medium/soil. Wild barley can potentially use their longer roots reaching deeper soil layers to search for water and obviously only suberize in the basal root parts where the soil is drying out faster. The measured water and solute permeability of the cultivar Morex are in agreement with earlier barley seminal root pressure probe measurements (Knipfer & Fricke, 2010, 2011; Ranathunge *et al.*, 2017). In response to osmotic stress Morex showed the same reactions as described for the cultivar Scarlett (Kreszies *et al.*, 2018b) with a three-fold decrease in hydrostatic hydraulic conductivity ($Lp_r$) (Table 1), which correlates well with the significant increase in aliphatic suberin (Fig. 6). With Morex it could also be shown as previously found for Scarlett that the osmotic $Lp_r$ describing the cell-to-cell path was not affected in response to stress conditions. Thus, the ratio of hydrostatic/osmotic $Lp_r$ indicates a shift from the apoplastic pathway to the cell-to-cell pathway to maintain further efficient water uptake (Kreszies *et al.*, 2018b). Different from Morex, the wild barley accession from Pakistan had a 1.5-fold significant greater hydrostatic $Lp_r$ under control conditions. Most surprisingly, in contrast to Morex there was no change in response to osmotic stress in hydrostatic $Lp_r$, and in addition even a significant two-fold increase in osmotic hydrostatic $Lp_r$ (Table 1). Pakistan had significantly longer roots (Fig. 2) and no significant increase in aliphatic suberin in zone B compared to Morex (Fig. 6) in response to osmotic stress. This fits to earlier descriptions that the maximal radial water uptake in barley roots occurs through this weakly-suberized younger zone that includes the root tip, whereas water uptake is significantly decreased in the strongly-suberized basal part of the root (Sanderson,

1983; Ranathunge *et al.*, 2017). The increase in osmotic $Lp_r$ in Pakistan also leads to a slight shift towards the cell-to-cell pathway, but since aliphatic suberin is not increasing the apoplastic pathway it is still dominant. The higher osmotic conductivity is most likely also the cause for the slightly increased hydrostatic conductivity in stressed plants. In Morex solute permeability ($P_{sr}$) is slightly reduced in osmotic stressed plants. This is not a very pronounced effect and it can be explained by the enhanced suberin lamellae. Different to Morex in Pakistan $P_{sr}$ was even slightly increased in osmotically stressed plants, which may be explained by the higher osmotic $Lp_r$ due to the fact that sodium ions might also move to a certain degree via ion channel or aquaporins. $P_{sr}$ is not changed significantly in response to osmotic stress which explains that the reflection coefficients ($\sigma_{sr}$) of Morex and Pakistan do not change in response to the osmotic stress (Table 1).

In conclusion this study showed that cultivated and wild barley roots show quite different responses to osmotic stress in the development of suberin lamellae and water transport. Cultivated barley shows a stronger earlier suberization under control conditions and in response to stress, which coincides with the decreased overall hydraulic $Lp_r$. Wild barley shows a delayed endodermal suberization under control conditions and in response to stress compared to cultivated barley and this coincides with higher hydraulic $Lp_r$ thus better securing plant water supply under water deficit conditions. Surprisingly in one of the wild type accessions Jordan investigated even the partial development of an exodermis was detected.

# 5 Summary

In future climate change will intensify extreme weather conditions, such as drought, which will lead to decreased yields of crops. A decrease in soil water potential is the first signal of potential drought stress for plants. In fact plant roots are the first organs sensing drought and water deficit in soil. At the same time plant roots are the main organ to take up water to supply shoots and leaves. Water uptake in roots is described by the composite transport model. The main components of the model are the apoplastic pathway (cell walls), which can be blocked by Casparian bands and the biopolymer suberin, and the cell-to-cell pathway which can be regulated by aquaporins.

The model plant Arabidopsis and crop plants such as rice and barley have very different root anatomical structures. This explains why a simple transfer of knowledge on root water transport from the model to the crop is not always valid. Especially the correlations between suberin amounts and water uptake require caution.

The response of barley seminal roots to different levels of low water potentials (-0.4, -0.8 and -1.2 MPa) induced by PEG8000 has been studied. In this approach, various experimental methods (histochemistry, analytical chemistry, transcriptomics and transport physiology) were used to test the hypothesis whether an increased suberization of barley roots could represent an efficient response to osmotic stress thus limiting uncontrolled, passive water loss from roots to the dry soil/medium. In response to osmotic stress, genes in the suberin biosynthesis pathway were up-regulated which correlated well with increased suberin amounts in the cell walls of the endodermis and overall reduction of hydraulic conductivity ($Lp_r$). In parallel, transcriptomic data indicated no or only weak effects of water stress on gene expression of aquaporins which are relevant for the cell-to-cell pathway.

Finally, the effect of osmotic stress on seminal roots of wild and cultivated barley was compared. Wild barley has a wider diversity than cultivated barley, which is also represented in the root response to water stress. In contrast to cultivated barley, the suberization of wild barley was delayed and not much affected in response to osmotic stress. Furthermore, $Lp_r$ of wild barley was not reduced in response to osmotic stress. Most remarkably, one wild barley accession from Jordan exhibited the formation of a suberized exodermis in about 20% of the seminal roots when exposed to osmotic stress. This was never observed in cultivated barley in all water stress conditions.

# 6 Bibliography

**Arifuzzaman M, Günal S, Bungartz A, Muzammil S, P. Afsharyan N, Léon J, Naz AA**. **2016**. Genetic Mapping Reveals Broader Role of Vrn-H3 Gene in Root and Shoot Development beyond Heading in Barley (R Papa, Ed.). *PLOS ONE* **11**: 1–16.

**Arifuzzaman M, Sayed MA, Muzammil S, Pillen K, Schumann H, Naz AA, Léon J**. **2014**. Detection and validation of novel QTL for shoot and root traits in barley (*Hordeum vulgare* L.). *Molecular Breeding* **34**: 1373–1387.

**Aroca R, Porcel R, Ruiz-Lozano JM**. **2012**. Regulation of root water uptake under abiotic stress conditions. *Journal of Experimental Botany* **63**: 43–57.

**Badr A, Müller K, Schäfer-Pregl R, Rabey H El, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F**. **2000**. On the Origin and Domestication History of Barley (*Hordeum vulgare*). *Molecular Biology and Evolution* **17**: 499–510.

**Barberon M, Vermeer JEM, De Bellis D, Wang P, Naseer S, Andersen TG, Humbel BM, Nawrath C, Takano J, Salt DE, *et al.* 2016**. Adaptation of Root Function by Nutrient-Induced Plasticity of Endodermal Differentiation. *Cell* **164**: 447–459.

**Baxter I, Hosmani PS, Rus A, Lahner B, Borevitz JO, Muthukumar B, Mickelbart MV, Schreiber L, Franke RB, Salt DE**. **2009**. Root Suberin Forms an Extracellular Barrier That Affects Water Relations and Mineral Nutrition in Arabidopsis (GP Copenhaver, Ed.). *PLoS Genetics* **5**: e1000492.

**Beisson F, Li Y, Bonaventure G, Pollard M, Ohlrogge JB**. **2007**. The Acyltransferase GPAT5 Is Required for the Synthesis of Suberin in Seed Coat and Root of Arabidopsis. *THE PLANT CELL ONLINE* **19**: 351–368.

**Benjamini Y, Hochberg Y**. **1995**. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* **57**: 289–300.

**Bernards MA**. **2002**. Demystifying suberin. *Canadian Journal of Botany* **80**: 227–240.

**Boyer JS**. **1982**. Plant Productivity and Environment. *Science* **218**: 443–448.

**Brundrett MC, Enstone DE, Peterson CA**. **1988**. A Berberine-Aniline Blue Fluorescent Staining Procedure for Suberin, Lignin, and Callose in Plant Tissue. *Protoplasma* **146**: 133–142.

**Brundrett MC, Kendrick B, Peterson CA**. **1991**. Efficient Lipid Staining in Plant

Material with Sudan Red 7B or Fluoral Yellow 088 in Polyethylene Glycol-Glycerol. *Biotechnic & Histochemistry* **66**: 111–116.

**Carpita NC**. **1996**. Structure and Biogenesis of the Cell Walls of Grasses. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**: 445–476.

**Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N**. **2014**. A meta-analysis of crop yield under climate change and adaptation. *Nature Climate Change* **4**: 287–291.

**Chen T, Cai X, Wu X, Karahara I, Schreiber L, Lin J**. **2011**. Casparian strip development and its potential function in salt tolerance. *Plant Signaling & Behavior* **6**: 1499–1502.

**Clark LH, Harris WH**. **1981**. Observations on the Root Anatomy of Rice (*Oryza sativa* L.). *American Journal of Botany* **68**: 154–161.

**Colmer TD, Flowers TJ, Munns R**. **2006**. Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany* **57**: 1059–1078.

**Colmer TD, Gibberd MR, Wiengweera A, Tinh TK**. **1998**. The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *Journal of Experimental Botany* **49**: 1431–1436.

**Compagnon V, Diehl P, Benveniste I, Meyer D, Schaller H, Schreiber L, Franke R, Pinot F**. **2009**. CYP86B1 Is Required for Very Long Chain -Hydroxyacid and , -Dicarboxylic Acid Synthesis in Root and Seed Suberin Polyester. *Plant Physiology* **150**: 1831–1843.

**Deng W, Nickle DC, Learn GH, Maust B, Mullins JI**. **2007**. ViroBLAST: a stand-alone BLAST web server for flexible queries of multiple databases and user's datasets. *Bioinformatics* **23**: 2334–2336.

**Domergue F, Vishwanath SJ, Joubes J, Ono J, Lee JA, Bourdon M, Alhattab R, Lowe C, Pascal S, Lessire R, *et al.* 2010**. Three Arabidopsis Fatty Acyl-Coenzyme A Reductases, FAR1, FAR4, and FAR5, Generate Primary Fatty Alcohols Associated with Suberin Deposition. *Plant Physiology* **153**: 1539–1554.

**Enstone DE, Peterson CA, Ma F**. **2002**. Root Endodermis and Exodermis: Structure, Function, and Responses to the Environment. *Journal of Plant Growth Regulation* **21**: 335–351.

**Franke R, Höfer R, Briesen I, Emsermann M, Efremova N, Yephremov A, Schreiber L**. **2009**. The DAISY gene from Arabidopsis encodes a fatty acid elongase condensing enzyme involved in the biosynthesis of aliphatic suberin in roots and the

chalaza-micropyle region of seeds. *The Plant Journal* **57**: 80–95.

**Franke R, Schreiber L**. **2007**. Suberin — a biopolyester forming apoplastic plant interfaces. *Current Opinion in Plant Biology* **10**: 252–259.

**Fraser CM, Chapple C**. **2011**. The Phenylpropanoid Pathway in Arabidopsis. *The Arabidopsis Book* **9**: e0152. doi: 10.1199/tab.0152

**Frolov A, Bilova T, Paudel G, Berger R, Balcke GU, Birkemeyer C, Wessjohann LA**. **2017**. Early responses of mature *Arabidopsis thaliana* plants to reduced water potential in the agar-based polyethylene glycol infusion drought model. *Journal of Plant Physiology* **208**: 70–83.

**Gambetta GA, Knipfer T, Fricke W, McElrone AJ**. **2017**. Aquaporins and Root Water Uptake IN: *Plant Aquaporins* (F Chaumont and SD Tyerman, Eds.). Cham: Springer International Publishing.

**Gou M, Hou G, Yang H, Zhang X, Cai Y, Kai G, Liu C-J**. **2017**. The MYB107 Transcription Factor Positively Regulates Suberin Biosynthesis. *Plant Physiology* **173**: 1045–1058.

**Graça J**. **2015**. Suberin: the biopolyester at the frontier of plants. *Frontiers in Chemistry* **3**: 62.

**Gunasekera D, Santakumari M, Glinka Z, Berkowitz GA**. **1994**. Wild and cultivated barley genotypes demonstrate varying ability to acclimate to plant water deficits. *Plant Science* **99**: 125–134.

**Harlan JR, Zohary D**. **1966**. Distribution of Wild Wheats and Barley. *Science* **153**: 1074–1080.

**Hoagland DR, Arnon DI**. **1950**. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* **347**: 1–32.

**Höfer R, Briesen I, Beck M, Pinot F, Schreiber L, Franke R**. **2008**. The Arabidopsis cytochrome P450 CYP86A1 encodes a fatty acid ω-hydroxylase involved in suberin monomer biosynthesis. *Journal of Experimental Botany* **59**: 2347–2360.

**van den Honert TH**. **1948**. Water transport in plants as a catenary process. *Discussions of the Faraday Society* **3**: 146.

**Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W**. **2001**. The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* **52**: 2245–2264.

**Hosmani PS, Kamiya T, Danku J, Naseer S, Geldner N, Guerinot ML, Salt DE**. **2013**. Dirigent domain-containing protein is part of the machinery required for formation of the lignin-based Casparian strip in the root. *Proceedings of the National*

*Academy of Sciences* **110**: 14498–14503.

**Jackson VG**. **1922**. Anatomical Structure of the Roots of Barley. *Annals of Botany* **36**: 21–40.

**Kaneko T, Horie T, Nakahara Y, Tsuji N, Shibasaka M, Katsuhara M**. **2015**. Dynamic Regulation of the Root Hydraulic Conductivity of Barley Plants in Response to Salinity/Osmotic Stress. *Plant and Cell Physiology* **56**: 875–882.

**Kang Y, Khan S, Ma X**. **2009**. Climate change impacts on crop yield, crop water productivity and food security – A review. *Progress in Natural Science* **19**: 1665–1674.

**Karahara I, Ikeda A, Kondo T, Uetake Y**. **2004**. Development of the Casparian strip in primary roots of maize under salt stress. *Planta* **219**: 41–47.

**Karahara I, Shibaoka H**. **1992**. Isolation of Casparian Strips from Pea Roots. *Plant and Cell Physiology* **33**: 555–561.

**Kawata S, Ishihara K, Shioya T**. **1964**. Studies on the Root Hairs of Lowland Rice Plants in the Upland Fields. *Japanese Journal of Crop Science* **32**: 250–253.

**Kersey PJ, Allen JE, Armean I, Boddu S, Bolt BJ, Carvalho-Silva D, Christensen M, Davis P, Falin LJ, Grabmueller C, *et al.* 2016**. Ensembl Genomes 2016: More genomes, more complexity. *Nucleic Acids Research* **44**: D574–D580.

**Kim YX, Ranathunge K, Lee S, Lee Y, Lee D, Sung J**. **2018**. Composite Transport Model and Water and Solute Transport across Plant Roots: an Update. *Frontiers in Plant Science* **9**: 193.

**Knipfer T, Fricke W**. **2010**. Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). *New Phytologist* **187**: 159–170.

**Knipfer T, Fricke W**. **2011**. Water uptake by seminal and adventitious roots in relation to whole-plant water flow in barley (*Hordeum vulgare L*.). *Journal of Experimental Botany* **62**: 717–733.

**Koevoets IT, Venema JH, Elzenga JTM, Testerink C**. **2016**. Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. *Frontiers in Plant Science* **07**: 1335.

**Kolattukudy PE, Agrawal VP**. **1974**. Structure and composition of aliphatic constituents of potato tuber skin (suberin). *Lipids* **9**: 682–691.

**Kolattukudy PE, Kronman K, Poulose AJ**. **1975**. Determination of Structure and Composition of Suberin from the Roots of Carrot, Parsnip, Rutabaga, Turnip, Red Beet, and Sweet Potato by Combined Gas-Liquid Chromatography and Mass Spectrometry.

*Plant Physiology* **55**: 567–573.

**Kosma DK, Murmu J, Razeq FM, Santos P, Bourgault R, Molina I, Rowland O**. **2014**. AtMYB41 activates ectopic suberin synthesis and assembly in multiple plant species and cell types. *The Plant Journal* **80**: 216–229.

**Kosová K, Vítámvás P, Prášil IT**. **2014**. Wheat and barley dehydrins under cold, drought, and salinity - what can LEA-II proteins tell us about plant stress response? *Frontiers in Plant Science* **5**: 343.

**Kotula L, Colmer TD, Nakazono M**. **2014**. Effects of organic acids on the formation of the barrier to radial oxygen loss in roots of *Hordeum marinum*. *Functional Plant Biology* **41**: 187.

**Kotula L, Ranathunge K, Schreiber L, Steudle E**. **2009a**. Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of rice (*Oryza sativa* L.) grown in aerated or deoxygenated solution. *Journal of Experimental Botany* **60**: 2155–2167.

**Kotula L, Ranathunge K, Steudle E**. **2009b**. Apoplastic barriers effectively block oxygen permeability across outer cell layers of rice roots under deoxygenated conditions: roles of apoplastic pores and of respiration. *New Phytologist* **184**: 909–917.

**Kotula L, Schreiber L, Colmer TD, Nakazono M**. **2017**. Anatomical and biochemical characterisation of a barrier to radial O2 loss in adventitious roots of two contrasting *Hordeum marinum* accessions. *Functional Plant Biology* **44**: 845.

**Kramer and Boyer**. **1995**. *Water Relations of Plants and Soils*. San Diego, USA Academic Press.

**Kreszies T, Schreiber L, Ranathunge K**. **2018a**. Suberized transport barriers in Arabidopsis, barley and rice roots: From the model plant to crop species. *Journal of Plant Physiology*. doi: 10.1016/j.jplph.2018.02.002

**Kreszies T, Shellakkutti N, Osthoff A, Baldauf J, Yu P, Zeisler-Diehl V, Hochholdinger F, Schreiber L**. **2018b**. Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses. *New Phytologist*. doi: 10.1111/nph.15351

**Krishnamurthy P, Ranathunge K, Franke R, Prakash HS, Schreiber L, Mathew MK**. **2009**. The role of root apoplastic transport barriers in salt tolerance of rice (*Oryza sativa* L.). *Planta* **230**: 119–134.

**Krishnamurthy P, Ranathunge K, Nayak S, Schreiber L, Mathew MK**. **2011**. Root apoplastic barriers block Na+ transport to shoots in rice (*Oryza sativa* L.). *Journal of*

*Experimental Botany* **62**: 4215–4228.

**Landsberg JJ, Fowkes ND**. **1978**. Water Movement Through Plant Roots. *Annals of Botany* **42**: 493–508.

**Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse USR**. **2010**. De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytologist* **185**: 577–588.

**Lashbrooke J, Cohen H, Levy-Samocha D, Tzfadia O, Panizel I, Zeisler V, Massalha H, Stern A, Trainotti L, Schreiber L, *et al.* 2016**. MYB107 and MYB9 Homologs Regulate Suberin Deposition in Angiosperms. *The Plant Cell* **28**: 2097–2116.

**Law CW, Chen Y, Shi W, Smyth GK**. **2014**. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* **15**: R29.

**Lee SB, Jung SJ, Go YS, Kim HU, Kim JK, Cho HJ, Park OK, Suh MC**. **2009**. Two Arabidopsis 3-ketoacyl CoA synthase genes, *KCS20* and *KCS2/DAISY*, are functionally redundant in cuticular wax and root suberin biosynthesis, but differentially controlled by osmotic stress. *Plant Journal* **60**: 462–475.

**Lehmann H, Stelzer R, Holzamer S, Kunz U, Gierth M**. **2000**. Analytical electron microscopical investigations on the apoplastic pathways of lanthanum transport in barley roots. *Planta* **211**: 816–822.

**Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Baud S, Bird D, DeBono A, Durrett TP, *et al.* 2013**. Acyl-Lipid Metabolism. *The Arabidopsis Book* **11**: e0161.doi: 10.1199/tab.0133

**Li Y, Beisson F, Koo AJ, Molina I, Pollard M, Ohlrogge J**. **2007**. Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 18339–18344.

**Lulai ECE, Corsini DL, Crop N**. **1998**. Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (*Solanum tuberosum* L.) wound-healing. *Physiological and Molecular Plant Pathology* **53**: 209–222.

**Lupoi JS, Singh S, Parthasarathi R, Simmons BA, Henry RJ**. **2015**. Recent innovations in analytical methods for the qualitative and quantitative assessment of lignin. *Renewable and Sustainable Energy Reviews* **49**: 871–906.

**Lynch JP**. **2011**. Root Phenes for Enhanced Soil Exploration and Phosphorus

Acquisition: Tools for Future Crops. *Plant Physiology* **156**: 1041–1049.

**Ma F, Peterson CA**. **2003**. Current insights into the development, structure, and chemistry of the endodermis and exodermis of roots. *Canadian Journal of Botany* **81**: 405–421.

**Man Y, Zhao Y, Ye R, Lin J, Jing Y**. **2018**. In vivo cytological and chemical analysis of Casparian strips using stimulated Raman scattering microscopy. *Journal of Plant Physiology* **220**: 136–144.

**Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, *et al.* 2017**. A chromosome conformation capture ordered sequence of the barley genome. *Nature* **544**: 427–433.

**Mascher M, Schuenemann VJ, Davidovich U, Marom N, Himmelbach A, Hübner S, Korol A, David M, Reiter E, Riehl S, *et al.* 2016**. Genomic analysis of 6,000-year-old cultivated grain illuminates the domestication history of barley. *Nature Genetics* **48**: 1089–1093.

**Maurel C, Boursiac Y, Luu D-T, Santoni V, Shahzad Z, Verdoucq L**. **2015**. Aquaporins in Plants. *Physiological Reviews* **95**: 1321–1358.

**Mayer KFX, Waugh R, Langridge P, Close TJ, Wise RP, Graner A, Matsumoto T, Sato K, Schulman A, Muehlbauer GJ, *et al.* 2012**. A physical, genetic and functional sequence assembly of the barley genome. *Nature* **491**: 711–716.

**Melillo JM, Richmond T, Yohe GW**. **2014**. *Higlights of Climate Change Impacts in the United States. :The Third National Climate Assessment.* U.S. Global Change Reasearch Program, 148 pp.

**Meyer CJ, Peterson CA**. **2013**. Structure and Function of Three Suberized Cell Layers: Epidermis, Exodermis, and Exodermis. In: Eshel A, Beeckman T, eds. Plant Roots: The Hidden Half, Fourth Edition. CRC Press, 5-1-5–20.

**Michel BE**. **1983**. Evaluation of the Water Potentials of Solutions of Polyethylene Glycol 8000 Both in the Absence and Presence of Other Solutes. *Plant Physiology* **72**: 66–70.

**Miyamoto N, Steudle E, Hirasawa T, Lafitte R**. **2001**. Hydraulic conductivity of rice roots. *Journal of Experimental Botany* **52**: 1835–1846.

**Molina I, Li-Beisson Y, Beisson F, Ohlrogge JB, Pollard M**. **2009**. Identification of an *Arabidopsis* feruloyl-coenzyme A transferase required for suberin synthesis. *Plant physiology* **151**: 1317–28.

**Naseer S, Lee Y, Lapierre C, Franke R, Nawrath C, Geldner N**. **2012**. Casparian

strip diffusion barrier in *Arabidopsis* is made of a lignin polymer without suberin. *Proceedings of the National Academy of Sciences* **109**: 10101–10106.

**Nawrath C, Schreiber L, Franke RB, Geldner N, Reina-Pinto JJ, Kunst L**. **2013**. Apoplastic Diffusion Barriers in Arabidopsis. *The Arabidopsis Book* **11**: e0167.

**Naz AA, Arifuzzaman M, Muzammil S, Pillen K, Léon J**. **2014**. Wild barley introgression lines revealed novel QTL alleles for root and related shoot traits in the cultivated barley (*Hordeum vulgare* L.). *BMC Genetics* **15**: 107.

**Naz AA, Ehl A, Pillen K, Léon J**. **2012**. Validation for root-related quantitative trait locus effects of wild origin in the cultivated background of barley (*Hordeum vulgare* L.). *Plant Breeding* **131**: 392–398.

**Nobel PS**. **2009**. *Physicochemical and Environmental Plant Physiology*. Academic Press, Oxford UK

**North GB, Nobel PS**. **1998**. Water uptake and structural plasticity along roots of a desert succulent during prolonged drought. *Plant, Cell and Environment* **21**: 705–713.

**North GB, Nobel PS**. **2000**. Heterogeneity in Water Availability Alters Cellular Development and Hydraulic Conductivity along Roots of a Desert Succulent. *Annals of Botany* **85**: 247–255.

**Opitz N, Marcon C, Paschold A, Malik WA, Lithio A, Brandt R, Piepho H-P, Nettleton D, Hochholdinger F**. **2016**. Extensive tissue-specific transcriptomic plasticity in maize primary roots upon water deficit. *Journal of Experimental Botany* **67**: 1095–1107.

**Panikashvili D, Shi JX, Bocobza S, Franke RB, Schreiber L, Aharoni A**. **2010**. The *Arabidopsis* DSO/ABCG11 Transporter Affects Cutin Metabolism in Reproductive Organs and Suberin in Roots. *Molecular Plant* **3**: 563–575.

**Peterson CA**. **1987**. The Exodermal Casparian Band of Onion Roots Blocks the Apoplastic Movement of Sulphate Ions. *Journal of Experimental Botany* **38**: 2068–2081.

**Peterson CA**. **1988**. Exodermal Casparian bands: their significance for ion uptake by roots. *Physiologia Plantarum* **72**: 204–208.

**Peterson CA, Cholewa E**. **1998**. Structural modifications of the apoplast and their potential impact on ion uptake. *Zeitschrift für Pflanzenernährung und Bodenkunde* **161**: 521–531.

**Peterson CA, Enstone DE**. **1996**. Functions of passage cells in the endodermis and exodermis of roots. *Physiologia Plantarum* **97**: 592–598.

**Ranathunge K, Kim YX, Wassmann F, Kreszies T, Zeisler V, Schreiber L**. **2017**. The composite water and solute transport of barley ( *Hordeum vulgare* ) roots: effect of suberized barriers. *Annals of Botany* **119**: 629–643.

**Ranathunge K, Kotula L, Steudle E, Lafitte R**. **2004**. Water permeability and reflection coefficient of the outer part of young rice roots are differently affected by closure of water channels (aquaporins) or blockage of apoplastic pores. *Journal of Experimental Botany* **55**: 433–447.

**Ranathunge K, Lin J, Steudle E, Schreiber L**. **2011a**. Stagnant deoxygenated growth enhances root suberization and lignifications, but differentially affects water and NaCl permeabilities in rice (*Oryza sativa* L.) roots. *Plant, Cell & Environment* **34**: 1223–1240.

**Ranathunge K, Schreiber L**. **2011**. Water and solute permeabilities of Arabidopsis roots in relation to the amount and composition of aliphatic suberin. *Journal of Experimental Botany* **62**: 1961–1974.

**Ranathunge K, Schreiber L, Bi Y-M, Rothstein SJ**. **2016**. Ammonium-induced architectural and anatomical changes with altered suberin and lignin levels significantly change water and solute permeabilities of rice (*Oryza sativa* L.) roots. *Planta* **243**: 231–249.

**Ranathunge K, Schreiber L, Franke R**. **2011b**. Suberin research in the genomics era-New interest for an old polymer. *Plant Science* **180**: 339–413.

**Ranathunge K, Schreiber L, Franke R**. **2011c**. Suberin research in the genomics era - New interest for an old polymer. *Plant Science* **180**: 399–413.

**Ranathunge K, Steudle E, Lafitte R**. **2003**. Control of water uptake by rice ( *Oryza sativa* L.): role of the outer part of the root. *Planta* **217**: 193–205.

**Ranathunge K, Thomas RH, Fang X, Peterson CA, Gijzen M, Bernards MA**. **2008**. Soybean Root Suberin and Partial Resistance to Root Rot Caused by *Phytophthora sojae*. *Phytopathology* **98**: 1179–1189.

**Rüdinger M, Hallgren SW, Steudle E, Schulze E-D**. **1994**. Hydraulic and osmotic properties of spruce roots. *Journal of Experimental Botany* **45**: 1413–1425.

**Sanderson J**. **1983**. Water Uptake by Different Regions of the Barley Root. Pathways of Radial Flow in Relation to Development of the Endodermis. *Journal of Experimental Botany* **34**: 240–253.

**Schreiber L**. **1996**. Chemical composition of Casparian strips isolated from *Clivia miniata* Reg. roots: evidence for lignin. *Planta* **199**: 596–601.

**Schreiber L**. **2010**. Transport barriers made of cutin, suberin and associated waxes. *Trends in plant science* **15**: 546–53.

**Schreiber L, Breiner H-W, Riederer M, Düggelin M, Guggenheim R**. **1994**. The Casparian Strip of *Clivia miniata* Reg. Roots: Isolation, Fine Structure and Chemical Nature. *Botanica Acta* **107**: 353–361.

**Schreiber L, Franke R, Hartmann K**. **2005a**. Effects of NO3 deficiency and NaCl stress on suberin deposition in rhizo- and hypodermal (RHCW) and endodermal cell walls (ECW) of castor bean (*Ricinus communis* L.) roots. *Plant and Soil* **269**: 333–339.

**Schreiber L, Franke R, Hartmann KD, Ranathunge K, Steudle E**. **2005b**. The chemical composition of suberin in apoplastic barriers affects radial hydraulic conductivity differently in the roots of rice (*Oryza sativa* L. cv. IR64) and corn (*Zea mays* L. cv. Helix). *Journal of Experimental Botany* **56**: 1427–1436.

**Schreiber L, Hartmann K, Skrabs M, Zeier J**. **1999**. Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. *Journal of Experimental Botany* **50**: 1267–1280.

**Shin-ichiro K, Ishihara K**. **1959**. Studies on the Root Hairs in Rice Plant. *Japanese Journal of Crop Science* **27**: 341–348.

**Shiono K, Ando M, Nishiuchi S, Takahashi H, Watanabe K, Nakamura M, Matsuo Y, Yasuno N, Yamanouchi U, Fujimoto M, *et al.* 2014a**. RCN1/OsABCG5, an ATP-binding cassette (ABC) transporter, is required for hypodermal suberization of roots in rice ( *Oryza sativa* ). *The Plant Journal* **80**: 40–51.

**Shiono K, Yamauchi T, Yamazaki S, Mohanty B, Malik AI, Nagamura Y, Nishizawa NK, Tsutsumi N, Colmer TD, Nakazono M**. **2014b**. Microarray analysis of laser-microdissected tissues indicates the biosynthesis of suberin in the outer part of roots during formation of a barrier to radial oxygen loss in rice (*Oryza sativa*). *Journal of experimental botany* **65**: 4795–4806.

**Singh C, Jacobsen L**. **1977**. The Radial and Longitudinal Path of Ion Movement in Roots. *Physiologia Plantarum* **41**: 59–64.

**Smyth GK**. **2004**. Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. *Statistical Applications in Genetics and Molecular Biology* **3**: 1–25.

**Smyth GK**. **2005**. limma: Linear Models for Microarray Data. In: Bioinformatics and Computational Biology Solutions Using R and Bioconductor. New York: Springer-Verlag, 397–420.

**Soler M, Serra O, Molinas M, Huguet G, Fluch S, Figueras M**. **2007**. A genomic approach to suberin biosynthesis and cork differentiation. *Plant Physiology* **144**: 419–31.

**Steudle E**. **1993**. Pressure probe techniques: basic principles and applications to studies of water and solute relations at the cell, tissue and organ level. In: JAC S, Griffiths H E, eds. Water deficits: plant responses from cell to community. Oxford: Bios Scientific Publishers, 5–36.

**Steudle E**. **1997**. Water transport across plant tissue: Role of water channels. *Biology of the Cell* **89**: 259–273.

**Steudle E**. **2000a**. Water uptake by roots: an integration of views. *Plant Soil* **226**: 45–56.

**Steudle E**. **2000b**. Water uptake by roots: effects of water deficit. *Journal of Experimental Botany* **51**: 1531–1542.

**Steudle E, Frensch J**. **1996**. Water transport in plants: Role of the apoplast. *Plant and Soil* **187**: 67–79.

**Steudle E, Heydt H**. **1997**. Water transport across tree roots. In: Rennenberg H, Eschrich W, Ziegler H, eds. Trees—contributions to modern tree physiology. The Netherlands: Backhuys Publishers, 235–255.

**Steudle E, Jeschke WD**. **1983**. Water transport in barley roots. *Planta* **158**: 237–248.

**Steudle E, Meshcheryakov AB**. **1996**. Hydraulic and osmotic properties of oak roots. *Journal of Experimental Botany* **47**: 387–401.

**Steudle E, Oren R, Schulze E-D**. **1987**. Water Transport in Maize Roots : Measurement of Hydraulic Conductivity, Solute Permeability, and of Reflection Coefficients of Excised Roots Using the Root Pressure Probe. *Plant Physiology* **84**: 1220–1232.

**Steudle E, Peterson CA**. **1998**. How does water get through roots? *Journal of Experimental Botany* **49**: 775–788.

**Steudle E, Ranathunge K**. **2007**. Apoplastic water transport in roots. In: The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions. Dordrecht: Springer Netherlands, 119–130.

**Suku S, Knipfer T, Fricke W**. **2013**. Do root hydraulic properties change during the early vegetative stage of plant development in barley (*Hordeum vulgare*)? *Annals of Botany* **113**: 385–402.

**Tanksley SD, McCouch SR**. **1997**. Seed Banks and Molecular Maps: Unlocking

Genetic Potential from the Wild. *Science* **277**: 1063–1066.

**Thomas R, Fang X, Ranathunge K, Anderson TR, Peterson CA, Bernards MA**. **2007**. Soybean root suberin: anatomical distribution, chemical composition, and relationship to partial resistance to *Phytophthora sojae*. *Plant Physiology* **144**: 299–311.

**Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z**. **2017**. agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Research* **45**: W122–W129.

**Tomos AD, Leigh RA**. **1999**. THE PRESSURE PROBE: A Versatile Tool in Plant Cell Physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 447–472.

**Tylová E, Pecková E, Blascheová Z, Soukup A**. **2017**. Casparian bands and suberin lamellae in exodermis of lateral roots: an important trait of roots system response to abiotic stress factors. *Annals of Botany* **120**: 71–85.

**Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K**. **2006**. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal* **45**: 523–539.

**Vishwanath SJ, Delude C, Domergue F, Rowland O**. **2015**. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. *Plant Cell Reports* **34**: 573–586.

**Wagner GP, Kin K, Lynch VJ**. **2012**. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theory in Biosciences* **131**: 281–285.

**Waßmann F**. **2014**. Suberin biosynthesis in *O. sativa*: characterisation of a cytochrome P450 monooxygenase. PhD-thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn (2014)

**Yadav V, Molina I, Ranathunge K, Castillo IQ, Rothstein SJ, Reed JW**. **2014**. ABCG transporters are required for suberin and pollen wall extracellular barriers in *Arabidopsis*. *The Plant cell* **26**: 3569–88.

**Yamaguchi M, Sharp RE**. **2010**. Complexity and coordination of root growth at low water potentials: Recent advances from transcriptomic and proteomic analyses. *Plant, Cell and Environment* **33**: 590–603.

**Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB**. **2012**. A Land-Plant-Specific Glycerol-3-Phosphate Acyltransferase Family in *Arabidopsis*: Substrate Specificity, sn-2 Preference, and Evolution. *Plant Physiology* **160**: 638–652.

**Zeier J, Ruel K, Ryser U, Schreiber L**. **1999**. Chemical analysis and immunolocalisation of lignin and suberin in endodermal and hypodermal/rhizodermal cell walls of developing maize (*Zea mays* L.) primary roots. *Planta* **209**: 1–12.

**Zeier J, Schreiber L**. **1997**. Chemical Composition of Hypodermal and Endodermal Cell Walls and Xylem Vessels lsolated from *Clivia miniata*. *Plant Physiology* **113**: 1223–1231.

**Zeier J, Schreiber L**. **1998**. Comparative investigation of primary and tertiary endodermal cell walls isolated from the roots of five monocotyledoneous species: chemical composition in relation to fine structure. *Planta* **206**: 349–361.

**Zhu GL, Steudle E**. **1991**. Water transport across maize roots. *Plant Physiology*. **95**: 305–315.

**Zimmermann HM, Hartmann K, Schreiber L, Steudle E**. **2000**. Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (Zea mays L.). *Planta* **210**: 302–311.

**Zimmermann HM, Steudle E**. **1998**. Apoplastic transport across young maize roots: effect of the exodermis. *Planta* **206**: 7–19.

**Zingaretti SM, Inácio MC, de Matos Pereira L, Antunes Paz T, de Castro França S**. **2013**. Water Stress and Agriculture. In Akinci DS, eds: Responses of Organisms to Water Stress. (InTechOpen) 151–179.

# 7 CV

# 8 List of publications

**Kreszies, T.**, Shellakkutti, N., Osthoff, A., Peng, Y., Baldauf, J.A., Zeisler-Diehl, V.V., Ranathunge, K., Hochholdinger, F., Schreiber, L., 2018 Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses. New Phytologist, doi: 10.1111/nph.15351

**Kreszies, T.**, Schreiber, L., Ranathunge, K., 2018. Suberized transport barriers in Arabidopsis, barley and rice roots: from the model plant to crop species. Journal of Plant Physiology, doi: 10.1016/j.jplph.2018.02.002

Even, M. Sabo, M., Meng, D., **Kreszies, T.**, Schreiber, L., Fricke, W., 2018 Night-time transpiration in barley (*Hordeum vulgare*) facilitates respiratory carbon dioxide release and is regulated during salt stress. Annals of Botany, doi: 10.1093/aob/mcy084

Ranathunge, K., Kim, Y.X., Wassmann, F., **Kreszies, T.**, Zeisler, V., Schreiber, L., 2017. The composite water and solute transport of barley (*Hordeum vulgare*) roots: effect of suberized barriers. Annals of Botany 119 (4), 629-643

# 9 Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig angefertigt habe. Ich habe keine anderen als die angegebenen Quellen und Hilfsmittel verwendet.

Die Arbeit wurde weder in gleicher, noch in ähnlicher Form einer anderen Prüfungsbehörde vorgelegt. Alle Veröffentlichungen sind angegeben.

Ort, Datum Tino Kreszies