Drought-induced processes in the rhizosphere of maize (*Zea mays* L.)

Dissertation to obtain the doctoral degree of Agricultural Sciences

(Dr. sc. agr.)

Faculty of Agricultural Sciences

University of Hohenheim

Institute of Crop Science

submitted by

Lena Maria Käsbauer

neé Werner

from Munich

2022

Die vorliegende Arbeit wurde am 05.05.2023 von der Fakultät Agrarwissenschaften der Universität Hohenheim als "Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften" angenommen.

Tag der mündlichen Prüfung: 05.05.2023

Leiter der Prüfung: Herr Prof. Dr. Thilo Streck

Berichterstatter, 1. Prüfer: Herr Prof. Dr. Christian Zörb

Mitberichterstatter, 2. Prüfer: Herr Prof. Dr. Gerd Patrick Bienert

weitere Berichterin bzw. Prüfer: Herr Prof. Dr. Uwe Ludewig

Contents

Chapter 1

General introduction

1. General introduction

The ongoing climate change has led to increasing water shortage worldwide, which has heightened the intensity and duration of drought (Dietz et al., 2021). About 55 million people are affected by drought yearly, according to the World Health Organization (2022b). The resulting crop losses lead to malnutrition and, subsequently, increased risk for infectious diseases (World Health Organization, 2022a). Thus far, several strategies by crops have been described to allocate and manage drought by plant shoot and root. For example, improving or limiting water uptake and increasing root density are already utilized for breeding strategies (Dietz et al., 2021; Tardieu et al., 2018).

Besides achieving drought adaption by root and shoot, research has recently focused on the rhizosphere, in detail the interplay of crop, roots and soil to improve stress responses and agricultural systems (Chen et al., 2022; Rolfe et al., 2019; Vetterlein et al., 2020; de Vries et al., 2020). According to Vetterlein et al. (2020), "the main knowledge gaps in rhizosphere research are related to the difficulty in mechanistically linking the physical, chemical, and biological processes taking place at different spatial and temporal scales in the rhizosphere (nm to cm and minutes to months) and then upscaling them to the root system and the soil profile." (Page 2) In other words, the rhizosphere is highly dynamic and complex (Hinsinger et al., 2009), and its spatiotemporal patterns must be identified (Vetterlein et al., 2020). However, accessing roots and the rhizosphere is challenging (Brunner et al., 2015; Vetterlein et al., 2020). Several methods are available to address these difficulties and have to be utilized in concert to achieve a complete picture of the rhizosphere (Oburger & Jones, 2018).

1.1. Drought and local drought

Drought can be classified as mild/sporadic or severe/extended, and plants react to the respective drought stress morphologically, biochemically, and physiologically (Zia et al., 2021). Several parameters are used to evaluate drought stress, including plant growth, relative water content, photosynthetic rate, transpiration, and stomata closure—each of which has a different sensitivity level (Yan et al., 2016). Plants confronted by a drought attempt to maintain a proper water relationship through different mechanisms (Dietz et al., 2021). For example, increased transpiration can cause turgor loss, to which the plant replies with stomatal closure (Tardieu et al., 2018). Plants can also accumulate osmolytes to adjust their osmotic potential and thereby maintain turgor (Blum, 2017; Trovato et al., 2008).

The crop's signaling pathway under drought is complex, and the function of roots in this pathway has been thoroughly researched (Blackman & Davies, 1985; Dietz et al., 2021). This research has utilized split root systems, in which only parts of the roots are drought-treated

(local drought) (Blackman & Davies, 1985; Saradadevi et al., 2014). Several split-root experiments have shown increased water-use efficiency due to local drought, likely caused by partial stomatal closure (Blackman & Davies, 1985; Campos et al., 2009; Iqbal et al., 2019; Schachtman & Goodger, 2008). However, Blackman and Davies (1985) measured unaffected turgor and abscisic acid content in their local drought experiment.

The cost of drought is a yield penalty and a reduced crop quality (Zia et al., 2021). Shoot growth is more affected by drought than root growth (Poorter et al., 2012). However, another mechanism by which plants maintain their water status is reorganizing their root architecture (Dietz et al., 2021). However, a too-quick allocation of the root system potentially risks suboptimal growth in the case of a restored water supply (Poorter et al., 2012). Therefore, adjustment of metabolites or water transporters at the beginning of a drought (Maurel & Nacry, 2020; Trovato et al., 2008) would be less risky. For instance, shaping the rhizosphere by root exudates as a quick and specific response to drought is currently discussed (Canarini et al., 2016; Gargallo-Garriga et al., 2018; Rolfe et al., 2019). However, whether rhizosphere shaping is promoted locally by individual roots' experiencing drought or whether it is caused by all roots regardless of water level remains unclear.

1.2. Rhizosphere and drought

Hinsinger et al. (2009) suggested, "The rhizosphere probably represents the most dynamic habitat on Earth." (Page 117) The rhizosphere is the soil surrounding roots, and it is a highly dynamic and active habitat (Hinsinger et al., 2009; Pang et al., 2017; Vetterlein et al., 2020). The expanse of the rhizosphere is not constant but rather depends on the measured parameter and its mobility (Kuzyakov & Razavi, 2019; Vetterlein et al., 2020). Furthermore, the rhizosphere is shaped by several factors, such as roots and root hair, mucilage, exudates, microorganisms, and soil properties, which in turn can be altered by the rhizosphere (Gahoonia & Nielsen, 1998; Vetterlein et al., 2020).

A highly hydrologically active region, the rhizosphere possesses different hydraulic properties from those of bulk soil (Bengough, 2012), and water is the key factor for transport processes and connectivity in the rhizosphere (Vetterlein et al., 2020). The flow of water in the rhizosphere can be affected by mucilage (see Section 1.2.2) (Ahmed et al., 2014; Kroener et al., 2014); especially in dry soils, mucilage can facilitate water uptake by roots (Ahmed et al., 2014). Mucilage also facilitates root growth by providing lubrication during soil penetration (Iijima et al., 2003). Furthermore, root hairs (see 1.2.1) support the root tip during soil penetration by providing grip, which can be increased by root exudation (Galloway et al., 2020; Rongsawat et al., 2021). Crops can adjust their root exudates under drought and therefore

influence the structure of the microbial community (Gargallo-Garriga et al., 2018; Munoz-Ucros et al., 2022). Therefore, root growth and type used to allocate water in the soil are especially relevant under dry conditions (Ahmed et al., 2018; Dietz et al., 2021).

1.2.1. Root hairs

A single epidermis cell (trichoblast) in the root elongation zone can differentiate and extend into a tubular outgrowth called "root hair" (Dolan, 2017; Gahoonia & Nielsen, 1998; Pang et al., 2017). Not all epidermal cells result in root hair formation, and the pattern of root hair cell distribution along the epidermis varies with species (Dolan, 2017).

Root hair research in *Zea mays* has been performed using hairless root mutants, such as the *rth3* mutant (Wen & Schnable, 1994). This mutant induces and initiates root hairs, but the elongation of these hairs is disturbed (Hochholdinger et al., 2008).

Root hairs have several functions and benefits, such as enlarging the root surface, anchoring the root, providing grip, forming rhizoheat, acquiring nutrients and water, increasing carbon exudation, and improving the microbial community (Galloway et al., 2020; Holz et al., 2018b; Robertson-Albertyn et al., 2017; Rongsawat et al., 2021). Root hairs increase the root-soil surface since the volume of the root hair cylinder is around 100 times greater than that of the root, enabling better acquisition of nutrients and water (Rongsawat et al., 2021). For instance, root hair density and length increase under phosphorous or boron deficiency (Martín-Rejano et al., 2011; Zhang et al., 2018). Under drought, maize root hairs lose their turgidity and shrink more quickly than roots due to drying soil (Duddek et al., 2022). In barley, wetted soil resulted in shorter root hairs (Haling et al., 2014). Therefore, increased root surface and the resultant greater root-soil contact seem beneficial for water acquisition (Gilroy & Jones, 2000).

1.2.2. Root exudation and mucilage

The release of root exudates can occur actively, for example, by the ABC or MATE transporter family, or passively by diffusion, ionic channels, or vesicles (Vives-Peris et al., 2020). Passive diffusion can also be performed selectively by opening membrane channels (Ryan et al., 2001). Furthermore, a reuptake of exudates is also possible (Oburger & Jones, 2018). Badri and Vivanco (2009) stated that the main exudation occurs in the area behind the root tip, though other and older root parts also seem to release exudates. Root mucilage in turn surrounds the root tip (Jones et al., 2009). It is secreted by the cap cells located at the root tip and is therefore called root cap mucilage (Carminati & Vetterlein, 2013). In addition, it is important to mention that often in literature, it is not distinguished between plant-derived root exudates and rhizodeposits, including cells from the root cap and mycorrhiza (Oburger & Jones, 2018).

Mucilage contains about 94% neutral and acid polysaccharides, as well as proteins, phenolic acids, and phospholipids (Bacic et al., 1986; Carminati & Vetterlein, 2013; Read et al., 2003).

Vives-Peris et al. (2020) summarized a broad variety of verified compounds in root exudates, including amino acids, sugars, organic acids, fatty acids, sterols, growth factors and vitamins, enzymes, flavonoids, nucleotides/purines, and several other substances, such as phytohormones and alcohols. Therefore, the compounds in root exudates are diverse, and their quality and quantity can be altered by external factors (e.g., drought, nutrient deficiency) or plant species and age (Badri & Vivanco, 2009; Carvalhais et al., 2011; Gargallo-Garriga et al., 2018; Neumann et al., 2014; Rolfe et al., 2019; Vives-Peris et al., 2020). Soil properties also affect exudation; for instance, exudates collected from lettuce roots growing in alluvial loam contained lower quantities of sugars and amino acids than exudates from roots growing in loess loam and diluvial sand (Neumann et al., 2014).

The physicochemical rhizosphere processes driven or affected by exudates are soil aggregation, water flow, weathering, nutrient mobilization, and detoxification (Oburger & Jones, 2018). Other processes that have been frequently discussed are communication via root exudates and signaling function or symbiosis with microorganisms, and subsequent adaptation to environmental conditions (Oburger & Jones, 2018; Rolfe et al., 2019). This symbiosis includes a release of carbon in the form of exudates, which is then consumed by microorganisms, resulting in increased microorganism activity and, thereby, the liberation of nutrients in the soil (Gargallo-Garriga et al., 2018; Kuzyakov & Domanski, 2000). Further biochemical processes affecting or driving root exudation are greenhouse gas emission, respiration, carbon and nutrient cycling, soil organic matter turnover, and carbon sequestration (Oburger & Jones, 2018).

A further process influencing root exudation is drought by altering the composition of root exudate compounds (Chen et al., 2022; Gargallo-Garriga et al., 2018). For example, under drought, holm oak (*Quercus ilex*) mainly exudes secondary metabolites (71%) but shifts to primary metabolites (81%) after recovery (Gargallo-Garriga et al., 2018). Furthermore, the duration of the drought event (in *Quercus ilex L.*) can increase the exuded carbon by up to 21% (Preece et al., 2018). Under extreme drought, root exudation decreases, likely due to resource redirection to maintain essential processes for survival (Gargallo-Garriga et al., 2018). In lupin, Carminati (2013) measured a wetter rhizosphere than in bulk soil during soil drying. However, after rewatering, a temporarily dryer rhizosphere was found (Carminati, 2013). This effect may have been due to mucilage's maintenance of the hydraulic conductivity of the soil, which keeps the soil close to the root wet (Ahmed et al., 2014). Further functions of mucilage are lubrication during root penetration, a role in rhizosphere hydraulics and root water uptake, and enables soil-

aggregate stabilization (Ahmed et al., 2014; Carminati et al., 2010; Iijima et al., 2003; Morel et al., 1991).

1.3. Root exudate and mucilage collection

Root exudate sampling is distinguished between sampling in hydroponic/*in vitro* cultures and soil-based approaches (Oburger & Jones, 2018; Vives-Peris et al., 2020). Hydroponic approaches can avoid exudate loss caused by soil sorption, alteration, and degradation by microorganisms (Oburger & Jones, 2018; Vives-Peris et al., 2020). Similarly, in aeroponic systems, roots grow in humid air, and exudates/mucilage can be collected directly from the root/tip (Holz et al., 2018a; Zickenrott et al., 2016). A further benefit of *in vitro* sterile and hydroponic systems is a higher reproducibility compared to soil collections in the field (Vives-Peris et al., 2020). However, the systems of these easily applicable approaches are highly artificial, and research questions considering the entire soil system or the impact of soil-relevant parameters can therefore not be answered (Oburger & Schmidt, 2016; Vranova et al., 2013).

For this reason, new soil-based approaches are constantly being invented. Oburger and Jones (2018) and Neumann et al. (2009) distinguished these approaches as systems extracting from the entire root system and systems sampling from individual root segments. Exudates from the entire root system growing in soil can be sampled using hybrid soil–hydroponic systems (Canarini et al., 2016; Lucas García et al., 2001). However, this method requires separating the roots from the soil by washing, and the unavoidable physical damage and thus bias by sampling cannot be excluded from the results (Oburger & Jones, 2018). Another approach is a "SOIL-REC" in which a root mat with a permeable membrane separates roots from the soil but allows a soil–solution exchange (Oburger et al., 2013; Oburger et al., 2014). A percolation or repeated leaching system also allows exudate collection in soils (Mimmo et al., 2011; Neumann & Römheld, 1999). In these setups, however, soil and microbial contamination cannot be eliminated (Oburger & Jones, 2018). By placing agar or absorbing filters or utilizing exudation traps on individual areas on roots, an undisturbed exudate sampling in soil of individual root segments is possible (Marschner et al., 1987; Neumann et al., 2014; Phillips et al., 2008). Nonetheless, information concerning the entire root system is likely lost by sampling individual segments (Oburger & Jones, 2018). An additional approach to collecting maize mucilage is rehydrating and then gathering the mucilage on airborne brace roots before the roots reach the soil (Ahmed et al., 2015). This method can only be applied for species with brace roots (Zickenrott et al., 2016). As shown above, all available sampling approaches have advantages and disadvantages (Oburger & Jones, 2018), and the precision of root sampling is hampered by the poor accessibility of roots and exudates (Brunner et al., 2015; Gargallo-Garriga et al., 2018).

1.4. Objectives

Identifying spatial and temporal patterns of chemical, physical, and biological processes in the rhizosphere, as well as the mechanisms underlying these processes, is highly relevant to discussing the role of the rhizosphere in drought resistance (Vetterlein et al., 2020; Vetterlein et al., 2021). Participants in these rhizosphere processes include root hairs, root exudation, and mucilage (Vetterlein et al., 2020). However, soil, rhizosphere, and root research is challenging because these parts of the plant–soil system are more difficult to access and sample (Oburger & Jones, 2018). Therefore, this thesis contributes to filling knowledge gaps regarding rhizosphere processes and enabling linkage between these gaps by discussing the following: *i*) the functions, behavior, and development of root hairs, considering nutrient availability and uptake (Chapter 2); *ii*) compensation and response to local drought in maize (Chapter 3); and *iii*) differences in the physico-chemical properties of two maize mucilage sampling strategies (Chapter 4).

This thesis seeks to answer the following questions:

- Are root hairs relevant for water uptake, and what role do they play under drought? (Chapter 2)
  - Root hairs are expected to be beneficial under drought stress.

- Does local drought in *Zea mays* result in a distinguishable systemic and local metabolic and physiological response to drought, as well as compensatory water uptake? (Chapter 3)
  - Local drought in *Zea mays* results in hydraulic redistribution, as well as local and systemic osmotic adjustment in *Zea mays*.

- Do the physico-chemical properties of *Zea mays* mucilage differ between two common collection systems? (Chapter 4)
  - The physico-chemical properties of *Zea mays* mucilage from different collection systems differ due to developmental stage, root type, and collection environment.

References

Ahmed, M. A., Holz, M., Woche, S. K., Bachmann, J., Carminati, A. (2015). Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. Journal of Plant Nutrition and Soil Science 178:821–824. doi:10.1002/jpln.201500177.

Ahmed, M.A., Kroener, E., Holz, M., Zarebanadkouki, M., Carminati, A. (2014). Mucilage exudation facilitates root water uptake in dry soils. Functional plant biology : FPB 41:1129–1137. doi:10.1071/FP13330.

Ahmed, M. A., Zarebanadkouki, M., Meunier, F., Javaux, M., Kaestner, A., Carminati, A. (2018). Root type matters: measurement of water uptake by seminal, crown, and lateral roots in maize. Journal of experimental botany 69:1199–1206. doi:10.1093/jxb/erx439.

Bacic, A., Moody, S. F., Clarke, A. E. (1986). Structural Analysis of Secreted Root Slime from Maize (Zea mays L.). Plant Physiology 80:771–777. doi:10.1104/pp.80.3.771.

Badri, D. V., Vivanco, J. M. (2009). Regulation and function of root exudates. Plant, cell & environment 32:666–681. doi:10.1111/j.1365-3040.2009.01926.x.

Bengough, A. G. (2012). Water Dynamics of the Root Zone: Rhizosphere Biophysics and Its Control on Soil Hydrology. Vadose Zone Journal 11:vzj2011.0111. doi:10.2136/vzj2011.0111.

Blackman, P. G., Davies, W. J. (1985). Root to Shoot Communication in Maize Plants of the Effects of Soil Drying. Journal of Experimental Botany 36:39–48. doi:10.1093/jxb/36.1.39.

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, cell & environment 40:4–10. doi:10.1111/pce.12800.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., Sperisen, C. (2015). How tree roots respond to drought. Frontiers in plant science 6:547. doi:10.3389/fpls.2015.00547.

Campos, H., Trejo, C., Peña-Valdivia, C. B., Ramírez-Ayala, C., Sánchez-García P (2009). Effect of partial rootzone drying on growth, gas exchange, and yield of tomato (Solanum lycopersicum L.). Scientia Horticulturae 120:493–499. doi:10.1016/j.scienta.2008.12.014.

Canarini, A., Merchant, A., Dijkstra, F. A. (2016). Drought effects on Helianthus annuus and Glycine max metabolites: from phloem to root exudates. Rhizosphere 2:85–97. doi:10.1016/j.rhisph.2016.06.003.

Carminati, A. (2013). Rhizosphere wettability decreases with root age: a problem or a strategy to increase water uptake of young roots? Frontiers in plant science 4:298. doi:10.3389/fpls.2013.00298.

Carminati, A., Moradi, A. B., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H.-J., Oswald, S. E. (2010). Dynamics of soil water content in the rhizosphere. Plant and Soil 332:163–176. doi:10.1007/s11104-010-0283-8.

Carminati, A., Vetterlein, D. (2013). Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Annals of botany 112:277–290. doi:10.1093/aob/mcs262.

Carvalhais, L. C., Dennis, P. G., Fedoseyenko, D., Hajirezaei, M.-R., Borriss, R., von Wirén, N. (2011). Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. Journal of Plant Nutrition and Soil Science 174:3–11. doi:10.1002/jpln.201000085.

Chen, Y., Yao, Z., Sun, Y., Wang, E., Tian, C., Sun, Y., Liu, J., Sun, C., Tian, L. (2022). Current Studies of the Effects of Drought Stress on Root Exudates and Rhizosphere Microbiomes of Crop Plant Species. International journal of molecular sciences 23. doi:10.3390/ijms23042374.

de Vries, F. T., Griffiths RI, Knight CG, Nicolitch O, Williams A (2020). Harnessing rhizosphere microbiomes for drought-resilient crop production. Science 368:270–274. doi:10.1126/science.aaz5192.

Dietz, K.-J., Zörb, C., Geilfus, C.-M. (2021). Drought and crop yield. Plant biology 23:881–893. doi:10.1111/plb.13304.

Dolan, L. (2017). Root hair development in grasses and cereals (Poaceae). Current opinion in genetics & development 45:76–81. doi:10.1016/j.gde.2017.03.009.

Duddek, P., Carminati, A., Koebernick, N., Ohmann, L., Lovric, G., Delzon, S., Rodriguez-Dominguez. C. M., King, A., Ahmed, M. A. (2022). The impact of drought-induced root and root hair shrinkage on root-soil contact. Plant physiology. doi:10.1093/plphys/kiac144.

Gahoonia, T. S., Nielsen, N.E. (1998). Direct evidence on participation of root hairs in phosphorus (32P) uptake from soil. Plant and Soil 198:147–152. doi:10.1023/A:1004346412006.

Galloway, A. F., Akhtar, J., Marcus, S. E., Fletcher, N., Field, K., Knox, P. (2020). Cereal root exudates contain highly structurally complex polysaccharides with soil-binding properties. The Plant journal : for cell and molecular biology 103:1666–1678. doi:10.1111/tpj.14852.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., Peñuelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. Scientific reports 8:12696. doi:10.1038/s41598-018-30150-0.

Gilroy, S., Jones, D. L. (2000). Through form to function: root hair development and nutrient uptake. Trends in plant science 5:56–60. doi:10.1016/S1360-1385(99)01551-4.

Haling, R. E., Brown, L. K., Bengough, A. G., Valentine, T. A., White, P. J., Young, I. M., George, T. S. (2014). Root hair length and rhizosheath mass depend on soil porosity, strength and water content in barley genotypes. Planta 239:643–651. doi:10.1007/s00425-013-2002-1.

Hinsinger, P., Bengough, A. G., Vetterlein, D., Young, I. M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant and Soil 321:117–152. doi:10.1007/s11104-008-9885-9.

Hochholdinger, F., Wen, T.-J., Zimmermann, R., Chimot-Marolle, P., Da Costa e Silva, O., Bruce, W., Lamkey, K.R., Wienand, U., Schnable, P. S. (2008). The maize (Zea mays L.) roothairless3 gene encodes a putative GPI-anchored, monocot-specific, COBRA-like protein that significantly affects grain yield. The Plant journal : for cell and molecular biology 54:888–898. doi:10.1111/j.1365-313X.2008.03459.x.

Holz, M., Leue, M., Ahmed, M. A., Benard, P., Gerke, H. H., Carminati A (2018a). Spatial Distribution of Mucilage in the Rhizosphere Measured With Infrared Spectroscopy. Frontiers in Environmental Science 6. doi:10.3389/fenvs.2018.00087.

Holz, M., Zarebanadkouki, M., Kuzyakov, Y., Pausch, J., Carminati, A. (2018b). Root hairs increase rhizosphere extension and carbon input to soil. Annals of botany 121:61–69. doi:10.1093/aob/mcx127.

Iijima, M., Higuchi, T., Barlow, P. W., Bengough, A. G. (2003). Root cap removal increases root penetration resistance in maize (Zea mays L). Journal of experimental botany 54:2105–2109. doi:10.1093/jxb/erg226.

Iqbal, N., Hussain, S., Raza, M.A., Yang, C.-Q., Safdar, M. E., Brestic, M., Aziz, A., Hayyat, M. S., Asghar, M. A., Wang, X. C., Zhang, J., Yang, W., Liu, J. (2019). Drought Tolerance of Soybean (Glycine max L. Merr.) by Improved Photosynthetic Characteristics and an Efficient Antioxidant Enzyme Activities Under a Split-Root System. Frontiers in physiology 10:786. doi:10.3389/fphys.2019.00786.

Jones, D. L., Nguyen, C., Finlay, R. D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant and Soil 321:5–33. doi:10.1007/s11104-009-9925-0.

Kroener, E., Zarebanadkouki, M., Kaestner, A., Carminati, A. (2014). Nonequilibrium water dynamics in the rhizosphere: How mucilage affects water flow in soils. Water Resources Research 50:6479–6495. doi:10.1002/2013WR014756.

Kuzyakov, Y., Domanski, G. (2000). Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163:421–431. doi:10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R.

Kuzyakov, Y., Razavi, B. S. (2019). Rhizosphere size and shape: Temporal dynamics and spatial stationarity. Soil Biology and Biochemistry 135:343–360. doi:10.1016/j.soilbio.2019.05.011.

Lucas García J. A., Barbas, C., Probanza, A., Barrientos, M. L., Gutierrez Mañero, F. J. (2001). Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. Phytochemical analysis : PCA 12:305–311. doi:10.1002/pca.596.

Marschner, H., Romheld, V., Kissel, M. (1987). Localization of phytosiderophore release and of iron uptake along intact barley roots. Physiologia Plantarum 71:157–162. doi:10.1111/j.1399-3054.1987.tb02861.x.

Martín-Rejano, E. M., Camacho-Cristóbal, J. J., Herrera-Rodríguez, M. B., Rexach, J., Navarro-Gochicoa, M. T., González-Fontes, A. (2011). Auxin and ethylene are involved in the responses of root system architecture to low boron supply in Arabidopsis seedlings. Physiologia plantarum 142:170–178. doi:10.1111/j.1399-3054.2011.01459.x.

Maurel, C., Nacry, P. (2020). Root architecture and hydraulics converge for acclimation to changing water availability. Nature plants 6:744–749. doi:10.1038/s41477-020-0684-5.

Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C. E., Puschenreiter, M. (2011). Time and substrate dependent exudation of carboxylates by Lupinus albus L. and Brassica napus L.

Plant physiology and biochemistry : PPB 49:1272–1278. doi:10.1016/j.plaphy.2011.08.012.

Morel, J. L., Habib, L., Plantureux, S., Guckert, A. (1991). Influence of maize root mucilage on soil aggregate stability. Plant and Soil 136:111–119. doi:10.1007/BF02465226.

Munoz-Ucros, J., Wilhelm, R. C., Buckley, D. H., Bauerle, T. L. (2022). Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. Plant and Soil 471:443–461. doi:10.1007/s11104-021-05227-x.

Neumann, G., Bott, S., Ohler, M. A., Mock, H.-P., Lippmann, R., Grosch, R., Smalla, K. (2014). Root exudation and root development of lettuce (Lactuca sativa L. cv. Tizian) as affected by different soils. Frontiers in microbiology 5:2. doi:10.3389/fmicb.2014.00002.

Neumann, G., George, T.S., Plassard, C. (2009). Strategies and methods for studying the rhizosphere—the plant science toolbox. Plant and Soil 321:431–456. doi:10.1007/s11104-009-9953-9.

Neumann, G., Römheld, V. (1999). Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil:121–130.

Oburger, E., Dell'mour, M., Hann, S., Wieshammer, G., Puschenreiter, M., Wenzel, W. W. (2013). Evaluation of a novel tool for sampling root exudates from soil-grown plants compared to conventional techniques. Environmental and Experimental Botany 87:235–247. doi:10.1016/j.envexpbot.2012.11.007.

Oburger, E., Gruber, B., Schindlegger, Y., Schenkeveld, W.D.C., Hann, S., Kraemer, S. M., Wenzel, W. W., Puschenreiter, M. (2014). Root exudation of phytosiderophores from soil-grown wheat. The New phytologist 203:1161–1174. doi:10.1111/nph.12868.

Oburger, E., Jones, D. L. (2018). Sampling root exudates – Mission impossible? Rhizosphere 6:116–133. doi:10.1016/j.rhisph.2018.06.004.

Oburger, E., Schmidt. H. (2016). New Methods To Unravel Rhizosphere Processes. Trends in plant science 21:243–255. doi:10.1016/j.tplants.2015.12.005.

Pang, J., Ryan, M. H., Siddique, K. H. M., Simpson, R. J. (2017). Unwrapping the rhizosheath. Plant and Soil 418:129–139. doi:10.1007/s11104-017-3358-y.

Phillips, R. P., Erlitz, Y., Bier, R., Bernhardt, E. S. (2008). New approach for capturing soluble root exudates in forest soils. Functional Ecology 22:990–999. doi:10.1111/j.1365-2435.2008.01495.x.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. The New phytologist 193:30–50. doi:10.1111/j.1469-8137.2011.03952.x.

Preece, C., Farré-Armengol, G., Llusià, J., Peñuelas, J. (2018). Thirsty tree roots exude more carbon. Tree physiology 38:690–695. doi:10.1093/treephys/tpx163.

Read, D. B., Bengough, A. G., Gregory, P. J., Crawford, J. W., Robinson, D., Scrimgeour, C. M., Young I. M., Zhang K, Zhang X (2003). Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytologist 157:315–326. doi:10.1046/j.1469-8137.2003.00665.x.

Robertson-Albertyn, S., Alegria Terrazas, R., Balbirnie, K., Blank, M., Janiak, A., Szarejko, I., Chmielewska, B., Karcz, J., Morris, J., Hedley, P. E., George, T.S., Bulgarelli, D. (2017). Root Hair Mutations Displace the Barley Rhizosphere Microbiota. Frontiers in plant science 8:1094. doi:10.3389/fpls.2017.01094.

Rolfe, S. A., Griffiths, J., Ton, J. (2019). Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current opinion in microbiology 49:73–82. doi:10.1016/j.mib.2019.10.003.

Rongsawat, T., Peltier, J.-B., Boyer, J.-C., Véry, A.-A., Sentenac, H. (2021). Looking for Root Hairs to Overcome Poor Soils. Trends in plant science 26:83–94. doi:10.1016/j.tplants.2020.09.001.

Ryan, P. R., Delhaize, E., Jones, D. L. (2001). Function and mechanism of organic anion exudation from plant roots. Annual review of plant physiology and plant molecular biology 52:527–560. doi:10.1146/annurev.arplant.52.1.527.

Saradadevi, R., Bramley, H., Siddique, K. H., Edwards, E., Palta, J. A. (2014). Reprint of "Contrasting stomatal regulation and leaf ABA concentrations in wheat genotypes when split root systems were exposed to terminal drought". Field Crops Research 165:5–14. doi:10.1016/j.fcr.2014.06.025.

Schachtman, D. P., Goodger, J. Q. D. (2008). Chemical root to shoot signaling under drought. Trends in plant science 13:281–287. doi:10.1016/j.tplants.2008.04.003.

Tardieu, F., Simonneau, T., Muller, B. (2018). The Physiological Basis of Drought Tolerance in Crop Plants: A Scenario-Dependent Probabilistic Approach. Annual review of plant biology 69:733–759. doi:10.1146/annurev-arplant-042817-040218.

Trovato, M., Mattioli, R., Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei 19:325–346. doi:10.1007/s12210-008-0022-8.

Vetterlein, D., Carminati, A., Kögel-Knabner, I., Bienert, G. P., Smalla, K., Oburger, E., Schnepf, A., Banitz, T., Tarkka, M. T., Schlüter, S. (2020). Rhizosphere Spatiotemporal Organization–A Key to Rhizosphere Functions. Frontiers in Agronomy 2. doi:10.3389/fagro.2020.00008.

Vetterlein, D., Lippold, E., Schreiter, S., Phalempin, M., Fahrenkampf, T., Hochholdinger, F., Marcon, C., Tarkka, M., Oburger, E., Ahmed, M., Javaux, M., Schlüter, S. (2021). Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. Journal of Plant Nutrition and Soil Science 184:35–50. doi:10.1002/jpln.202000079.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R. M. (2020). Root exudates: from plant to rhizosphere and beyond. Plant cell reports 39:3–17. doi:10.1007/s00299-019-02447-5.

Vranova, V., Rejsek, K., Skene, K. R., Janous, D., Formanek, P. (2013). Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. Journal of Plant Nutrition and Soil Science 176:175–199. doi:10.1002/jpln.201000360.

Wen, T.-J., Schnable, P. S. (1994). Analyses of Mutants of Three Genes that Influence Root Hair Development in Zea mays (Gramineae) Suggest that Root Hairs are Dispensable. American Journal of Botany 81:833. doi:10.2307/2445764.

World Health Organization (2022a). Drought - Impact, https://www.who.int/health-topics/drought#tab=tab_2 (last checked 20.09.2022).

World Health Organization (2022b). Drought - Overview, https://www.who.int/health-topics/drought#tab=tab_1 (last checked 20.09.2022).

Yan, W., Zhong, Y., Shangguan, Z. (2016). Evaluation of physiological traits of summer maize under drought stress. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 66:133–140. doi:10.1080/09064710.2015.1083610.

Zhang, C., Simpson, R. J., Kim, C. M., Warthmann, N., Delhaize, E., Dolan, L., Byrne, M. E., Wu, Y., Ryan, P. R. (2018). Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic Brachypodium distachyon lines overexpressing endogenous RSL genes. The New phytologist 217:1654–1666. doi:10.1111/nph.14980.

Zia, R., Nawaz, M. S., Siddique, M. J., Hakim, S., Imran, A. (2021). Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiological research 242:126626. doi:10.1016/j.micres.2020.126626.

Zickenrott, I.-M., Woche, S. K., Bachmann, J., Ahmed, M. A., Vetterlein, D. (2016). An efficient method for the collection of root mucilage from different plant species—A case study on the effect of mucilage on soil water repellency. Journal of Plant Nutrition and Soil Science 179:294–302. doi:10.1002/jpln.201500511.

Chapter 2

Root hairs: the villi of plants

Bienert, M. D., Werner, L. M., Wimmer, M. A., Bienert, G. P.

Biochemical Society Transactions 49 (3) (2021) 1133-1146

https://doi.org/10.1042/BST20200716

Biochemical Society Transactions (2021)
https://doi.org/10.1042/BST20200716

Review Article

Root hairs: the villi of plants

Manuela Désirée Bienert[1], Lena M. Werner[2], Monika A. Wimmer[2] and Gerd Patrick Bienert[1]

[1]Crop Physiology, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany; [2]Department Quality of Plant Products, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany

Correspondence: Gerd Patrick Bienert (patrick.bienert@tum.de) or Monika A. Wimmer (m.wimmer@uni-hohenheim.de)

Strikingly, evolution shaped similar tubular structures at the μm to mm scale in roots of sessile plants and in small intestines of mobile mammals to ensure an efficient transfer of essential nutrients from 'dead matter' into biota. These structures, named root hairs (RHs) in plants and villi in mammals, numerously stretch into the environment, and extremely enlarge root and intestine surfaces. They are believed to forage for nutrients, and mediate their uptake. While the conceptional understanding of plant RH function in hydromineral nutrition seems clear, experimental evidence presented in textbooks is restricted to a very limited number of reference-nutrients. Here, we make an element-by-element journey through the periodic table and link individual nutrient availabilities to the development, structure/shape and function of RHs. Based on recent developments in molecular biology and the identification of mutants differing in number, length or other shape-related characteristics of RHs in various plant species, we present comprehensive advances in (i) the physiological role of RHs for the uptake of specific nutrients, (ii) the developmental and morphological responses of RHs to element availability and (iii) RH-localized nutrient transport proteins. Our update identifies crucial roles of RHs for hydro-mineral nutrition, mostly under nutrient and/or water limiting conditions, and highlights the influence of certain mineral availabilities on early stages of RH development, suggesting that nutritional stimuli, as deficiencies in P, Mn or B, can even dominate over intrinsic developmental programs underlying RH differentiation.

Plastic root hairs increase the capability of root surfaces to physically and chemically interact with the soil

Mineral nutrients can be essential, beneficial or toxic for plants and animals. At least fourteen and seventeen essential elements are described for vascular plants and animals, respectively [1,2]. Several are essential for both groups (N, P, K, Mg, Ca, S, Fe, Zn, Cu, Mo, Mn, Ni, Cl), while others are necessary for organismal-specific functions (e.g. Co for mammals or B for plants). Worldwide, insufficient nutrient supply is one of the most limiting resources in crop production [3], and human mal- and undernutrition are the main risk factors for human disorders [4,5]. Both nutrient-related challenges are interlinked and highlight the essentiality of nutrient uptake processes for life on earth.

Interestingly, both animals and plants evolved comparable anatomical structures to regulate nutrient uptake [6], although they face inverse conditions regarding uptake location (internal in the intestine of animals, external at the root surface of plants) and nutrient availability (relatively constant versus widely fluctuating forageable volume and environment for animals and plants, respectively). In humans, multicellular tubular structures of 0.5–1.5 mm length, the so-called villi, extend into the lumen of the small intestine, where most minerals are absorbed. Each villus bears a multitude of ≈1 μm long extensions named microvilli. Villi and microvilli increase the surface area of the intestinal walls by 30- and 600-fold, respectively [7]. The plant counterpart to animal villi are root hairs (RHs), which are short-lived tubular outgrowths of single epidermal cells, the so-called trichoblasts, apical to

Received: 28 February 2021
Revised: 16 April 2021
Accepted: 23 April 2021

Version of Record published:
18 May 2021

Biochemical Society Transactions (2021)
https://doi.org/10.1042/BST20200716

the root elongation zone [8]. The high plasticity of RHs [9,10] is reflected by variations in diameter (5–17 μm) and length (80–1500 μm) [11,12]. The genetic and molecular regulations of RH development were covered in detail in recent reviews (e.g. [13,14]).

Both villi and RHs not only increase the contact area to the nutrient bearing environment, but also hold a multitude of nutrient and water transport proteins [7,8,15]. While in animals these uptake transporters are well characterized, little is known about morphological adaptations of (micro)villi in response to variations of nutrient availability. In plants, RH responses to mineral imbalances are widely observed, but quantitative contributions to the influx and efflux of specific nutrients is largely unknown.

This mini-review presents an update on the development, plasticity and functional role of RHs by assessing the (i) contribution of RHs to the uptake of individual mineral nutrients and water, (ii) morphological responses of RHs to element availability and (iii) RH-localized nutrient transport proteins.

Nitrogen (N)

Among all mineral nutrients, N is needed in largest quantities because it is a crucial constituent of numerous primary and secondary metabolites. Under N-limiting conditions, roots possess a N-foraging behavior, and adaptation of the root system architecture contributes significantly to N nutrition [16]. While RH length and density negatively correlate with homogeneous nitrate (NO_3^-) supply [17–20], local NO_3^- supply increases RH density [17,20]. This increase in RHs was attributed to a reduced trichoblast length, resulting in more cells per unit length of root, but not due to ectopic RH development. Accordingly, RH development is controlled by both local and systemic NO_3^--signaling effects [16]. In the Arabidopsis RH mutants *rhd6-3* and *cpc*, root NO_3^- concentration was reduced by ∼58% under NO_3^--sufficient and deficient conditions, implying a significant role of RHs in NO_3^- uptake [17].

In water-logged and low pH soils, ammonium (NH_4^+) is often the main inorganic N source. Compared with NO_3^-, NH_4^+ had only minor effects on RH density in Arabidopsis [20], while RH length increased with decreasing NH_4^+ levels [21,22]. Supraoptimal NH_4^+ concentrations (1.25 to 20 mM) stimulated the formation of branched RHs and inhibited RH elongation in Arabidopsis [23].

Several NO_3^- and NH_4^+ transporting proteins, which are crucial for N uptake, quantitatively respond to the external N availability and localize to RHs (Figure 1 and Table 1). This collectively indicates that RHs are important for both NO_3^- and NH_4^+ uptake under N-sufficient and deficient growth conditions.

Potassium (K)

Potassium fulfills manifold important roles in plant water relations and in the plants' metabolism. Amongst others, K^+-ions drive crucial physiological and biochemical processes such as cell extension and growth, stomatal- and osmoregulation, enzyme activation, membrane transport, photosynthesis, and cation-anion balance ([24,25] and references herein). In RHs, K^+-dependent turgor control is pivotal for the incorporation of membrane and cell wall components during tip growth [26,27]. Positive correlations exist between RH length, K^+ uptake rates and shoot K^+ concentrations [24,28–30]. Under low K^+ availability, RH length increased in many crop species but not in rice [30,31], suggesting a species- and genotype-dependent plasticity of RH traits controlled by K^+ supply. RHs express a range of K^+-responsive K^+ uptake transporters (Figure 1 and Table 1). Furthermore, the expression of TRH1, a RH-localized high affinity K^+ transporter, is essential for proper RH development [26]. Together, these results support the importance of RHs for K^+ acquisition at least under K^+ deficiency, as well as an essential function of an efficient K^+ uptake for RH elongation in general.

Calcium (Ca)

Calcium is an essential element for plant growth with multiple importance in the plant body, ranging from stabilizing cell wall and membrane integrity to the function as a secondary messenger in stress responses and developmental processes [32,33]. In RH elongation, Ca is a central player since an oscillating tip-focused Ca^{2+} gradient is essential for tip growth of RHs, as also evidenced in pollen tubes (reviewed in: [32,34,35]). These essential roles of Ca for RH outgrowth have been extensively reviewed (e.g. [32,33]). However, little is known about the contribution of RHs to the overall Ca uptake, and observed RH responses to Ca supply are inconsistent. While Ca deficiency increased RH length and density in trifoliate orange [36], it reduced RH formation in lettuce [37] and oat, where it also caused RH deformations [38]. In Arabidopsis, RH-expressed Ca-permeable cation channels of the CNGC family are important for cell expansion during RH elongation (Figure 1 and

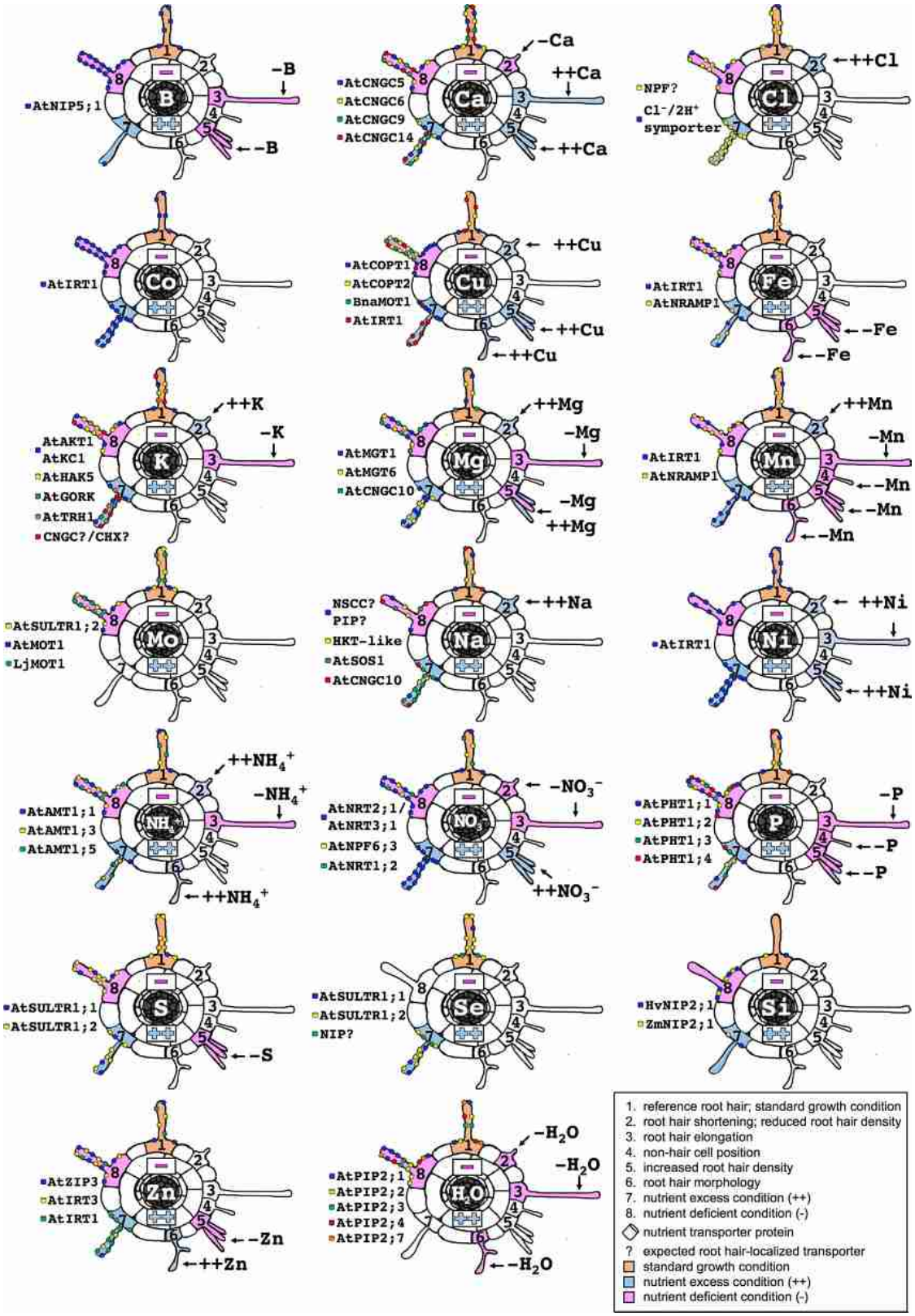


Figure 1. Effects of water and mineral nutrient availabilities on transport proteins and developmental processes. Part 1 of 2
Effects of water and mineral nutrient availabilities on transport proteins and developmental processes are shown on the left and right part of each root-cross-section-illustration, respectively. Molecular, developmental or morphological statuses of root hairs

Biochemical Society Transactions (2021)
https://doi.org/10.1042/BST20200716

Figure 1. Effects of water and mineral nutrient availabilities on transport proteins and developmental processes. Part 2 of 2

caused by sufficient (orange), deficient (magenta and/or '−') or excess (blue and/or '++') local availability of the indicated nutrient on transporter expression (1, 7, 8), on root hair shortening (2), on root hair elongation (3), at non-hair cell position (4), on root hair density (2 = reduced density; 5 = increased density) or on root hair morphology (6) is indicated by the numbers 1 to 8. Displayed developmental or morphological root hair alterations are based on data extracted from a literature search (B, [83,84]; Ca, [32,33,39,45,120]; Cl, [70–73]; Cu, [45,92–94]; Fe, [45,49,60,61,63,157]; K, [24,28–30,45,73,157]; Mg, [41,157]; Mn, [37,45,76,77,157]; Na, [70–73]; Ni, [93,101]; NH_4^+, [20–23]; NO_3^-, [17,20,23]; P, [44,45,157]; S, [45,51]; Zn, [45,62,81]; H_2O, [14,111]). References providing expression data of individual transporters are listed in Table 1. The orange-colored root hair (1) should reflect a reference root hair, with respect to transporter expression and morphology, which forms under sufficient supply of the indicated nutrient. Root hair-localized plasma membrane spanning transport proteins mediating the uptake of the indicated nutrient/water are displayed as blue, yellow, green, red and orange cylindric symbols. The level of expression of each listed transporter under nutrient deficient (8) or excess (7) conditions is compared with the sufficient nutrient supply condition (1) and depicted by the different numbers of displayed cylindric symbols. A '?' following a transporter indicates a suggested root hair-localized transport protein which is however not experimentally confirmed yet. If a numbered root hair cell remains white-colored, it either means that the respective root hair status was not described in a performed experiment or that the experiment which would allow a conclusion has not been performed yet.

Table 1) [39]. Whether these RH-localized transporters aid in Ca uptake under Ca-deficient growth conditions or whether the Ca uptake has merely local effects in RH growth and development remains to be identified.

Magnesium (Mg)

Magnesium fulfils essential functions in the photosynthetic apparatus and in stabilizing nucleotides [40]. In Arabidopsis, low Mg supply resulted in increased RH formation and length, related to the initiation of new trichoblast files and differentiations, while high Mg supply reduced RH initiation, density and length [41]. This is substantiated by the Mg deficiency-induced up-regulation of many cell fate-determining morphogenetic H-genes which increase the probability that epidermal cells develop into trichoblast (=higher RH density), as well as of cell wall organization genes that likely modulate cell wall plasticity and thereby the elongation ability of RHs (=longer RHs) [41]. Consistently, under high Mg supply, the same two classes of genes are down-regulated causing the reduced RH initiation, density and length [41]. Moreover, Mg availability regulates the expression of Mg-specific uptake transporters in RHs (Figure 1 and Table 1).

Phosphorus (P)

Phosphorus is essential for key processes of life including energy metabolism and phosphorylation-based signaling pathways, and is a constituent of many molecules [42], but is often unavailable for plants due to its restricted mobility in most soils [43]. Plant RH density and length are the main drivers to improve P uptake especially under P deficiency (summarized in [44]). Higher RH density was caused largely (66%) by an increased likelihood of trichoblasts to form RHs, and partly (33%) by an increased amount of trichoblast files [45]. A significantly reduced P uptake in Arabidopsis and barley mutants lacking proper RHs further confirms the relevance of RHs for P uptake [46–48]. It is interesting that Arabidopsis mutants lacking regularly shaped RHs under sufficient P supply, develop normally shaped RHs under P deficiency [49]. This suggests that the plants' P status is perceived at an early stage of rhizodermal cell development, by the SPX-domain-containing protein pathway sensing the amount of inositol pyrophosphate molecules (PP-InsP), a proxy for cellular P-content [14]. A low PP-InsP concentration further triggers signaling processes affecting RH growth and fate thereby overruling endogenous developmental-dependent differentiation [14]. Together with the constitutive and modulative expression of P transporters in RHs (Figure 1 and Table 1) [44], all these results suggest that RHs are essential for the regulation of the P nutritional status in plants, and that P deficiency triggers the development (more) and shape (longer) of RHs.

Sulfur (S) and selenium (Se)

Sulfur plays a central role in plant metabolism, stress resistance, and crop quality [50]. Under S deficiency, RH number increased in Arabidopsis [51]. High-affinity S transporters are constitutively expressed in RHs of diverse plant species [52–55], and a functional relevance of RH-localized transporters such as AtSultr1;1 and

Table 1 Mineral nutrient and water transport proteins which are expressed in root hairs and their transcript regulation by the corresponding nutrient or water availability Part 1 of 2

Mineral element	Transporter name	Transporter expression level in root hairs (RH) or non-hair rhizodermal cells (RC) under variable nutrient availabilities			(d) Nutrient uptake ability in transporter knockout or antisense plants
		(a) DEFICIENT	(b) SUFFICIENT	(c) EXCESS	
Boron B	AtNIP5;1	↑ [84]	RH ?/RC ✓ [84]	↓ [84]	↓ [84]
Calcium Ca	AtCNGC5	● [120]	RH ✓ [120]	● [120]	?
	AtCNGC6	● [120]	RH ✓ [120]	● [120]	?
	AtCNGC9	● [120]	RH ✓ [120]	● [120]	?
	AtCNGC14	● [121]	RH ✓ [121]	● [121]	?
Chloride Cl	Cl^-/H^+ symporter	● [68,69]	RH ✓ [68,69]	● [68,69]	?
	NPF	● [68,69]	RH ✓ [68,69]	↑ [68,69]	?
Cobalt Co	AtIRT1	? ↑ (–Fe) [98]	RH ✓ [98,122]	? ↑ (++Fe) [98]	↓ (–Fe) [122]
Copper Cu	AtIRT1	● [122]	RH ✓ [122]	↑ [122]	↓ [122]
	AtCOPT1	↑ [96,123]	RC ✓ [96,123]	↑ [96,123]	↓ [96,124]
	AtCOPT2	↑ [96,123]	RH ✓ [96,123]	↓ [96,123]	● [96,123]
Iron Fe	AtIRT1	↑ [122,125]	RH ✓ [122,125]	↑ [122,125]	↓ [122]
	AtNRAMP1	↑ [125]	RH ✓ [125]	● [125]	● [125]
Potassium K	AtAKT1	● [8,126]	RH ✓ [8,126]	● [8,126]	↓ [27]
	AtHAK5	↑ [8,126]	RH ✓ [8,126]	↓ [8,126]	↓ [127]
	AtGORK	- [8,126]	RH ✓ [8,126]	↑ [8,126]	↑ (+stress) [128]
	AtTRH1	● [26,27,129]	RH ✓ [26,27,129]	● [26,27,129]	↓ [26,129,130]
	CNGC	● [126]	RH ✓ [126]	↑ [126]	?
	CHX	● [126]	RH ✓ [126]	↑ [126]	?
Magnesium Mg	AtMGT1	↑ [131]	RH ✓ [131]	↑ [131]	● [132]
	AtMGT6	↑ [131,133]	RH ✓ [131,133]	↑ [131,133]	↓ [133]
	AtCNGC10	↑ [134]	RH ✓ [134]	- [134]	↓ [134]
Manganese Mn	AtIRT1	● [75]	RH ✓ [75]	↑ [75]	↓ (–Fe) [122]
	AtNRAMP1	↑ [75]	RH ✓ [75]	- [75]	↓ [135]
Molybdenum Mo	AtMOT1	↑ [103,105]	RH ?/RC ✓ [103,105]	?	↓ [105]
	AtSULTR1;2	● [105]	RH ✓ [57,136]	?	?
Sodium Na	AtSOS1	● [137,138]	RH ✓ [137,138]	↑ [137,138]	↑ (++Na) [139]
	AtCNGC10	● [138,140]	RH ✓ [138,140]	● [138,140]	↓ (++Na) [141]
	HKT-like	● [137]	RH ✓ [137]	● [137]	?
	PIP	● [137]	RH ✓ [137]	● [137]	?
	NSCC	● [137]	RH ✓ [137]	● [137]	?
Nickel Ni	AtIRT1	● [93,99,100,142]	RH ✓ [93,99,100,142]	↑ [93,99,100,142]	● [99]
Nitrogen Ammonium NH_4^+	AtAMT1;1	↑ [143,144]	RH ✓ [143,144]	● [143,144]	↓ [143,144]
	AtAMT1;3	↑ [144,145]	RH ✓ [144,145]	● [144,145]	↓ [144]
	AtAMT1;5	↑ [145]	RH ✓ [145]	● [145]	?
Nitrogen Nitrate NO_3-	AtNRT1;2	● [146]	RH ✓ [146]	↓ [146]	↓ [146]
	AtNRT2;1	↑ [146]	RH ✓ [146]	↑ [146]	↓ [146]
	AtNPF6;3	● [146]	RH ✓ [146]	↓ [146]	↓ [146]
Phosphorous P	AtPHT1;1	↑ [147]	RH ✓ [147]	● [147]	↓ [148]
	AtPHT1;2	↑ [147]	RH ✓ [147]	● [147]	↓ [149]
	AtPHT1;3	↑ [147]	RH ✓ [147]	● [147]	↓ [149]
	AtPHT1;4	↑ [147]	RH ✓ [147]	● [147]	↓ [148]
Sulfur S	AtSULTR1;1	↑ [51,55,135]	RH ✓ [51,55,135]	↑ [51,55,135]	↓ [136,150]
	AtSULTR1;2	● [51,55,135]	RH ✓ [51,55,135]	● [51,55,135]	↓ [136,150]

Continued

Biochemical Society Transactions (2021)
https://doi.org/10.1042/BST20200716

Table 1 Mineral nutrient and water transport proteins which are expressed in root hairs and their transcript regulation by the corresponding nutrient or water availability Part 2 of 2

Mineral element	Transporter name	Transporter expression level in root hairs (RH) or non-hair rhizodermal cells (RC) under variable nutrient availabilities			(d) Nutrient uptake ability in transporter knockout or antisense plants
		(a) DEFICIENT	(b) SUFFICIENT	(c) EXCESS	
Selenium Se	AtSULTR1;1	?	RH ✓ [57,136]	↑ [57,136]	↓ [151]
	AtSULTR1;2	?	RH ✓ [57,136]	● [57,136]	● [151]
	NIP	?	RC ✓ [152,153]	?	↓ [59]
Silicon Si	HvNIP2;1	↑ [152]	RC ✓ [152]	● [152]	ne
	ZmNIP2;1	↑ [153]	RC ✓ [153]	● [153]	ne
Zinc Zn	AtIRT1	● [154,155]	RH ✓ [154,155]	↑ [154,155]	↓ (–Fe) [122]
	AtIRT3	↑ [154,155]	RH ✓ [154,155]	● [154,155]	?
	AtZIP3	↑ [154,155]	RH ✓ [154,155]	↓ [154,155]	?
Water H_2O	AtPIP2;1	↑ [156]	RH ✓ [156]	?	● [156]
	AtPIP2;2	↑ [156]	RH ✓ [156]	?	● [156]
	AtPIP2;3	● [156]	RH ✓ [156]	?	● [156]
	AtPIP2;4	● [156]	RH ✓ [156]	?	● [156]
	AtPIP2;7	● [156]	RH ✓ [156]	?	● [156]

Transporter expression levels in root hairs (RH) or non-hair rhizodermal cells (RC) in plants grown under nutrient or water deficient- or excess conditions are compared with those of plants grown under nutrient or water sufficient conditions (a, b, c). Nutrient or water uptake abilities of transporter knockout or antisense plants are compared with wildtype plants (d). (●, no change in expression or nutrient/water status; ↑, major significant transporter up-regulation or major significant higher nutrient level; ↑, significant transporter up-regulation or significant higher nutrient level; ↓, major significant transporter down-regulation or major significant lower nutrient level; ↓, significant transporter down-regulation or significant lower nutrient level; ✓, expression detected; ?, no information; ne, not existent).

AtSultr1;2 for S uptake under S-limited conditions was confirmed in Arabidopsis (Figure 1 and Table 1). Despite this established role of RH-localized S transporters, the number and length of RHs do not seem to contribute significantly to total plant S uptake in Arabidopsis under both sufficient and deficient S supply [51].

Selenium is a beneficial element for plants when present at low concentrations but becomes rapidly toxic at supraoptimal supply [56]. The plants' Se acquisition seems to depend on the S and Si nutrition, because S and Si transporters represent incidental transmembrane sneak-entry-points for selenate and selenite [57–59]. Otherwise, to our knowledge, no studies elucidating the role of RHs for Se uptake are available.

Iron (Fe) and cobalt (Co)

Based on its redox activity, Fe is an important enzymatic cofactor in many key redox reactions governing respiration and photosynthesis. Under limited Fe supply, plants respond with an altered root system architecture, and an enhanced RH density [60,61]. This is in line with an Fe deficiency-dependent up-regulation of the *RTH5* gene in maize roots, which is essential for RH formation [62]. Additionally, Fe deficiency causes branched RHs and ectopic RH formation in atrichoblastic cells [49,61,63].

In general, plants have adopted two different strategies to countervail Fe deficiency in the growth medium. Strategy-I is mainly based on an enhanced net excretion of protons and coumarins to dissolve Fe from soil particles followed by the reduction in Fe(III) to Fe(II) by a plasma membrane-bound reductase. Fe (II) is then taken up by Fe transporters such as IRT1s [1,64,65]. Strategy-I is employed by all plant species except of *Poaceae*. The latter taxa makes use of strategy-II, that is an active biosynthesis and release of so-called phytosiderophores, which highly efficiently form chelates with Fe(III) which are then taken up by plant Yellow stripe like transporters [1]. In the strategy-I plant Arabidopsis, the formation of RHs under Fe deficiency is accompanied by the transcript induction of RH-localized *AtIRT1* and *AtNRAMP1* Fe transporters (Figure 1 and Table 1). In Fe deficiency stressed strategy-II cereals, it is unclear whether transporter-related or anatomical RH responses have a significant effect on Fe uptake, since the RH-less barley mutant *brb* has a comparable release of phytosiderophores, and an unchanged Fe content compared with the wildtype (WT) upon Fe depletion [66].

The Fe uptake transporter machinery seems to additionally regulate the plant content of the beneficial element Co, which is suggested to be taken up in a Co-non-selective and non-targeted manner [67].

Chlorine (Cl) and sodium (Na)

Chlorine plays essential regulatory roles in transpiration and photosynthesis [68]. To the best of our knowledge, data regarding RH responses to Cl deficiency are missing. Whereas Cl toxicity frequently occurs under saline conditions, its effects on RHs are rarely distinguished from those of Na, which is often the salt counter-cation [69]. Generally, high NaCl levels reduced the number, length and density of RHs in a dose-dependent manner in Arabidopsis and wheat [70–73] and even causes bulbous RHs [73]. The reduction in RH number was probably due to downsized numbers of epidermal cells that differentiate into trichoblasts. Similar phenotypes are caused by excess KCl or LiCl, but not by mannitol, indicating an ionic rather than an osmotic signal driving RH responses [73]. Differently, 100 mM NaCl increased length and density of RHs in flowering rapeseeds [74], indicating species- or development-specific RH responses.

Under non-saline conditions, Cl uptake into RHs is thought to occur via 'active' mechanisms, e.g. NRT2-type transporters and/or Cl^-/H^+ symport by a not yet identified transport protein (Figure 1 and Table 1) [68,69]. Under high external Cl concentrations, passive uptake into root cortical cells and RHs is facilitated by molecularly not yet specified anion channels (reviewed by [69]).

Manganese (Mn)

Manganese is an essential redox-active cofactor for various plant proteins [75]. Under Mn-deficient conditions, RH length and density are stimulated, and RHs form in normally atrichoblastic cells [37,45], indicating reprogramming of rhizodermal cells [76]. Interestingly, the RH-less phenotype of the Arabidopsis *cpc* mutant is partly rescued by Mn deficiency, demonstrating that the nutritional trigger alters cell fate differently from the endogenous developmental differentiation [76]. When grown on excess Mn, RHs of the *eca1* mutant fail to elongate [77].

Members of the Mn-transporting NRAMP and ZIP protein families are expressed in RHs or root epidermal cells and their up-regulation (e.g. AtNRAMP1 and HvIRT1) is detected under Mn limitations (see review [75]) (Figure 1 and Table 1). A significant effect of increased RH density and length on Mn uptake remains to be demonstrated.

Zinc (Zn)

Zinc is essentially required for the structure and function of many proteins [78]. Under Zn deficiency, Arabidopsis and maize significantly increased RH density [45,62]. In contrast, RH length and density were not affected by Zn deficiency in barley, though total root length was increased [79]. Despite this lack of RH response to Zn supply, the RH-less barley mutant *brb* had a significantly lower Zn uptake compared with the WT [79,80]. These results indicate that a Zn status-dependent RH plasticity is species- and genotype-specific. Arabidopsis plants grown on Zn excess form branched and abnormally shaped RHs, possibly related to reduced levels of RHD3 and MRH5/SHV3 proteins, both involved in RH morphogenesis [81]. When exposed to Zn deprivation, several genes involved in RH morphogenesis and Zn uptake were up-regulated in RHs of Arabidopsis, amongst them key Zn uptake and translocation regulators (Figure 1 and Table 1).

Overall, data regarding RH response to Zn supply are fragmentary, but suggest that RH shape and physiology are modified by external Zn levels. Whether RH-localized Zn transporters or an increased root surface upon Zn deficiency is the major player influencing Zn uptake remains an open question.

Boron (B)

The main established function of B is the stabilization of plant cell walls by crosslinking rhamnogalacturonan-II molecules [82]. Under B deficiency, increases in RH number, length, and density are observed in many plant species, and growth of additional RHs close to the tip occurs after only one day of low B supply [83,84]. The Arabidopsis RH-less *rhd2* mutant is able to develop 'normal' RHs under B deficiency [85], suggesting that the plants' B status dominates over endogenous developmental differentiation processes, similar to what is observed under Mn- and P-deficient conditions. The role of RHs in B uptake is unclear [86]. Since under sufficient B supply, B is taken up by passive membrane diffusion [87], RHs may affect B uptake simply by increasing the contact area between roots and soil (-water). Under B deficiency, B uptake is mediated by two cooperating transport protein family types [84,88–90]. The striking up-regulation of the essential B uptake channel, AtNIP5;1, in the RH zone under B limitation suggests a putative function of this channel for B uptake in RHs (Figure 1 and Table 1).

Biochemical Society Transactions (2021)
https://doi.org/10.1042/BST20200716

Copper (Cu)

Copper is an important redox-active cofactor for many proteins [91]. To the best of our knowledge, significant effects of Cu deficiency on RH density and length were neither observed nor were they specifically studied [45,92]. Under Cu excess, RH density was significantly increased, but it was not analyzed whether additional RHs developed, or whether RHs appeared denser due to inhibited rhizodermal cell elongation [93,94]. At very high Cu concentrations (100 μM), misshapen, short and obtuse RHs form [94]. The contribution of RHs to total Cu uptake is not fully resolved. Under sufficient supply, both RH length and density correlated with shoot Cu content in wheat cultivars [95], and the Cu content of the Arabidopsis RH-less *NR23* mutant was lower compared with its WT [48]. Both studies suggest a role of RHs for Cu uptake. While in Arabidopsis the RH-localized AtCOPT2-type transporter was significantly up-regulated under Cu deficiency, its contribution to Cu uptake seemed minor. On the other hand, the high-affinity COPT1-type transporter, which is crucial for overall Cu acquisition, is only expressed in atrichoblastic rhizodermis cells (Figure 1 and Table 1) [96].

Together these data suggest that RHs add to Cu acquisition of plants, while the contribution to total uptake seems not substantial.

Nickel (Ni)

Nickel is a vital constituent of several metallo-enzymes [97]. While no Ni-specific uptake transporters were identified yet, Ni enters roots via the RH-localized Fe uptake transporter IRT1, and probably additional yet unspecified divalent metal transporting proteins [98–100]. This is further supported by Ni accumulation in RHs and epidermal cells of Fe-deficient Arabidopsis WT, but not *irt1* mutant plants [99]. Under Ni toxicity, RHs appeared more abundant close to the root tip due to an inhibited axial cell elongation in Arabidopsis [93]. In barley, RH length correlated with Ni toxicity symptoms [101].

Taken together, these data suggest the involvement of RHs in Ni uptake under certain nutritional conditions. Whether RH phenotypes are direct responses to the sensing of Ni availability, or side-effects of imbalances in other nutrients such as Fe or Zn remains an open question.

Molybdenum (Mo)

Molybdenum is the element with the lowest abundancy in plants, but nevertheless is an essential cofactor in enzymes driving N metabolism [102]. Mo is taken up by plants as molybdate, which shares physico-chemical characteristics with sulfate potentially leading to its non-specific uptake via RH-localized sulfate transporters (Figure 1 and Table 1). In addition, Mo-specific transport proteins (MOT1s) are expressed in trichoblasts and atrichoblasts in *Lotus japonicus* and Arabidopsis, respectively (Figure 1 and Table 1) [103–105].

So far, the contribution of RHs to Mo uptake into plants is far from being solved and might be influenced by an impaired N metabolism in addition to a direct Mo deficiency effect.

Silicon (Si)

Silicon is a beneficial element for vascular plants and alleviates stress symptoms under several biotic and abiotic stresses [106]. Plants take up Si as silicic acid via two types of cooperating transport protein families, namely the Nodulin26-like Intrinsic Proteins (NIPs) and the Lsi2-type transporters, which represent bidirectional channels and efflux transporters, respectively [106]. There was no significant difference in Si uptake, shoot Si concentration, and number of leaf silica bodies between *RH2*, a rice mutant, defective in the formation of RHs, and WT plants [107]. Si uptake was comparable at the root tip (without RHs in WT and *RH2*) and the mature zone (without RHs in *RH2*) in both genotypes. Moreover, none of the NIP-specific antibodies targeting Si channels in various crops have yet resulted in RH-localized signals [108]. All these results indicate that RHs may not contribute to Si uptake, at least in rice.

Water (H_2O)

RHs greatly extend the contact area between roots and soil, which is beneficial for H_2O acquisition [109]. In mature root systems, RH traits such as length and density seem to vary in response to soil moisture variations only in newly forming but not in existing roots [110]. The underlying physiological reasons are unknown. The question of whether RHs relevantly contribute to H_2O uptake or not, has been a long-lasting one, and different studies came to different conclusions. While the density and length of RHs correlated with H_2O uptake in diverse plant species [111], H_2O uptake was reduced in RH-less Arabidopsis mutants and oat [48,112], but did

not differ between RH-less barley and rice mutants and their corresponding WTs [113,114]. Possibly the beneficial effect of RHs is evident only under H_2O or nutrient limitations, as no benefit of RHs was seen in high-fertility humid soils [115,116]. For example, a more efficient H_2O uptake was observed in the WT compared with a RH-less *brb* barley mutant in a moderately dry, but not in a humid soil [115]. Similarly, a study comparing WT to a RH-defective *rth3* maize mutant demonstrated that the lack of RHs clearly reduced shoot growth under drought conditions independent of whether plants were sufficiently or deficiently supplied with P [117]. In another study, the tolerance of a population of barley RH mutants to an extreme combined P and H_2O deficit depended on the presence of RHs [46]. By analyzing five barley genotypes exhibiting variations in RH length and density in field experiments for two consecutive years under contrasting climate conditions and different soil textures, a recent study demonstrated that beneficial effects of RHs for plant performance were only evident in dry growth seasons [118]. Nevertheless, it is not yet clear, whether RHs take up enough H_2O to influence leaf H_2O potential and transpiration [115]. Aquaporins are H_2O channeling proteins regulating the H_2O homeostasis in plants [119]. Plasma membrane Intrinsic Protein (PIP) aquaporins are expressed in RHs of different plant species (Figure 1 and Table 1), but little is known about their RH-specific regulation upon drought stress.

Perspectives

- **Importance to the field:** An increasing world population, agricultural land pollution and climatic challenges lead to the use of suboptimal soils (nutrient- and water-limited) for crop production. A better understanding of root traits including root hair contributions to nutrient/water uptake is needed to maintain or even improve crop yield and quality.
- **Current thinking:** Root hairs are generally accepted to be of importance for nutrient/water uptake, but the real contribution for individual elements is not well known.
- **Future directions:** We are convinced that a more detailed knowledge on genetic regulations and functions of root hairs will pave the way for the generation of more resilient agricultural and horticultural crops and sustain crop yields even under suboptimal climatic and soil conditions.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

M.D.S, L.M.W., M.A.W., and G.P.B. prepared figures and wrote the article.

Acknowledgements

G.P.B. and M.A.W. acknowledge funding by the DFG (German Research Foundation) within the framework of the priority program 2089 'Rhizosphere spatiotemporal organization-a key to rhizosphere functions' (BI 1668/4-1 and WI 2685/3-1). We thank all scientists for uncovering the exciting nature of plant root hairs. We apologize to all authors whose contributions to this research area could not have been mentioned due to Journal guidelines.

Abbreviations

MOT1s, Mo-specific transport proteins; NIPs, Nodulin26-like Intrinsic Proteins; PIP, plasma membrane intrinsic protein; RH, root hair; WT, wildtype.

References

1 Marschner, H. (2012) *Marschner's Mineral Nutrition of Higher Plants*, 3rd edn, Academic Press
2 Chellan, P. and Sadler, P.J. (2015) The elements of life and medicines. *Philos. Trans. R. Soc.* **373**, 20140182 https://doi.org/10.1098/rsta.2014.0182

3 Müller, C., Elliott, J., Pugh, T.A.M., Ruane, A.C., Ciais, P., Balkovic, J. et al. (2018) Global patterns of crop yield stability under additional nutrient and water inputs. *PLoS ONE* **13**, e0198748 https://doi.org/10.1371/journal.pone.0198748
4 Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A. and Lombi, E. (2019) Soil and the intensification of agriculture for global food security. *Environ. Int.* **132**, 105078 https://doi.org/10.1016/j.envint.2019.105078
5 Ebert, A.W. (2020) The role of vegetable genetic resources in nutrition security and vegetable breeding. *Plants* **9**, 736 https://doi.org/10.3390/plants9060736
6 Barberon, M. and Geldner, N. (2014) Radial transport of nutrients: the plant root as a polarized epithelium. *Plant Phys.* **166**, 528–537 https://doi.org/10.1104/pp.114.246124
7 Kiela, P.R. and Ghishan, F.K. (2016) Physiology of intestinal absorption and secretion. *Best Pract. Res. Clin. Gastroenterol.* **30**, 145–159 https://doi.org/10.1016/j.bpg.2016.02.007
8 Rongsawat, T., Peltier, J.B., Boyer, J.C., Véry, A.A. and Sentenac, H. (2021) Looking for root hairs to overcome poor soils. *Trends Plant Sci.* **26**, 83–94 https://doi.org/10.1016/j.tplants.2020.09.001
9 Motte, H., Vanneste, S. and Beeckman, T. (2019) Molecular and environmental regulation of root development. *Annu. Rev. Plant Biol.* **29**, 465–488 https://doi.org/10.1146/annurev-arplant-050718-100423
10 Schneider, H.M. and Lynch, J.P. (2020) Should root plasticity be a crop breeding target? *Front. Plant Sci.* **11**, 546 https://doi.org/10.3389/fpls.2020.00546
11 Dittmer, H.J. (1949) Root hair variations in plant species. *Am. J. Bot.* **36**, 152–155 https://doi.org/10.2307/2437782
12 Choi, H.S. and Cho, H.T. (2019) Root hairs enhance Arabidopsis seedling survival upon soil disruption. *Sci. Rep.* **9**, 11181 https://doi.org/10.1038/s41598-019-47733-0
13 Shibata, M. and Sugimoto, K. (2019) A gene regulatory network for root hair development. *J. Plant Res.* **132**, 301–309 https://doi.org/10.1007/s10265-019-01100-2
14 Vissenberg, K., Claeijs, N., Balcerowicz, D. and Schoenaers, S. (2020) Hormonal regulation of root hair growth and responses to the environment in *Arabidopsis*. *J. Exp. Bot.* **71**, 2412–2427 https://doi.org/10.1093/jxb/eraa048
15 Goff, J.P. (2018) Invited review: mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Int. J. Dairy Sci.* **101**, 2763–2813 https://doi.org/10.3168/jds.2017-13112
16 Jia, Z. and von Wirén, N. (2020) Signaling pathways underlying nitrogen-dependent changes in root system architecture: from model to crop species. *J. Exp. Bot.* **71**, 4393–4404 https://doi.org/10.1093/jxb/eraa033
17 Canales, J., Contreras-López, O., Álvarez, J.M. and Gutiérrez, R.A. (2017) Nitrate induction of root hair density is mediated by TGA1/TGA4 and CPC transcription factors in *Arabidopsis thaliana*. *Plant J.* **92**, 305–316 https://doi.org/10.1111/tpj.13656
18 Foehse, D. and Jungk, A. (1983) Influence of phosphate and nitrate sup-ply on root hair formation of rape, spinach and tomato plants. *Plant Soil* **74**, 359–368 https://doi.org/10.1007/BF02181353
19 Robinson, D. and Rorison, I.H. (1983) A comparison of the responses of *Lolium perenne* L., *Holcus lanatus* L. and *Deschampsia flexuosa* (L.) Trin. to a localized supply of nitrogen. . *New Phytol.* **94**, 263–273 https://doi.org/10.1111/j.1469-8137.1983.tb04499.x
20 Vatter, T., Neuhäuser, B., Stetter, M. and Ludewig, U. (2015) Regulation of length and density of Arabidopsis root hairs by ammonium and nitrate. *J. Plant Res.* **128**, 839–848 https://doi.org/10.1007/s10265-015-0733-8
21 Bloch, D., Monshausen, G., Singer, M., Gilroy, S. and Yalovsky, S. (2011) Nitrogen source interacts with ROP signaling in root hair tip-growth. *Plant Cell Environ.* **34**, 76–88 https://doi.org/10.1111/j.1365-3040.2010.02227.x
22 Zhu, C., Yang, N., Guo, Z., Qian, M. and Gan, L. (2016) An ethylene and ROS-dependent pathway is involved in low ammonium-induced root hair elongation in Arabidopsis seedlings. *Plant Physiol. Biochem.* **105**, 37–44 https://doi.org/10.1016/j.plaphy.2016.04.002
23 Yang, N., Zhu, C., Gan, L., Ng, D. and Xia, K. (2011) Ammonium-stimulated root hair branching is enhanced by methyl jasmonate and suppressed by ethylene in *Arabidopsis thaliana*. *J. Plant Biol.* **54**, 92–100 https://doi.org/10.1007/s12374-011-9147-x
24 Sustr, M., Soukup, A. and Tylova, E. (2019) Potassium in root growth and development. *Plants* **8**, 435 https://doi.org/10.3390/plants8100435
25 Nieves-Cordones, M., Al Shiblawi, F.R. and Sentenac, H. (2016) Roles and transport of sodium and potassium in plants. In *The Alkali Metal Ions: Their Role for Life. Metal Ions in Life Sciences* (Sigel, A., Sigel, H. and Sigel, R., eds), vol. **16**, 291–324, Springer, Cham
26 Rigas, S., Debrosses, G., Haralampidis, K., Vicente-Agullo, F., Feldmann, K.A., Grabov, A. et al. (2001) TRH1 encodes a potassium transporter required for tip growth in Arabidopsis root hairs. *Plant Cell* **13**, 139–151 https://doi.org/10.1105/tpc.13.1.139
27 Desbrosses, G., Josefsson, C., Rigas, S., Hatzopoulos, P. and Dolan, L. (2003) *AKT1* and *TRH1* are required during root hair elongation in *Arabidopsis*. *J. Exp. Bot.* **54**, 781–788 https://doi.org/10.1093/jxb/erg066
28 Gahoonia, T.S., Ali, O., Sarker, A., Nielsen, N.E. and Rahman, M.M. (2006) Genetic variation in root traits and nutrient acquisition of lentil genotypes. *J. Plant Nutr.* **29**, 643–655 https://doi.org/10.1080/01904160600564378
29 Gahoonia, T.S., Ali, R., Malhotra, R.S., Jahoor, A. and Rahman, M.M. (2007) Variation in root morphological and physiological traits and nutrient uptake of chickpea genotypes. *J. Plant Nutr.* **30**, 829–841 https://doi.org/10.1080/15226510701373213
30 Hogh-Jensen, H. and Pedersen, M.B. (2003) Morphological plasticity by crop plants and their potassium use efficiency. *J. Plant Nut.* **26**, 969–984 https://doi.org/10.1081/PLN-120020069
31 Klinsawang, S., Sumranwanich, T., Wannaro, A. and Saengwilai, P. (2018) Effects of root hair length on potassium acquisition in rice (*Oryza sativa* L.). *Appl. Ecol. Environ. Sci.* **16**, 1609–1620 https://doi.org/10.15666/aeer/1602_16091620
32 Mendrinna, A. and Persson, S. (2015) Root hair growth: it's a one way street. *F1000Prime Rep.* **7**, 23 https://doi.org/10.12703/p7-23
33 Thor, K. (2019) Calcium—Nutrient and messenger. *Trends Plant Sci.* **10**, 440 https://doi.org/10.3389/fpls.2019.00440
34 Grierson, C. and Schiefelbein, J. (2002) Root hairs. *Arabidopsis Book* **1**, e0060 https://doi.org/10.1199/tab.0060
35 Schoenaers, S., Balcerowicz, D. and Vissenberg, K. (2017) Molecular mechanisms regulating root hair tip growth: A comparison with pollen tubes. In *Pollen Tip Growth* (Obermeyer, G. and Feijó, J., eds), 167–243, Springer, Cham
36 Cao, X., Chen, C., Zhang, D., Shu, B., Xiao, J. and Xia, R. (2013) Influence of nutrient deficiency on root architecture and root hair morphology of trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings under sand culture. *Sci. Hort.* **162**, 100–105 https://doi.org/10.1016/j.scienta.2013.07.034

37 Konno, M., Ooishi, M. and Inoue, Y. (2003) Role of manganese in low-pH-induced root hair formation in *Lactuca sativa* cv. grand rapids seedlings. *J. Plant Res.* **116**, 301–307 https://doi.org/10.1007/s10265-003-0100-z
38 Tanaka, Y. and Woods, F.W. (1973) Root and root hair growth of oats: replaceability of calcium. *Can. J. Bot.* **51**, 1655–1659 https://doi.org/10.1139/b73-212
39 Brost, C., Studtrucker, T., Reimann, R., Denninger, P., Czekalla, J., Krebs, M. et al. (2019) Multiple cyclic nucleotide-gated channels coordinate calcium oscillations and polar growth of root hairs. *Plant J.* **99**, 910–923 https://doi.org/10.1111/tpj.14371
40 Gerendás, J. and Führs, H. (2013) The significance of magnesium for crop quality. *Plant Soil* **368**, 101–128 https://doi.org/10.1007/s11104-012-1555-2
41 Niu, Y., Chai, R., Liu, L., Jin, G., Liu, M., Tang, C. et al. (2014) Magnesium availability regulates the development of root hairs in *Arabidopsis thaliana* (L.) Heynh. *Plant Cell Environ.* **37**, 2795–2813 https://doi.org/10.1111/pce.12362
42 Cho, H., Bouain, N., Zheng, L. and Rouached, H. (2021) Plant resilience to phosphate limitation: current knowledge and future challenges. *Crit. Rev. Biotechnol.* **41**, 63–71 https://doi.org/10.1080/07388551.2020.1825321
43 Schachtman, D.P., Reid, R.J. and Ayling, S.M. (1998) Phosphorus uptake by plants: From soil to cell. *Plant Phys.* **116**, 447–453 https://doi.org/10.1104/pp.116.2.447
44 Zhang, C., Simpson, R.J., Kim, C.M., Warthmann, N., Delhaize, E., Dolan, L. et al. (2018) Do longer root hairs improve phosphorus uptake? testing the hypothesis with transgenic *Brachypodium distachyon* lines overexpressing endogenous RSL genes. *New Phytol.* **217**, 1654–1666 https://doi.org/10.1111/nph.14980
45 Ma, Z., Bielenberg, D.G., Brown, K.M. and Lynch, J.P. (2001) Regulation of root hair density by phosphorous availability in *Arabidopsis thaliana*. *Plant Cell Environ.* **24**, 459–467 https://doi.org/10.1046/j.1365-3040.2001.00695.x
46 Brown, L.K., George, T.S., Thompson, J.A., Wright, G., Lyon, J., Dupuy, L. et al. (2012) What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (*Hordeum vulgare*)? *Ann. Bot.* **110**, 319–328 https://doi.org/10.1093/aob/mcs085
47 Gahoonia, T.S., Nielsen, N.E., Joshi, P.A. and Jahoor, A. (2001) A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake. *Plant Soil* **235**, 211–219 https://doi.org/10.1023/A:1011993322286
48 Tanaka, N., Kato, M., Tomioka, R., Kurata, R., Fukao, Y., Aoyama, T. et al. (2014) Characteristics of a root hair-less line of *Arabidopsis thaliana* under physiological stresses. *J. Exp. Bot.* **65**, 1497–1512 https://doi.org/10.1093/jxb/eru014
49 Müller, M. and Schmidt, W. (2004) Environmentally induced plasticity of root hair development in Arabidopsis. *Plant Phys.* **134**, 409–419 https://doi.org/10.1104/pp.103.029066
50 Bouranis, D.L., Malagoli, M., Avice, J.C. and Bloem, E. (2020) Advances in plant sulfur research. *Plants* **9**, 256 https://doi.org/10.3390/plants9020256
51 Kimura, Y., Ushiwatari, T., Suyama, A., Tominaga-Wada, R., Wada, T. and Maruyama-Nakashita, A. (2019) Contribution of root hair development to sulfate uptake in *Arabidopsis*. *Plants* **8**, 106 https://doi.org/10.3390/plants8040106
52 Buchner, P., Parmar, S., Kriegel, A., Carpentier, M. and Hawkesford, M.J. (2010) The sulfate transporter family in wheat: tissue-specific gene expression in relation to nutrition. *Mol. Plant* **3**, 374–389 https://doi.org/10.1093/mp/ssp119
53 Lan, P., Li, W., Lin, W.D., Santi, S. and Schmidt, W. (2013) Mapping gene activity of Arabidopsis root hairs. *Genome Biol.* **14**, R67 https://doi.org/10.1186/gb-2013-14-6-r67
54 Moon, S., Chandran, A.K.N., An, G., Lee, C. and Jung, K.H. (2018) Genome-wide analysis of root hair-preferential genes in rice. *Rice* **11**, 48 https://doi.org/10.1186/s12284-018-0241-2
55 Takahashi, H. (2019) Sulfate transport systems in plants: functional diversity and molecular mechanisms underlying regulatory coordination. *J. Ex. Bot.* **70**, 4075–4087 https://doi.org/10.1093/jxb/erz132
56 Schiavon, M., Nardi, S., Dalla Vecchia, F. and Ertani, A. (2020) Selenium biofortification in the 21st century: status and challenges for healthy human nutrition. *Plant Soil* **16**, 1–26 https://doi.org/10.1007/s11104-020-04635-9
57 Barberon, M., Berthomieu, P., Clairotte, M., Shibagaki, N., Davidian, J.C. and Gosti, F. (2008) Unequal functional redundancy between the two *Arabidopsis thaliana* high-affinity sulphate transporters SULTR1;1 and SULTR1;2. *New Phytol.* **180**, 608–619 https://doi.org/10.1111/j.1469-8137.2008.02604.x
58 El Mehdawi, A.F., Jiang, Y., Guignardi, Z.S., Esmat, A., Pilon, M., Pilon-Smits, E. et al. (2018) Influence of sulfate supply on selenium uptake dynamics and expression of sulfate/selenate transporters in selenium hyperaccumulator and nonhyperaccumulator Brassicaceae. *New Phytol.* **217**, 194–205 https://doi.org/10.1111/nph.14838
59 Zhao, X.Q., Mitani, N., Yamaji, N., Shen, R.F. and Ma, J.F. (2010) Involvement of silicon influx transporter OsNIP2;1 in selenite uptake in rice. *Plant Phys.* **153**, 1871–1877 https://doi.org/10.1104/pp.110.157867
60 Graziano, M. and Lamattina, L. (2007) Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots. *Plant J.* **52**, 949–960 https://doi.org/10.1111/j.1365-313X.2007.03283.x
61 Schmidt, W. and Schikora, A. (2001) Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. *Plant Phys.* **125**, 2078–2084 https://doi.org/10.1104/pp.125.4.2078
62 Li, Z., Phillip, D., Neuhäuser, B., Schulze, W.X. and Ludewig, U. (2015) Protein dynamics in young maize root hairs in response to macro- and micronutrient deprivation. *J. Proteome Res.* **14**, 3362–3371 https://doi.org/10.1021/acs.jproteome.5b00399
63 Santi, S. and Schmidt, W. (2008) Laser microdissection-assisted analysis of the functional fate of iron deficiency-induced root hairs in cucumber. *J. Exp. Bot.* **59**, 697–704 https://doi.org/10.1093/jxb/erm351
64 Schmid, N.B., Giehl, R.F., Döll, S., Mock, H.P., Strehmel, N., Scheel, D. et al. (2014) Feruloyl-CoA 6′-Hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in Arabidopsis. *Plant Physiol.* **164**, 160–172 https://doi.org/10.1104/pp.113.228544
65 Rajniak, J., Giehl, R.F.H., Chang, E., Murgia, I., von Wirén, N. and Sattely, E.S. (2018) Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nat Chem. Biol.* **14**, 442–450 https://doi.org/10.1038/s41589-018-0019-2
66 Zuchi, S., Cesco, S., Gottardi, S., Pinton, R., Römheld, V. and Astolfi, S. (2011) The root-hairless barley mutant *brb* used as model for assessment of role of root hairs in iron accumulation. *Plant Physiol. Biochem.* **49**, 506–512 https://doi.org/10.1016/j.plaphy.2010.12.005

67 Morrissey, J., Baxter, I.R., Lee, J., Li, L., Lahner, B., Grotz, N. et al. (2009) The ferroportin metal efflux proteins function in iron and cobalt homeostasis in Arabidopsis. *Plant Cell* **21**, 3326–3338 https://doi.org/10.1105/tpc.109.069401
68 Li, B., Tester, M. and Gilliham, M. (2017) Chloride on the move. *Trends Plant Sci.* **22**, 236–248 https://doi.org/10.1016/j.tplants.2016.12.004
69 Geilfus, C.M. (2018) Chloride: from nutrient to toxicant. *Plant Cell Physiol.* **59**, 877–886 https://doi.org/10.1093/pcp/pcy071
70 Halperin, S.J. and Lynch, J.P. (2003) Effects of salinity on cytosolic Na^+ and K^+ in root hairs of *Arabidopsis thaliana*: in vivo measurements using the fluorescent dyes SBFI and PBFI. *J. Exp. Bot.* **54**, 2035–2043 https://doi.org/10.1093/jxb/erg219
71 Lv, S., Yu, D., Sun, Q. and Jiang, J. (2018) Activation of gibberellin 20-oxidase 2 undermines auxin-dependent root and root hair growth in NaCl-stressed Arabidopsis seedlings. *Plant Growth Regul.* **84**, 225–236 https://doi.org/10.1007/s10725-017-0333-9
72 Robin, A.H., Matthew, C., Uddin, M.J. and Bayazid, K.N. (2016) Salinity-induced reduction in root surface area and changes in major root and shoot traits at the phytomer level in wheat. *J. Exp. Bot.* **67**, 3719–3729 https://doi.org/10.1093/jxb/erw064
73 Wang, Y., Zhang, W., Li, K., Sun, F., Han, C., Wang, Y. et al. (2008) Salt-induced plasticity of root hair development is caused by ion disequilibrium in *Arabidopsis thaliana*. *J. Plant Res.* **121**, 87–96 https://doi.org/10.1007/s10265-007-0123-y
74 Arif, M.R., Islam, M.T. and Robin, A.H.K. (2019) Salinity stress alters root morphology and root hair traits in *Brassica napus*. *Plants* **8**, 192 https://doi.org/10.3390/plants8070192
75 Alejandro, S., Höller, S., Meier, B. and Peiter, E. (2020) Manganese in plants: from acquisition to subcellular allocation. *Front. Plant Sci.* **11**, 300 https://doi.org/10.3389/fpls.2020.00300
76 Yang, T.J., Perry, P.J., Ciani, S., Pandian, S. and Schmidt, W. (2008) Manganese deficiency alters the patterning and development of root hairs in Arabidopsis. *J. Exp. Bot.* **59**, 3453–3464 https://doi.org/10.1093/jxb/ern195
77 Wu, Z., Liang, F., Hong, B., Young, J.C., Sussman, M.R., Harper, J.F. et al. (2002) An endoplasmic reticulum-bound Ca^{2+}/Mn^{2+} pump, ECA1, supports plant growth and confers tolerance to Mn^{2+} stress. *Plant Phys.* **130**, 128–137 https://doi.org/10.1104/pp.004440
78 Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and Lux, A. (2007) Zinc in plants. *New Phytol.* **173**, 677–702 https://doi.org/10.1111/j.1469-8137.2007.01996.x
79 Genc, Y., Huang, C.Y. and Langridge, P. (2007) A study of the role of root morphological traits in growth of barley in zinc-deficient soil. *J. Exp. Bot.* **58**, 2775–2784 https://doi.org/10.1093/jxb/erm142
80 Gahoonia, T.S. and Nielsen, N.E. (2003) Phosphorus (P) uptake and growth of a root hairless barley mutant (*bald root barley*, *brb*) and wild type in low-and high-P soils. *Plant Cell Environ.* **26**, 1759–1766 https://doi.org/10.1046/j.1365-3040.2003.01093.x
81 Fukao, Y., Ferjani, A., Tomioka, R., Nagasaki, N., Kurata, R., Nishimori, Y. et al. (2011) ITRAQ analysis reveals mechanisms of growth defects due to excess zinc in Arabidopsis. *Plant Phys.* **155**, 1893–1907 https://doi.org/10.1104/pp.110.169730
82 O'Neill, M.A., Ishii, T., Albersheim, P. and Darvill, A.G. (2004) Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annu. Rev. Plant Biol.* **55**, 109–139 https://doi.org/10.1146/annurev.arplant.55.031903.141750
83 Martín-Rejano, E.M., Camacho-Cristóbal, J.J., Herrera-Rodríguez, M.B., Rexach, J., Navarro-Gochicoa, M.T. and González-Fontes, A. (2011) Auxin and ethylene are involved in the responses of root system architecture to low boron supply in Arabidopsis seedlings. *Physiol. Plant.* **142**, 170–178 https://doi.org/10.1111/j.1399-3054.2011.01459.x
84 Takano, J., Wada, M., Ludewig, U., Schaaf, G., von Wirén, N. and Fujiwara, T. (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* **18**, 1498–1509 https://doi.org/10.1105/tpc.106.041640
85 Bassil, E., Nijo, G., Baluska, F., Volkmann, D., Menzel, D., Goldbach, H. et al. (2005) Boron deficiency rescues the *Arabidopsis thaliana* rhd2 root hair phenotype: are reactive oxygen species involved? *Eur. J. Cell Biol.* **84**, 63 https://doi.org/10.1016/j.ejcb.2005.02.001
86 Hey, S., Baldauf, J., Opitz, N., Lithio, A., Pasha, A., Provart, N. et al. (2017) Complexity and specificity of the maize (*Zea mays* L.) root hair transcriptome. *J. Exp. Bot.* **68**, 2175–2185 https://doi.org/10.1093/jxb/erx104
87 Brdar-Jokanović, M. (2020) Boron toxicity and deficiency in agricultural plants. *Int. J. Mol. Sci.* **21**, 1424 https://doi.org/10.3390/ijms21041424
88 Takano, J., Tanaka, M., Toyoda, A., Miwa, K., Kasai, K., Fuji, K. et al. (2010) Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. *Proc. Natl Acad. Sci. U.S.A.* **107**, 5220–5225 https://doi.org/10.1073/pnas.0910744107
89 Miwa, K., Tanaka, M., Kamiya, T. and Fujiwara, T. (2010) Molecular mechanisms of boron transport in plants: Involvement of Arabidopsis NIP5;1 and NIP6;1. *Adv. Exp. Med. Biol.* **679**, 83–96 https://doi.org/10.1007/978-1-4419-6315-4_7
90 Wang, S., Yoshinari, A., Shimada, T., Hara-Nishimura, I., Mitani-Ueno, N., Ma, J.F. et al. (2017) Polar localization of the NIP5;1 boric acid channel is maintained by endocytosis and facilitates boron transport in Arabidopsis roots. *Plant Cell* **29**, 824–842 https://doi.org/10.1105/tpc.16.00825
91 Printz, B., Lutts, S., Hausman, J.-F. and Sergeant, K. (2016) Copper trafficking In plants and its implication on cell wall dynamics. *Front. Plant Sci.* **7**, 601 https://doi.org/10.3389/fpls.2016.00601
92 He, Z., Ma, Z., Brown, K.M. and Lynch, J.P. (2005) Assessment of inequality of root hair density in *Arabidopsis thaliana* using the Gini coefficient: a close look at the effect of phosphorus and its interaction with ethylene. *Ann. Bot.* **95**, 287–293 https://doi.org/10.1093/aob/mci024
93 Leškovi, A., Zvarí, K.M., Araya, T. and Giehl, R.F.H. (2020) Nickel toxicity targets cell wall-related processes and pin2-mediated auxin transport to inhibit root elongation and gravitropic responses in Arabidopsis. *Plant Cell Physiol.* **61**, 519–535 https://doi.org/10.1093/pcp/pcz217
94 Pasternak, T., Rudas, V., Potters, G. and Jansen, M.A.K. (2005) Morphogenic effects of abiotic stress: reorientation of growth in *Arabidopsis thaliana* seedlings. *Environ. Exp. Bot.* **53**, 299–314 https://doi.org/10.1016/j.envexpbot.2004.04.009
95 Wang, Y., Thorup-Kristensen, K., Jensen, L.S. and Magid, J. (2016) Vigorous root growth is a better indicator of early nutrient uptake than root hair traits in spring wheat grown under low fertility. *Front. Plant Sci.* **7**, 865 https://doi.org/10.3389/fpls.2016.00865
96 Puig, S. (2014) Function and regulation of the plant COPT family of high-affinity copper transport proteins. *Adv. Bot.* **2014**, e476917 https://doi.org/10.1155/2014/476917
97 Li, Y. and Zamble, D.B. (2009) Nickel homeostasis and nickel regulation: an overview. *Chem. Rev.* **109**, 4617–4643 https://doi.org/10.1021/cr900010n
98 Barberon, M., Dubeaux, G., Kolb, C., Isono, E., Zelazny, E. and Vert, G. (2014) Polarization of IRON-REGULATED TRANSPORTER 1 (IRT1) to the plant-soil interface plays crucial role in metal homeostasis. *Proc. Natl Acad. Sci. U.S.A.* **111**, 8293–8298 https://doi.org/10.1073/pnas.1402262111
99 Nishida, S., Tsuzuki, C., Kato, A., Aisu, A., Yoshida, J. and Mizuno, T. (2011) AtIRT1, the primary iron uptake transporter in the root, mediates excess nickel accumulation *in Arabidopsis thaliana*. *Plant Cell Physiol.* **52**, 1433–1442 https://doi.org/10.1093/pcp/pcr089

100 Nishida, S., Kato, A., Tsuzuki, C., Yoshida, J. and Mizuno, T. (2015) Induction of nickel accumulation in response to zinc deficiency in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **16**, 9420–9430 https://doi.org/10.3390/ijms16059420
101 Lin, Y., Allen, H.E. and Di Toro, D.M. (2016) Barley root hair growth and morphology in soil, sand, and water solution media and relationship with nickel toxicity. *Environ. Toxicol. Chem.* **35**, 2125–2133 https://doi.org/10.1002/etc.3389
102 Tejada-Jiménez, M., Chamizo-Ampudia, A., Galván, A., Fernández, E. and Llamas, Á. (2013) Molybdenum metabolism in plants. *Metallomics* **5**, 1191–1203 https://doi.org/10.1039/c3mt00078h
103 Tomatsu, H., Takano, J., Takahashi, H., Watanabe-Takahashi, A., Shibagaki, N. and Fujiwara, T. (2007) An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil. *Proc. Natl Acad. Sci. U.S.A.* **104**, 18807–18812 https://doi.org/10.1073/pnas.0706373104
104 Baxter, I., Muthukumar, B., Park, H.C., Buchner, P., Lahner, B., Danku, J. et al. (2008) Variation in molybdenum content across broadly distributed populations *of Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (*MOT1*). *PLoS Genet.* **4**, e1000004 https://doi.org/10.1371/journal.pgen.1000004
105 Duan, G., Hakoyama, T., Kamiya, T., Miwa, H., Lombardo, F., Sato, S. et al. (2017) LjMOT1, a high-affinity molybdate transporter from *Lotus japonicus*, is essential for molybdate uptake, but not for the delivery to nodules. *Plant J.* **90**, 1108–1119 https://doi.org/10.1111/tpj.13532
106 Coskun, D., Deshmukh, R., Sonah, H., Menzies, J.G., Reynolds, O., Ma, J.F. et al. (2018) The controversies of silicon's role in plant biology. *New Phytol.* **221**, 67–85 https://doi.org/10.1111/nph.15343
107 Ma, J.F., Goto, S., Tamai, K. and Ichii, M. (2001) Role of root hairs and lateral roots in silicon uptake by rice. *Plant Phys.* **127**, 1773–1780 https://doi.org/10.1104/pp.010271
108 Ma, J.F. and Yamaji, N. (2015) A cooperative system of silicon transport in plants. *Trends Plant Sci.* **20**, 435–442 https://doi.org/10.1016/j.tplants.2015.04.007
109 Gilroy, S. and Jones, D.L. (2000) Through form to function: root hair development and nutrient uptake. *Trends Plant Sci.* **5**, 56–60 https://doi.org/10.1016/s1360-1385(99)01551-4
110 Mackay, A.D. and Barber, S.A. (1987) Effect of cyclic wetting and drying of a soil on root hair growth of maize roots. *Plant Soil* **104**, 291–293 https://doi.org/10.1007/BF02372544
111 Marzec, M., Melzer, M. and Szarejko, I. (2015) Root hair development in the grasses: what we already know and what we still need to know. *Plant Phys.* **168**, 407–414 https://doi.org/10.1104/pp.15.00158
112 Cailloux, M. (1972) Metabolism and the absorption of water by root hairs. *Can. J. Bot.* **50**, 557–573 https://doi.org/10.1139/b72-069
113 Dodd, I.C. and Diatloff, E. (2016) Enhanced root growth of the *brb* (bald root barley) mutant in drying soil allows similar shoot physiological responses to soil water deficit as wild-type plants. *Funct. Plant Biol.* **43**, 199–206 https://doi.org/10.1071/fp15303
114 Suzuki, N., Taketa, S. and Ichii, M. (2003) Morphological and physiological characteristics of a root-hairless mutant in rice (*Oryza sativa* L.). *Plant Soil* **255**, 9–17 https://doi.org/10.1023/A:1026180318923
115 Carminati, A., Passioura, J.B., Zarebanadkouki, M., Ahmed, M.A., Ryan, P.R., Watt, M. et al. (2017) Root hairs enable high transpiration rates in drying soils. *New Phytol.* **216**, 771–781 https://doi.org/10.1111/nph.14715
116 Wen, T.J. and Schnable, P.S. (1994) Analyses of mutants of three genes that influence root hair development in *Zea mays* (Gramineae) suggest that root hairs are dispensable. *Am. J. Bot.* **81**, 833–842 https://doi.org/10.1002/j.1537-2197.1994.tb15564.x
117 Klamer, F., Vogel, F., Li, X., Bremer, H., Neumann, G., Neuhäuser, B. et al. (2019) Estimating the importance of maize root hairs in low phosphorus conditions and under drought. *Ann Bot.* **124**, 961–968 https://doi.org/org10.1093/aob/mcz011
118 Marin, M., Feeney, D.S., Brown, L.K., Naveed, M., Riuz, S., Koebernick, N. et al. (2020) Significance of root hairs for plant performance under contrasting field conditions and water deficit. *Ann. Bot.*, mcaa181 https://doi.org/10.1093/aob/mcaa181
119 Maurel, C., Boursiac, Y., Luu, D.T., Santoni, V., Shahzad, Z. and Verdoucq, L. (2015) Aquaporins in plants. *Physiol. Rev.* **95**, 1321–1358 https://doi.org/10.1152/physrev.00008.2015
120 Tan, Y.Q., Yang, Y., Zhang, A., Fei, C.F., Gu, L.L., Sun, S.J. et al. (2020) Three CNGC family members, CNGC5, CNGC6, and CNGC9, are required for constitutive growth of Arabidopsis root hairs as Ca^{2+}-permeable channels. *Plant Commun.* **1**, 100001 https://doi.org/10.1016/j.xplc.2019.100001
121 Zhang, S., Pan, Y., Tian, W., Dong, M., Zhu, H., Luan, S. et al. (2017) Arabidopsis CNGC14 mediates calcium influx required for tip growth in root hairs. *Mol. Plant* **10**, 1004–1006 https://doi.org/10.1016/j.molp.2017.02.007
122 Vert, G., Grotz, N., Dédaldéchamp, F., Gaymard, F., Guerinot, M.L., Briat, J.F. et al. (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14**, 1223–1233 https://doi.org/10.1105/tpc.001388
123 Perea-García, A., Garcia-Molina, A., Andrés-Colás, N., Vera-Sirera, F., Pérez-Amador, M.A., Puig, S. et al. (2013) Arabidopsis copper transport protein COPT2 participates in the cross talk between iron deficiency responses and low-phosphate signaling. *Plant Phys.* **162**, 180–194 https://doi.org/10.1104/pp.112.212407
124 Sancenón, V., Puig, S., Mateu-Andrés, I., Dorcey, E., Thiele, D.J. and Peñarrubia, L. (2004) The Arabidopsis copper transporter COPT1 functions in root elongation and pollen development. *J. Biol. Chem.* **279**, 15348–15355 https://doi.org/10.1074/jbc.M313321200
125 Castaings, L., Caquot, A., Loubet, S. and Curie, C. (2016) The high-affinity metal transporters NRAMP1 and IRT1 team up to take up iron under sufficient metal provision. *Sci. Rep.* **6**, 37222 https://doi.org/10.1038/srep37222
126 Ragel, P., Raddatz, N., Leidi, E.O., Quintero, F.J. and Pardo, J.M. (2019) Regulation of K^+ nutrition in plants. *Front Plant Sci.* **10**, 281 https://doi.org/10.3389/fpls.2019.00281
127 Qi, Z., Hampton, C.R., Shin, R., Barkla, B.J., White, P.J. and Schachtman, D.P. (2008) The high affinity K^+ transporter AtHAK5 plays a physiological role in planta at very low K^+ concentrations and provides a caesium uptake pathway in Arabidopsis. *J. Exp. Bot.* **59**, 595–607 https://doi.org/10.1093/jxb/erm330
128 Demidchik, V., Straltsova, D., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A. and Yurin, V. (2014) Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. *J. Exp. Bot.* **65**, 1259–1270 https://doi.org/10.1093/jxb/eru004

129 Rigas, S., Ditengou, F.A., Ljung, K., Daras, G., Tietz, O., Palme, K. et al. (2013) Root gravitropism and root hair development constitute coupled developmental responses regulated by auxin homeostasis in the Arabidopsis root apex. *New Phytol.* **197**, 1130–1141 https://doi.org/10.1111/nph.12092

130 Lee, R.D. and Cho, H.T. (2013) Auxin, the organizer of the hormonal/environmental signals for root hair growth. *Front. Plant Sci.* **4**, 448 https://doi.org/10.3389/fpls.2013.00448

131 Gebert, M., Meschenmoser, K., Svidová, S., Weghuber, J., Schweyen, R., Eifler, K. et al. (2009) A root-expressed magnesium transporter of the MRS2/MGT gene family in *Arabidopsis thaliana* allows for growth in low-Mg^{2+} environments. *Plant Cell* **21**, 4018–4030 https://doi.org/10.1105/tpc.109.070557

132 Visscher, A.M., Paul, A.L., Kirst, M., Guy, C.L., Schuerger, A.C. and Ferl, R.J. (2010) Growth performance and root transcriptome remodeling of Arabidopsis in response to Mars-like levels of magnesium sulfate. *PLoS ONE* **5**, e12348 https://doi.org/10.1371/journal.pone.0012348

133 Mao, D., Chen, J., Tian, L., Liu, Z., Yang, L., Tang, R. et al. (2014) Arabidopsis transporter MGT6 mediates magnesium uptake and is required for growth under magnesium limitation. *Plant Cell* **26**, 2234–2248 https://doi.org/10.1105/tpc.114.124628

134 Guo, K.M., Babourina, O., Christopher, D.A., Borsic, T. and Rengel, Z. (2010) The cyclic nucleotide-gated channel AtCNGC10 transports Ca^{2+} and Mg^{2+} in Arabidopsis. *Physiol. Plant* **139**, 303–312 https://doi.org/10.1111/j.1399-3054.2010.01366.x

135 Cailliatte, R., Schikora, A., Briat, J.F., Mari, S. and Curie, C. (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for Arabidopsis growth in low manganese conditions. *Plant Cell* **22**, 904–917 https://doi.org/10.1105/tpc.109.073023

136 Yoshimoto, N., Takahashi, H., Smith, F.W., Yamaya, T. and Saito, K. (2002) Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis roots. *Plant J.* **29**, 465–473 https://doi.org/10.1046/j.0960-7412.2001.01231.x

137 Isayenkov, S.V. and Maathuis, F. (2019) Plant salinity stress: many unanswered questions remain. *Front Plant Sci.* **10**, 80 https://doi.org/10.3389/fpls.2019.00080

138 Apse, M.P. and Blumwald, E. (2007) Na^+ transport in plants. *FEBS Lett.* **581**, 2247–2254 https://doi.org/10.1016/j.febslet.2007.04.014

139 Wang, Q., Guan, C., Wang, P., Ma, Q., Bao, A.K., Zhang, J.L. et al. (2019) The effect of AtHKT1;1 or AtSOS1 mutation on the expressions of Na^+ or K^+ transporter genes and ion homeostasis in *Arabidopsis thaliana* under salt stress. *Int J Mol Sci.* **20**, 1085 https://doi.org/10.3390/ijms20051085

140 Guo, K.M., Babourina, O., Christopher, D.A., Borsics, T. and Rengel, Z. (2008) The cyclic nucleotide-gated channel, AtCNGC10, influences salt tolerance in Arabidopsis. *Physiol Plant.* **134**, 499–507 https://doi.org/10.1111/j.1399-3054.2008.01157.x

141 Jin, Y., Jing, W., Zhang, Q. and Zhang, W. (2015) Cyclic nucleotide gated channel 10 negatively regulates salt tolerance by mediating Na^+ transport in Arabidopsis. *J. Plant Res.* **128**, 211–220 https://doi.org/10.1007/s10265-014-0679-2

142 Nishida, S., Tanikawa, R., Ishida, S., Yoshida, J., Mizuno, T., Nakanishi, H. et al. (2020) Elevated expression of vacuolar nickel transporter gene ireg2 is associated with reduced root-to-shoot nickel translocation in *Noccaea japonica*. *Front. Plant Sci.* **11**, 610 https://doi.org/10.3389/fpls.2020.00610

143 Kaiser, B.N., Rawat, S.R., Siddiqi, M.Y., Masle, J. and Glass, A.D. (2002) Functional analysis of an Arabidopsis T-DNA 'knockout' of the high-affinity NH_4^+ transporter AtAMT1;1. *Plant Phys.* **130**, 1263–1275 https://doi.org/10.1104/pp.102.010843

144 Loqué, D., Yuan, L., Kojima, S., Gojon, A., Wirth, J., Gazzarrini, S. et al. (2006) Additive contribution of AMT1;1 and AMT1;3 to high-affinity ammonium uptake across the plasma membrane of nitrogen-deficient Arabidopsis roots. *Plant J.* **48**, 522–534 https://doi.org/10.1111/j.1365-313X.2006.02887.x

145 Yuan, L., Loqué, D., Ye, F., Frommer, W.B. and von Wirén, N. (2007) Nitrogen-dependent posttranscriptional regulation of the ammonium transporter AtAMT1;1. *Plant Phys.* **143**, 732–744 https://doi.org/10.1104/pp.106.093237

146 Wang, Y.Y., Cheng, Y.H., Chen, K.E. and Tsay, Y.F. (2018) Nitrate transport, signaling, and use efficiency. *Annu. Rev. Plant. Biol.* **69**, 85–122 https://doi.org/10.1146/annurev-arplant-042817-040056

147 Gu, M., Chen, A., Sun, S. and Xu, G. (2016) Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: What is missing? *Mol. Plant* **9**, 396–416 https://doi.org/10.1016/j.molp.2015.12.012

148 Shin, R. and Schachtman, D.P. (2004) Hydrogen peroxide mediates plant root cell response to nutrient deprivation. *Proc. Natl Acad. Sci. U.S.A.* **101**, 8829–8832 https://doi.org/10.1073/pnas.0401707101

149 Ayadi, A., David, P., Arrighi, J.F., Chiarenza, S., Thibaud, M.C., Nussaume, L. et al. (2015) Reducing the genetic redundancy of Arabidopsis PHOSPHATE TRANSPORTER1 transporters to study phosphate uptake and signaling. *Plant Phys.* **167**, 1511–1526 https://doi.org/10.1104/pp.114.252338

150 El-Zohri, M., Odjegba, V., Ma, L. and Rathinasabapathi, B. (2015) Sulfate influx transporters in *Arabidopsis thaliana* are not involved in arsenate uptake but critical for tissue nutrient status and arsenate tolerance. *Planta* **241**, 1109–1118 https://doi.org/10.1007/s00425-015-2241-4

151 Shibagaki, N., Rose, A., McDermott, J.P., Fujiwara, T., Hayashi, H., Yoneyama, T. et al. (2002) Selenate-resistant mutants of *Arabidopsis thaliana* identify Sultr1;2, a sulfate transporter required for efficient transport of sulfate into roots. *Plant J.* **29**, 475–486 https://doi.org/10.1046/j.0960-7412.2001.01232.x

152 Chiba, Y., Mitani, N., Yamaji, N. and Ma, J.F. (2009) Hvlsi1 is a silicon influx transporter in barley. *Plant J.* **57**, 810–818 https://doi.org/10.1111/j.1365-313X.2008.03728.x

153 Mitani, N., Yamaji, N. and Ma, J.F. (2009) Identification of maize silicon influx transporters. *Plant Cell Physiol.* **50**, 5–12 https://doi.org/10.1093/pcp/pcn110

154 van de Mortel, J.E., Villanueva, L.A., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland, P.D. et al. (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Phys.* **142**, 1127–1147 https://doi.org/10.1104/pp.106.082073

155 Shanmugam, V., Lo, J.C., Wu, C.L., Wang, S.L., Lai, C.C., Connolly, E.L. et al. (2011) Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana*–the role in zinc tolerance. *New Phytol.* **190**, 125–137 https://doi.org/10.1111/j.1469-8137.2010.03606.x

156 Da Ines, O. (2008) Functional analysis of PIP2 aquaporins in *Arabidopsis thaliana*. Dissertationen (Dissertation, LMU München) https://doi.org/10.5282/edoc.12029

157 Giehl, R.F. and von Wirén, N. (2014) Root nutrient foraging. *Plant Physiol.* **166**, 509–517 https://doi.org/10.1104/pp.114.245225

Chapter 3

Local and systemic metabolic adjustments to drought in maize: Hydraulic redistribution in a split-root system

Werner, L. M., Hartwig, R. P., Engel, I., Franzisky, B. L., Wienkoop, S., Brenner, M., Preiner, J., Repper, D., Hartung, J., Zörb, C., Wimmer, M. A.

Plant Nutrition and Soil Science 185 (5) (2022) 632-642

https://doi.org/10.1002/jpln.202200279

Received: 11 August 2022 | Accepted: 3 September 2022

DOI: 10.1002/jpln.202200279

RESEARCH ARTICLE

Local and systemic metabolic adjustments to drought in maize: Hydraulic redistribution in a split-root system

Lena Maria Werner[1] | Roman Paul Hartwig[1] | Isabelle Engel[1] | Bastian Leander Franzisky[2] | Stefanie Wienkoop[3] | Martin Brenner[3,4] | Julian Preiner[3] | Dagmar Repper[1] | Jens Hartung[5] | Christian Zörb[1] | Monika Andrea Wimmer[1]

[1]Institute of Crop Science, Department Quality of Plant Products, University of Hohenheim, Stuttgart, Germany

[2]Department Soil Science and Plant Nutrition, Hochschule Geisenheim University, Geisenheim, Germany

[3]Unit of Molecular Systems Biology, Department Functional and Evolution Ecology, University of Vienna, Vienna, Austria

[4]Department of Pharmaceutical Sciences, University of Vienna, Vienna, Austria

[5]Institute of Crop Science, Department Biostatistics, University of Hohenheim, Stuttgart, Germany

Correspondence
Monika A. Wimmer, Institute of Crop Science, Department Quality of Plant Products, University of Hohenheim (340e), Emil-Wolff-Str. 25, 70599 Stuttgart, Germany.
Email: m.wimmer@uni-hohenheim.de

This article has been edited by Klaus Dittert.

Funding information
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)

Abstract

Background: It is yet unknown how maize plants respond to a partial root drying under conditions of a limited total water supply, and which adaptation mechanisms are triggered under these conditions.

Aims: The aims of this study were to assess whether partial root drying results in distinguishable local and systemic physiological and metabolic drought responses, and whether compensatory water uptake and/or alteration of root architecture occurs under these conditions.

Methods: Maize plants were grown in a split-root system. When plants were 20 days old, the treatments 'well-watered', 'local drought' and 'full drought' were established for a period of 10 days. Shoot length and gas exchange were measured non-destructively, root exudates were collected using a filter system and biomass, relative water content, osmolality and proline content were determined destructively at final harvest.

Results: Local drought triggered stress responses such as reduced biomass, shoot length, relative water content and increased osmolality. Maintained root growth was systemically achieved by hydraulic redistribution rather than by altering root architecture. Local and systemic osmolyte adjustments contributed to this hydraulic redistribution.

Conclusions: Both local and systemic metabolic responses helped the plants to induce hydraulic redistribution, enhance water availability and in consequence plant water relations. This resulted in a surprisingly well-maintained root growth even in the drought stressed root compartment.

KEYWORDS
exudate collection, local drought, partial root drying, rhizosphere, root exudates

1 | INTRODUCTION

A consequence of climate change is water scarcity in many agricultural regions. To cope with drought, plants use several strategies, including stomatal closure to reduce transpiration, thereby limiting water loss and stabilizing the carbon status (Tardieu et al., 2018). Another strategy is osmotic adjustment by accumulation of compatible solutes in order to maintain a gradient in water potential between the bulk soil and the plant, thus upholding water movement and cell turgor (Blum, 2017), which helps in preserving root growth and reorganizing root

architecture to allow access to water in deeper soil layers (Dietz et al., 2021). However, the synthesis of compatible solutes is energy-consuming and can lead to suboptimal plant growth especially in case of a quickly restored water supply (Poorter et al., 2012). Thus, maintaining water relations by other mechanisms would represent a less costly adaptation strategy. One option is hydraulic redistribution (HR), which is the passive movement of water from moister to drier soil regions following a gradient in the soil water potential, using the roots as a conduit (Burgess et al., 1998). Such HR includes hydraulic lift, that is, the uplift of water from deeper to shallower soil layers (Caldwell et al., 1998), but also refers to lateral or downward water movement (Hafner et al., 2017).

Drought responses are triggered locally at the cellular level (*e.g.*, production of osmolytes), but systemic signalling results in responses of the whole plant. For example, drought perception in roots stimulates the production of abscisic acid (ABA), which is transported to the shoot and induces stomatal closure (Tardieu et al., 2018). Depending on plant species, other signalling components include brassinosteroids, strigolactones, ethylene, sap pH or the small peptide CLE25, which moves through the vasculature to plant leaves where it activates ABA production via NCED3 (Gupta et al., 2020). It is to our knowledge not yet clear whether partial root drying results only in local metabolic responses, or also sets off measurable systemic adaptations of the whole plant.

The quantity and composition of root exudates can also vary in response to water deficit (Gargallo-Garriga et al., 2018). Carbon skeletons derived from photosynthetic CO_2 fixation serve as precursors for synthesis of compound classes detected in exudates, such as sugars, amino acids, organic acids, fatty acids, sterols, vitamins, growth factors, enzymes, flavonoids, nucleotides and purines (Vives-Peris et al., 2020). The mechanism of CO_2 concentration also affects plant exudate production, as C_4 plants release more amino acids compared to C_3 plants (Vranova et al., 2013).

Drought stress alters not only the amount but also the composition of root exudates (Chen et al., 2022). This may improve the contact of the root movement through and the nutrient uptake from the soil (Gargallo-Garriga et al., 2018). Following osmotic adjustment of the plant, concentrations of metabolites in exudates can increase (Gargallo-Garriga et al., 2018), thus lowering the water potential of the rhizosphere and improving water flow to the root. Since exudates also attract beneficial microorganisms promoting plant recovery after a stress event (Munoz-Ucros et al., 2022), a better understanding of root exudation in response to drought could contribute to secure crop production. Despite known effects of root exudates in the rhizosphere, it is—to our knowledge—not clear if changes in exudate composition can be triggered under local stress in roots. Given that roots directly sense the water content in soil (Schachtman & Goodger, 2008), such a local response might be feasible.

Split-root settings are suitable to induce partial root-zone drying by irrigating just half of the root system. They are excellent systems to assess local responses to drought and were used in several studies, for example, to demonstrate increases in the water use efficiency under partial drought for several species (reviewed in Schachtman & Goodger, 2008). For maize, partial root-zone drying induced a compensatory increase in the total water uptake from the irrigated root half (Hu et al., 2011). However, all these studies provided unlimited water to maintain an optimum soil water content in the irrigated root compartment. Here, using a split-root system, the overall aim of this study was to assess whether local and locally induced systemic physiological drought responses can be distinguished under conditions of partial root drying combined with limited total water supply. Specifically, we addressed two hypotheses: (1) plants exposed to partial root drying respond locally by compensatory increased water uptake from the watered root half and/or compensatory root growth; (2) partial root drying induces local and systemic acclimation strategies which act synergistically to improve water relations in the plant.

2 | MATERIALS AND METHODS

2.1 | Plant growth conditions

The *Zea mays* line B73 was used in all experiments. All seeds were provided by the group Crop Functional Genomics of the University of Bonn. Seeds were sterilized with 10% H_2O_2, rinsed in water and soaked for 4 h in saturated $CaSO_4$ solution. Seeds were then germinated between layers of filter paper (REF 150010; MN: 710; Macherey-Nagel, Germany) and imbibed with 4 mM $CaSO_4$ solution in a dark climate chamber (24°C, 65% relative humidity [rH]; WeißTechnik Fitotron HGC 0714). When primary roots reached a minimum length of 1 cm, they were cut off, and seedlings were kept between filter paper soaked with 2 mM $CaSO_4$ solution until the shoot emerged (day 4 after sowing [DAS]), then they were exposed to light (350 μM m^{-2} s^{-1} PAR; 12 h per day), with roots covered. On DAS 7, seedlings were transferred to soil filled split-root rhizoboxes (Figure 1B). Soil sieving, filling and fertilization were performed as described in Vetterlein et al. (2021). Emerging lateral roots were evenly distributed between the root compartments. Boxes were covered with a black sheet and placed at a 52° angle. The experiment was conducted in a greenhouse at the University of Hohenheim (48°42'39.2"N, 9°11'53.0"E) with an average temperature of 24.6°C, rH of 51% and LED lights adjusted to 400 μM m^{-2} s^{-1} PAR for 12 h per day. Beneficial insects were used for pest control.

2.2 | Experimental setup and watering regime

Until DAS 19, all plants were watered daily with filtered rainwater (120 mesh/130 micron, Netafim, Germany) to a volumetric soil water content (VWC) of 22% (v/v), determined by weighing the rhizoboxes. On DAS 20 (day of treatment [DOT] 0), the three treatments, well-watered (WW), full drought (FD), and local drought (LD), were established. WW plants were kept at 22% VWC in both root compartments. In LD, one root compartment (LD_{wet}) was supplied with half of the amount of water of WW, while the other compartment did not receive any water (LD_{dry}). In FD, water supply was completely stopped. Plants were harvested at DOT 10.

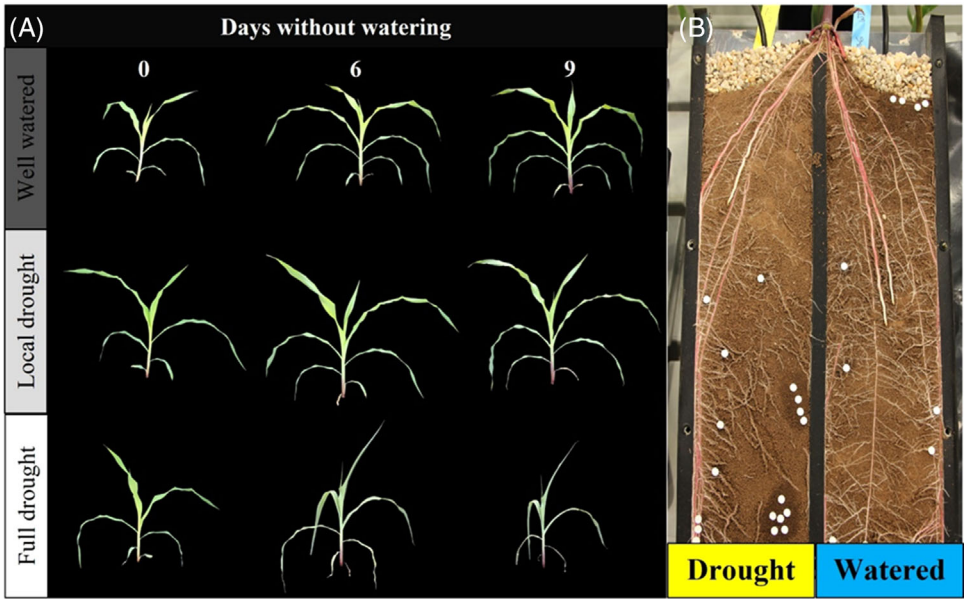


FIGURE 1 Shoots of *Zea mays* at 0, 6 and 9 days of the treatments well-watered, local and full drought (A) and roots after 9 days of local drought treatment with the watered right side and the non-watered left side of the rhizobox (B). Filters for exudate collection were placed consistently on larger roots (50%) and on smaller roots (50%), and additionally on root-free bulk soil in the lower half of the rhizobox.

One-third of the water was always supplied from the top, and two thirds via angle arrow droppers (Netafim) fixed to the centre of each root compartment and connected to an irrigation system (multi-control duo, Gardena, Germany). Preliminary tests validated a consistent water supply accuracy and even water distribution.

2.3 | Non-destructive measurements of shoot length and gas exchange

Shoot length and gas exchange were determined daily 2 h after turning the lights on. Photosynthetic rate (A) and transpiration rate (E) were measured on the second youngest fully elongated leaf (FEL) (20 cm from the leaf tip) using a leaf chamber/soil respiration analysis system (L.MAN-LCI; ADC BioScientific Ltd. Hoddesdon, Herts, EN11 0DB).

2.4 | Sampling and analysis of root exudates and rhizosphere compounds

Root exudates and rhizosphere metabolites were collected on DOT 9 with filter papers according to Neumann et al. (2014). Sampling started 2.5 h after lights were turned on and lasted for 3 h. Briefly, 10 sorption filters (diameter 0.5 cm; MN818; Macherey-Nagel, pre-rinsed in 80% methanol and MilliQ water) per root compartment were placed on exposed roots in the lower rhizobox half to ensure that sampled roots had indeed grown during (and not before) the treatment period (Figure 1B). Filters were kept moist, and roots were protected from light by a black foil. After removal, they were immediately frozen (−80°C) and freeze-dried before analysis. Rhizoboxes were returned to their positions for 24 h before harvest on DOT 10.

Metabolites were extracted from filters in two steps by incubating in 80% methanol and 80% ethanol (each at 95°C for 30 min). Derivatization was carried out according to Mehmeti et al. (2013). In brief, 20 μL of methoxyamin hydrochlorid in pyridine (40 g L^{-1}) was added to each sample/standard, the pellet completely dissolved and samples were incubated at 30°C for 90 min in a shaker (700 rpm). Subsequently, they were silylated by adding 80 μL MSTFA (95%–100%, Macherey-Nagel) and incubating at 37°C for 30 min (700 rpm).

GC-MS measurement was performed as described in Turetscheck et al. (2017), with an Agilent 7890B GC coupled to a LECO Pegasus® BT GC-TOFMS (LECO Corporation, Michigan, USA). Raw data were processed with the LECO Chroma-TOF® software (LECO® Corporation, Michigan, USA).

Metabolites were identified using MS-Dial (ver. 4.60) (Tsugawa et al., 2020). Data were exported as centroid and nominal masses and converted utilizing Reifycs Abf Converter. Settings were chosen as follows: smoothing level 3, average peak width 20, minimum peak height 1 × 10e4, mass slice width 0.5 and mass accuracy 0.5. Measured alkanes were used for retention index calculation, with a retention index tolerance set to 20. Gap filling by compulsion was activated and sample max/blank average filter was set to 5. All metabolites were normalized to the internal standards phenyl ß-D-glucopyranoside and pentaerythritol, according to their minimum distance of retention index (Weiszmann et al., 2020). Confirmation of level 1 identification (Schymanski et al., 2014) was given by measuring a mixture of standard compounds in different concentrations within each batch. Relative quantification of metabolites was done by normalized peak intensities of the quant masses of all target metabolites. These relative values were utilized for further statistical data analysis.

Even though the filter collection method is established for sampling of root exudates (Neumann et al., 2014), some shortcomings

should be considered. First, because the volume of collected solution cannot be determined, absolute quantification of metabolites is not possible. To assess treatment effects, the sum of all detected peak intensities was calculated, and each compound was expressed in percent of the total sum. For construction of the heatmap, mean values of the treatments were further expressed as fold-change relative to the WW conditions. Secondly, collected samples represent a mixture of root exudates and metabolites present in the rhizosphere before the sampling (*e.g.*, possibly also produced by microorganisms), and the contribution of each source cannot be distinguished. For reasons of simplicity, we consciously use the term 'exudate' throughout this study even though strictly speaking it should be 'exudates and surrounding rhizosphere compounds'.

2.5 | Final destructive harvest

On DOT 10, all plants were harvested to determine root and shoot biomass, relative water content (RWC), osmolality and proline. Additionally, soil water content was determined. The harvest started 2 h after turning the lights on and was performed within 3 h to avoid bias resulting from diurnal variance (Hachez et al., 2008). Each parameter was determined in defined parts of the plants, that is, the second FEL (distal 25 cm) was used for osmolality, the third FEL for proline extraction and the fourth FEL (distal 15 cm) for RWC. In addition, approximately 1 g of roots from the lower half of the rhizobox were also used for proline extraction. Fresh weight of all sampled material was determined.

Leaf RWC was determined according to Wedeking et al. (2016), using two 4 cm long leaf segments without midrib. Osmolality was determined in cell sap collected by repeated freezing/thawing of leaves and centrifuging (5 min, 3600 × *g*). Duplicates were analyzed using a vapour pressure osmometer (Vapro, Model 5600, ELITech). For proline extraction, leaves and roots were cut, washed twice in deionized water (roots) and ground in liquid N_2. Extraction was performed with 30 mg FW in 1.5 mL 70% (v/v) ethanol (80°C, 20 min) and centrifugation (RT, 5 min, 18,800 × *g*). The supernatant was mixed (1:1) with a ninhydrin solution (1% [w/v] ninhydrin in 60% [v/v] acetic acid and 20% [v/v] ethanol), heated (95°C, 20 min), cooled on ice and measured photometrically (TECAN infinite M nano) at 520 nm (modified from Chinard, 1952). Remaining roots were washed, blotted and oven-dried (65°C) together with the remaining shoot for dry weight (DW) determination.

Soil samples from three different depths (0–10, 10–20, and 20–30 cm) of each root compartment were mixed, weighed (FW), dried at 105°C and reweighed (DW) to determine gravimetric soil water content (GWC).

2.6 | Experimental design and statistics

Treatments were randomized to pots/plants according to a resolvable row-column design with six replicates, two rows and six columns per replicate. Additionally, columns of two subsequent replicates were latinized, resulting in additional complete blocks. Replicates were allocated side-by-side, forming two rows and 36 columns. Thus, units of 1 × 12 and 2 × 6 form complete blocks.

Data were analyzed according to the design with the following mixed model:

$$y_{ijklmn} = \mu + b_k + d_l + r_{lk} + c_{mk} + p_{lmk} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + e_{ijklmn}, \quad (1)$$

where y_{ijklmn} is the observation of genotype *i* treated with watering treatment level *j* in side *n* of row *k*, column *m* with the complete blocks *k* and *l*; μ is the intercept; b_k and d_l are the fixed effects of complete block *k* and *l*; r_{lk}, c_{mk} and p_{lmk} are the random effects of row *l*, column *m* and pot *lm* within replicate *k*; τ_i, φ_j and $(\tau\varphi)_{ij}$ are the fixed main and interaction effects of genotype *i* and treatment *j*; and e_{ijklmn} is the error of y_{ijklmn} associated with the side. The error variance was allowed to be genotype, treatment or genotype-by-treatment specific if this increased model fit was measured via AIC (Wolfinger, 1993). Note that WW, LD and FD treatment resulted in compartments treated with four treatment levels: WW, LD_{wet}, LD_{dry}, and FD. Normal distributed and homogeneous variance of residuals were checked graphically via residual plots. If necessary, data were square-root, log or logit transformed prior to analysis to fulfil these pre-requirements. Adjusted means were back-transformed for presentation purpose only. Standard errors were back-transformed using the delta method.

The current study considers a single genotype and data from pots harvested at DAS 40, even though the experiment included another genotype and harvest time. All available data were used to adjust means and to estimate variances. Afterwards, results were limited to the genotype and harvest of interest.

For gas exchange measurements, only two-thirds of the pots were randomly measured. As information about row and column effects per day is sparse and can cause convergence problems, both effects were dropped from the model. Additionally, blocks are incomplete now and thus were fitted as random.

3 | RESULTS

3.1 | Plant growth and water uptake

Ten days of LD did not lead to visual drought stress symptoms, while such symptoms (*e.g.*, wilting) were observed in the FD treatment after 6 DOT (Figure 1A). Growth was significantly reduced beginning after 5 DOT (shoot length, Figure 2A) and after 10 DOT (shoot dry weight, Figure 3A), respectively. At the final harvest, shoot DW was reduced by 26% (LD) and by 59% (FD) in comparison to WW, respectively (Figure 3A). The root dry weight did not differ between LD_{wet}, LD_{dry} and WW and was also similar in LD_{wet} and LD_{dry}, but was significantly reduced under FD (Figure 3B). The ratio between root and shoot dry weight was significantly increased in both LD and FD (Figure 3C).

Significant differences were observed in photosynthetic (A) and transpiration (E) rates between LD and FD (Figure 2B,C).

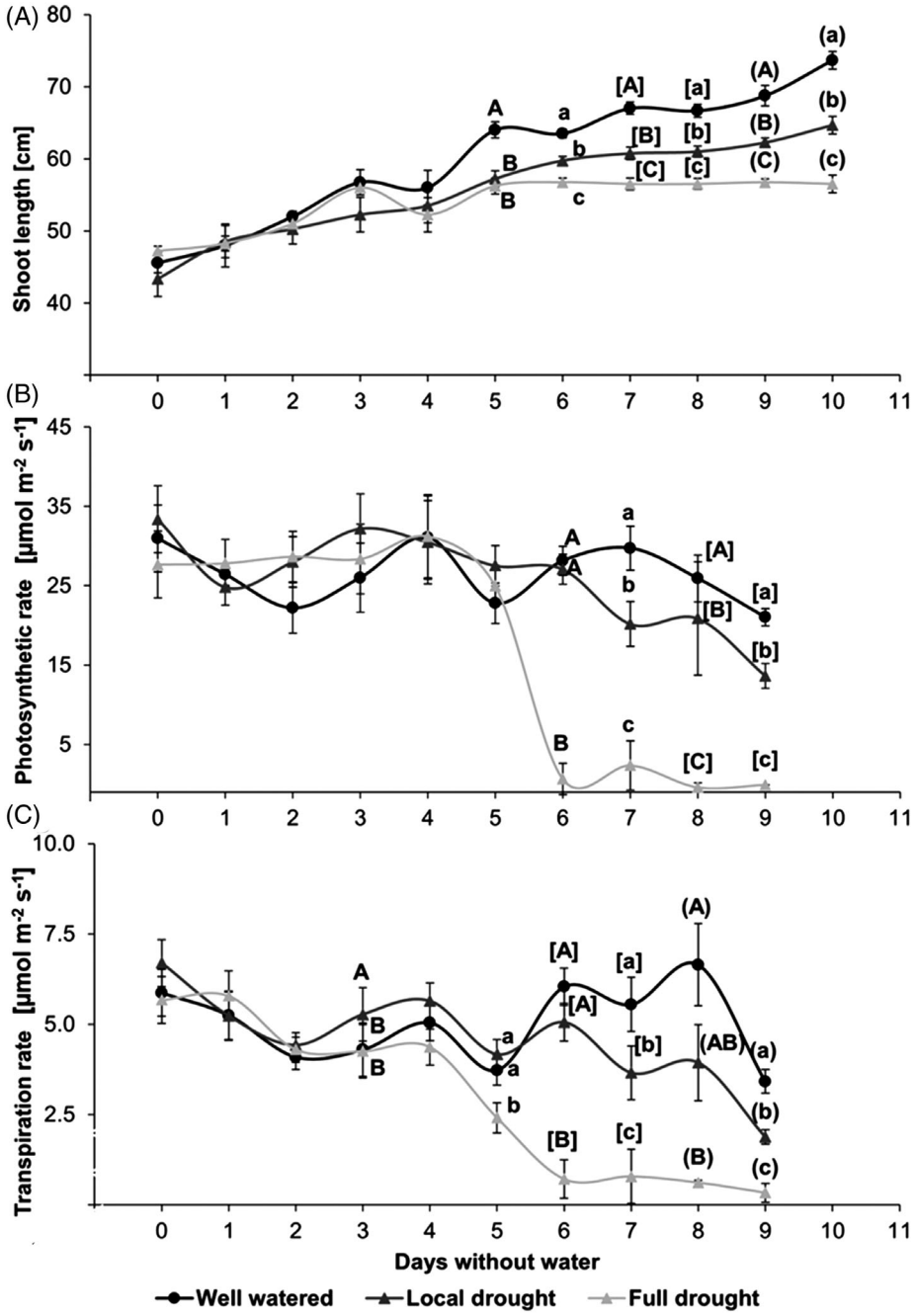


FIGURE 2 Shoot length (A), photosynthetic rate (B) and transpiration rate (C) of *Zea mays* during 10 days of well-watered (filled circles, black), local drought (triangles, dark grey) and full drought (triangles, light grey) treatments. Values represent adjusted means, and error bars indicate the corresponding standard errors ($n = 4$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments well-watered, local drought and full drought. Significance was tested for each individual day. Significance per day is indicated by capital and lowercase letters and non or differing brackets.

Under FD conditions, transpiration rate decreased steadily starting at DOT 5, while photosynthetic rate dropped drastically on DOT 6 (Figure 2B,C). Afterwards, both E and A were almost neglectable. On the other hand, LD resulted in a significant reduction of both E and A from DOT 7 onwards. However, both rates were maintained at a (at least partly) functional level, still reaching 65% (A) and 56% (E) of the WW plants on DOT 9 (Figure 2B,C).

At DOT 10, the soil in FD contained less water (6% GWC) than that of the WW treatment (15% GWC). Under LD conditions, the soil GWC was significantly reduced in both root compartments, but in addition LD_{wet} was significantly wetter (10% GWC) than LD_{dry} (7% GWC) (Figure 3D). Interestingly, the soil in LD_{dry} contained slightly, but significantly, more water than FD soil. Root water content did not differ significantly between LD_{wet} and LD_{dry}, between WW and LD_{wet}, as well as between LD_{dry} and FD conditions (Figure 3E).

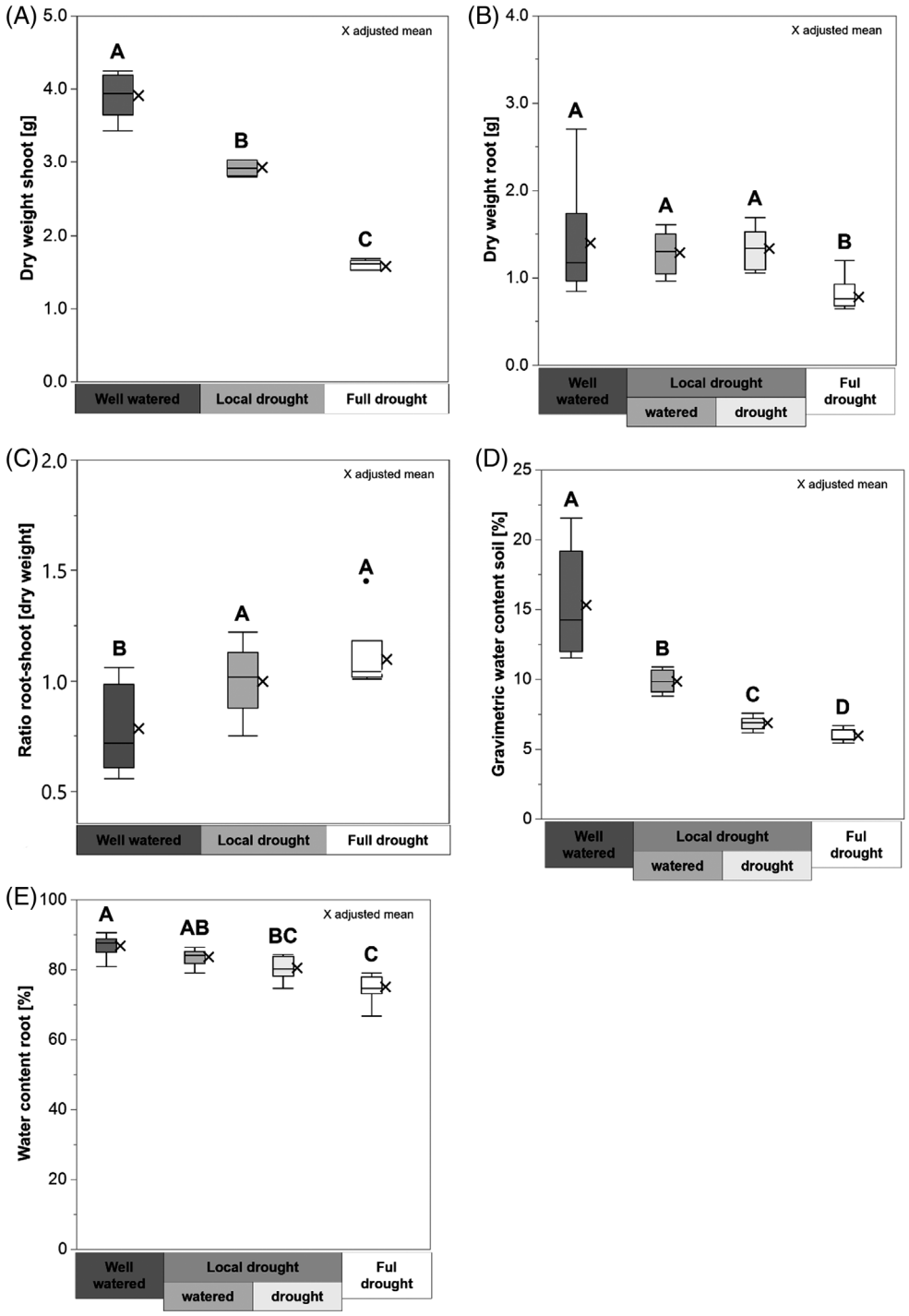


FIGURE 3 Boxplots of dry weight of roots (A) and shoots (B), gravimetric water content of the soil (C), root-shoot ratio (D) and root water content (E) of *Zea mays* after 10 days of well-watered, local drought and full drought treatments. The cross indicates the adjusted mean within the range ($n = 6$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments.

3.2 | Plant water relations and accumulation of osmotic solutes

Under WW conditions, the average RWC in the shoot at the final harvest was 98%. It was slightly but significantly reduced to 93% in LD and strongly reduced to 56% in FD (Figure 4A). Similarly, shoot osmolality was moderately increased by 25% compared to WW plants in LD, and by 113% in FD (Figure 4B), and shoot proline concentration was increased by 19% (though not significant) in LD, and by 244% in FD (Figure 4C). Root proline concentrations were significantly increased

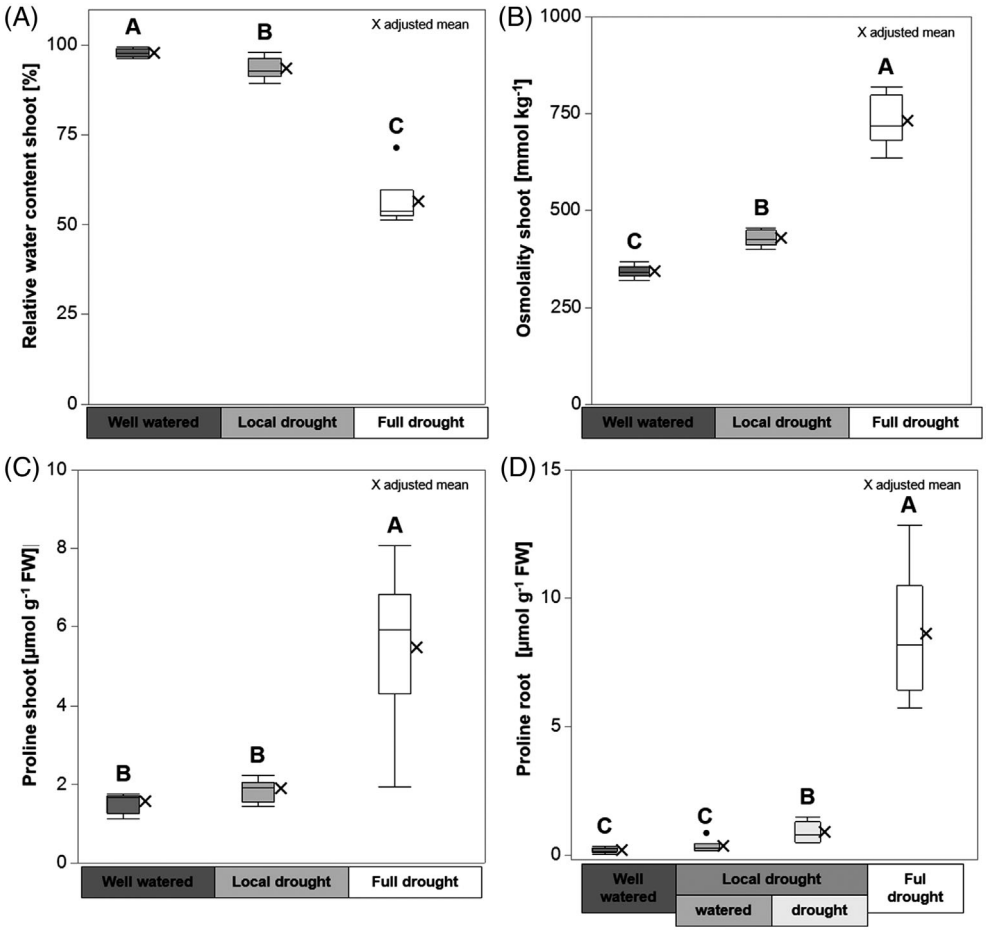


FIGURE 4 Boxplots of relative water content (A), osmolality (B), proline concentration in shoots (C) and roots (D) of *Zea mays* after 10 days of well-watered, local drought and full drought treatments. The cross indicates the adjusted mean within the range, and ($n = 6$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments.

in FD, as well as in LD_{dry}, even though the latter increase was less pronounced (Figure 4D).

3.3 | Composition of root exudates

In total, 39 metabolites were identified in root exudates and were classified into five categories (Figure 5). Irrespective of the treatment, sugars represented by far the largest fraction (on average 66% across all treatments), followed by organic acids (18%), amino acids (9%), sugar alcohols (1.6%) and amines (0.8%).

The largest impact on exudate fractions was triggered by FD. Compared to WW, fractions of proline, putrescine, maltose and trehalose significantly increased under FD (Figure 5). In LD_{dry}, these metabolites also showed a trend of higher values, but only putrescine was significantly increased compared to WW. A tendency towards higher values under drought was also observed for the organic acids fumaric acid, threonic acid, gluconic acid and the amine spermidine, as well as the amino acids leucine, glycine and proline. A significant reduction compared to WW conditions was observed in FD for malic acid and glucose, while tyrosine, phenylalanine, tryptophane, glutamate, lysine, ornithine, glutamine and fructose showed a trend towards lower values. Between the two sides of LD, fractions of 2-oxoglutaric acid and phenylalanine were lower in LD_{dry} than in LD_{wet}. However, a similar difference of these compounds was not observed between FD and WW (Figure 5).

4 | DISCUSSIONS

4.1 | Local maintenance of root water content is systemically achieved by hydraulic redistribution rather than by locally altered root architecture

One hypothesis of this study was that plants perceiving a local drought stress would respond by compensatory increased water uptake from the watered root half and/or compensatory root growth.

Hydraulic redistribution, that is, the movement of water from moist to drier soil using the plant roots as a conduit (Burgess et al., 1998), is a well-known process especially in arid or semi-arid ecosystems, and

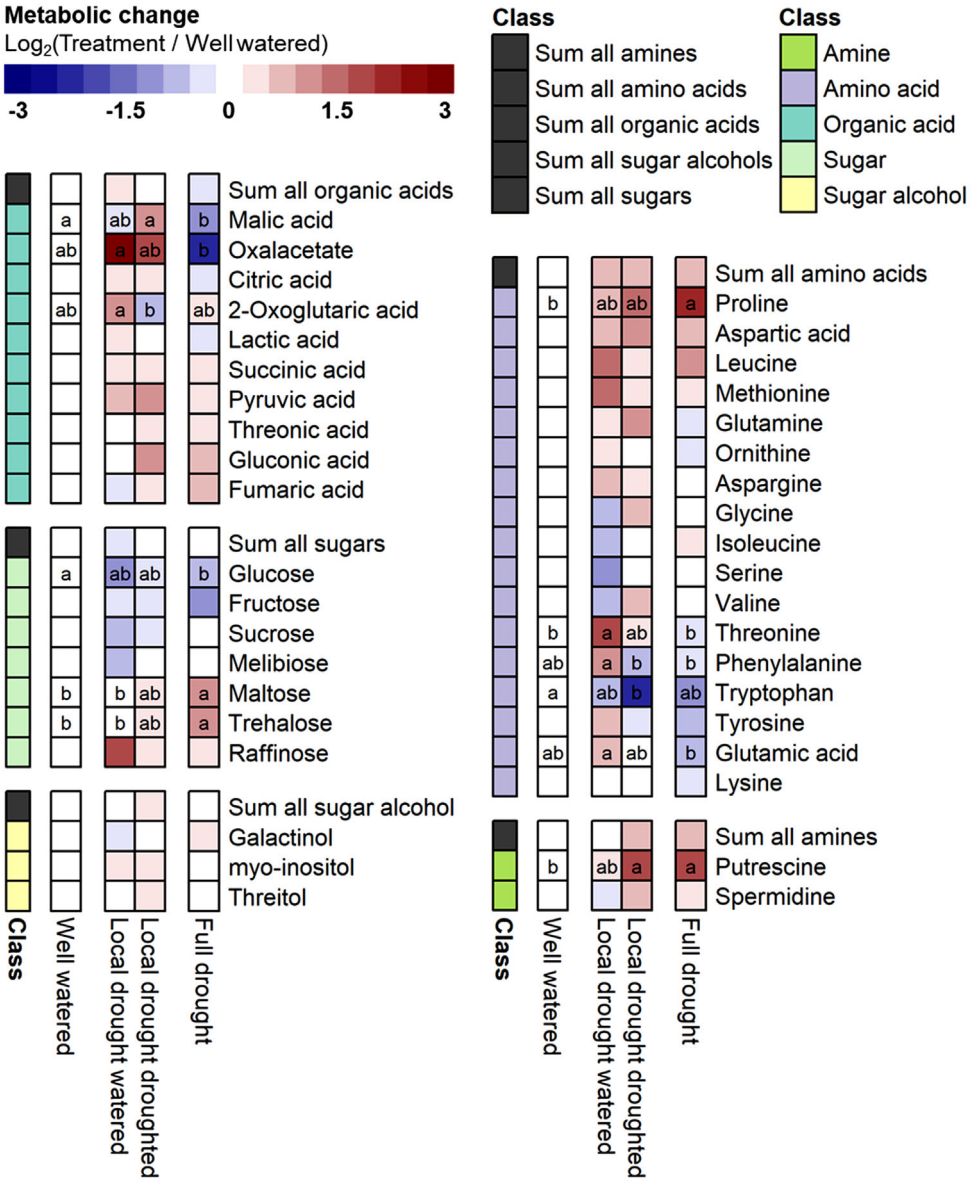


FIGURE 5 Metabolic changes in root exudates of *Zea mays* after 10 days of (left to right) well-watered, local drought watered, local drought drought stressed and full drought treatments. Metabolic changes are presented as means of each treatment, normalized to the well-watered control, in log2 scale. Colours indicate increases (red) and decreases (blue) compared to well-watered conditions. For each metabolite, values with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test). Metabolites without letters did not show significant differences between treatments (data provided in Table S1).

most common in trees and shrubs (Hafner et al., 2017), but was not yet described for annual crops. It has significant ecological implications not only by providing water to the stressed root parts, but also by releasing water into the dryer soil which can be used by neighbouring plants and root-associated microorganisms (Hafner et al., 2017). In the following discussion, we use the term 'hydraulic redistribution' in the strict sense, that is, only when referring to water movement via the roots, while we use the term 'water movement' for water flow within the soil but without root contribution. The significantly higher soil water content (GWC) in LD_{dry} compared to FD could be the result of HR, or alternatively of a lower water uptake in LD_{dry}, or both. However, in combination with the lower GWC in LD_{wet} compared to WW, and the similar root water content in both root halves of LD, a hydraulic redistribution of water from the watered to the drought stressed root side is more likely. This indicates that the locally stressed plants were able to replenish water in the stressed root half. It is important that no direct water movement via the soil was possible from one root compartment to the other. Aquaporins such as PIP1.2, PIP2.1 or PIP2.5 have an important role in water uptake regulation of maize (Hachez et al., 2006). However, gene expression levels of PIP1.2, PIP2.1, and PIP2.5 in root tissues were not significantly different between the drought conditions, with the exception of PIP2.5, which was expressed less in LD_{dry} compared to FD (Tables S2 and S3). Since it was, however, similarly low in LD_{wet}, this down-regulation is unlikely to explain the observed differ-

ences in GWC of the soil. It seems that enhanced water movement via osmotic adaptation in combination with HR was sufficient to maintain root growth, even though we cannot exclude the possibility of a change in water uptake via altered aquaporin activity rather than expression.

Reorganization of the root system is also important for fostering the water supply during water limitation (Dietz et al., 2021). At least under moderate drought, shoot growth is usually more rapidly reduced than root growth (Poorter et al., 2012), which can be even enhanced to access deeper soil layers (Dietz et al., 2021). On the other hand, in drying soils, root elongation can be limited by a reduced hydrostatic pressure in the root-tip cells (K. Jin et al., 2013). Thus, an increased root-shoot ratio is reported under moderate drought (Poorter et al., 2012). In the present study, a small but significant increase in root-shoot ratio was observed in both LD and FD, even though it was not different between the two drought treatments (Figure 2D). This is in line with the fact that in LD, shoot growth was significantly reduced, while root biomass was not altered (Figure 2A,B), resulting in a larger root–shoot ratio. The lack of a further increase in root–shoot ratio in FD can be explained by the onset of significant root growth inhibition. It is noteworthy that we did not observe any compensatory root growth in either root side of LD. Indeed, root growth was equally maintained in both root compartments despite a final GWC of only 7% in LD_{dry} (Figure 2A,C), which is close to the permanent wilting point for the loamy soil used in this study (6% GWC or 8% VWC: Vetterlein et al., 2021). This surprising lack of root growth inhibition would again be in line with the suggested HR described above.

Collectively, these data indicate that local maintenance of root water relations was achieved at least in part by HR between the watered and the stressed root halves, that is, a systemic response of the plants, rather than by a local compensatory change of root growth or architecture.

4.2 | Local and locally induced systemic adjustments of osmolytes in roots and exudates contribute to hydraulic redistribution

We also wanted to know whether the partial root drying resulted in local stress responses only in the drought stressed root half, or whether the whole plant responded systemically with metabolic and physiological acclimation strategies.

Maintaining water uptake in a drying soil depends on the ability of the plant to maintain a sufficient gradient in water potential between soil and plant, which can be achieved by osmotic adjustment (Dietz et al., 2021). In the present study, despite no visual drought symptoms under LD conditions (Figure 1A), plants clearly experienced drought stress as indicated by reduced transpiration and assimilation rates (Figure 2), inhibited shoot growth (Figure 3) and accumulation of osmolytes and proline in leaves and roots (Figure 4). The lack of wilting symptoms correlates well with the shoot RWC, indicating that water availability to one root half was sufficient to maintain almost normal water relations in the plants. Nevertheless, these plants responded with a metabolic adaptation of the whole plant, that is, a systemic metabolic response, as indicated by an increased osmolality in the shoot. Thus, partial root drying resulted in a systemic increase in shoot osmotic potential, thus likely enhancing water movement from the bulk soil to the root in both root compartments. Since half of the roots had access to water, this effect is likely to have a positive effect on the whole plant water relations.

Proline increases under drought stress (Ilahi & Dorffling, 1982), and not only serves as osmolyte but also protects cell membranes from damage by reactive oxygen species (Trovato et al., 2008). The strong observed increase in proline in both shoots and roots in FD indicated severe stress in the whole plant. To a much lower extent, but still significantly, proline concentration was also elevated in roots of LD_{dry} but not of LD_{wet}, indicating an additional local response (Figure 4D). In this setting, we were concerned that the determination of osmolality in roots would be flawed by the necessary extensive washing of the roots to remove adhering soil. We thus determined proline content in roots assuming that it would correlate with total osmolality. Indeed, in the shoot, proline and total osmolality correlated significantly (R^2 = 0.9406), even though proline, on average, represented less than 1% of the total osmolality (Figure 4B,C). We would thus suggest that other osmolytes (not determined here) were likely also increased locally in LD_{dry} roots.

This local metabolic adjustment to drought was further supported by changes of exudate composition in LD_{dry} and FD (Figure 5). Overall, more pronounced effects in FD compared to LD_{dry} (including a significant decrease of malic acid and glucose in FD) confirm that some metabolic changes occur at the transition from moderate to severe drought stress (Schneider et al., 2019) and correlate with shutdown of assimilation (Ulrich et al., 2022). The trend of increasing proline, maltose and trehalose, however, correlated with decreasing soil water content, supporting an effect of drought intensity on exudate composition and specifically osmolytes (Gargallo-Garriga et al., 2018). In addition to osmotic effects, especially carbohydrates may also contribute to a better movement of the roots through the drying soil (Gargallo-Garriga et al., 2018), and proline, putrescine, trehalose and maltose are known to enhance beneficial microorganisms under drought (Y. Jin et al., 2019; Kuiper et al., 2001; Vílchez et al., 2000; Vílchez et al., 2016). Whether their increasing trend has a measurable effect on microbiomes of locally stressed root parts still needs to be clarified. Many root exudates, particularly sugars, are released mainly by passive or facilitated diffusion (Li et al., 2018), and the composition of root exudates seems to reflect that of the root tissue (Gargallo-Garriga et al., 2018). It is thus likely that the observed increases in exudate metabolites in LD_{dry} roots reflect corresponding increases in the root tissue. Such local accumulation of osmolytes would lower the water potential in the drought stressed root half, with two possible consequences. First, this would further contribute to a steeper gradient in water potential between bulk soil and root and enhance water movement towards the roots. A local increase of osmolyte concentrations in root exudates would intensify this effect by lowering the water potential in the rhizosphere. Second, it would result in an increase in water potential gradient between the two root halves, and thus enhance RH from the wetter to the dryer root compartment via the roots.

Altogether, our data indicate that local and locally induced systemic osmolyte accumulation acts synergistically under partial root drying by inducing hydraulic redistribution, enhancing water availability and in consequence plant water relations and root growth under conditions of local drought stress.

5 | CONCLUSIONS

The locally drought stressed root side experienced a stress level sufficient to trigger both local (*e.g.*, osmolyte accumulation) and locally induced systemic (*e.g.*, osmolyte accumulation in the shoot, stomatal closure) responses, even though no difference in root growth was observed compared to the watered side. These adaptation mechanisms collectively helped the plants to not only improve water movement from soil to root but also to distribute water from the watered to the drought stressed side via the roots, resulting in a surprisingly well-maintained root growth even in the dried compartment despite a very low soil water content (similar to that of a full drought stress). Systemically, osmotic adjustment increased the water potential gradient between soil and plant to enhance water movement despite partial closure of stomata. Locally, osmotic adjustment and concurrent reduction in water potential of the drought stressed root half probably contributed to water movement from soil into roots as well as hydraulic redistribution from the watered to the drought stressed root compartment. Whole-plant water relations were little affected as long as parts of the root system still had access to water. Additional local and systemic changes in root exudate composition were observed and may possibly have lasting effects on the microbiome structure of the rhizosphere.

ACKNOWLEDGEMENTS

This study was conducted within the *priority program 2089*, funded by the *Deutsche Forschungsgemeinschaft* (*DFG*, *German Research Foundation*). Seeds of the maize genotype B73 were kindly provided by *Caroline Macron* and *Frank Hochholdinger* (University of Bonn). We thank *Prof. Asch* (University Hohenheim) for the Licor instrument, and gratefully acknowledge excellent technical support by *Christiane Beierle*, staff members of the Phytotechnikum and the TZ workshop of the University of Hohenheim.

Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Monika Andrea Wimmer https://orcid.org/0000-0002-7456-5667

REFERENCES

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment*, *40*(1), 4–10.

Burgess, S. S., Adams, M. A., Turner, N. C., & Ong, C. K. (1998). The redistribution of soil water by tree root systems. *Oecologia*, *115*(3), 306–311.

Caldwell, M. M., Dawson, T. E., & Richards, J. H. (1998). Hydraulic lift: Consequences of water efflux from the roots of plants. *Oecologia*, *113*(2), 151–161.

Chen, Y., Yao, Z., Sun, Y., Wang, E., Tian, C., Sun, Y., Liu, J., Sun, C., & Tian, L. (2022). Current studies of the effects of drought stress on root exudates and rhizosphere microbiomes of crop plant species. *International Journal of Molecular Sciences*, *23*(4), 2374. https://doi.org/10.3390/ijms23042374

Chinard, F. P. (1952). Photometric estimation of proline and ornithine. *Journal of Biological Chemistry*, *199*, 91–95.

Dietz, K. J., Zörb, C., & Geilfus, C. M. (2021). Drought and crop yield. *Plant Biology*, *23*(6), 881–893.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., & Peñuelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific Reports*, *8*(1), 1–15.

Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, *368*(6488), 266–269.

Hachez, C., Heinen, R. B., Draye, X., & Chaumont, F. (2008). The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. *Plant Molecular Biology*, *68*(4), 337–353.

Hachez, C., Moshelion, M., Zelazny, E., Cavez, D., & Chaumont, F. (2006). Localization and quantification of plasma membrane aquaporin expression in maize primary root: A clue to understanding their role as cellular plumbers. *Plant Molecular Biology*, *62*(1), 305–323.

Hafner, B. D., Tomasella, M., Häberle, K. H., Goebel, M., Matyssek, R., & Grams, T. E. (2017). Hydraulic redistribution under moderate drought among English oak, European beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment. *Tree Physiology*, *37*(7), 950–960.

Hu, T., Kang, S., Li, F., & Zhang, J. (2011). Effects of partial root-zone irrigation on hydraulic conductivity in the soil–root system of maize plants. *Journal of Experimental Botany*, *62*(12), 4163–4172.

Ilahi, I., & Dörffling, K. (1982). Changes in abscisic acid and proline levels in maize varieties of different drought resistance. *Physiologia Plantarum*, *55*(2), 129–135.

Jin, K., Shen, J., Ashton, R. W., Dodd, I. C., Parry, M. A., & Whalley, W. R. (2013). How do roots elongate in a structured soil? *Journal of Experimental Botany*, *64*(15), 4761–4777.

Jin, Y., Zhu, H., Luo, S., Yang, W., Zhang, L., Li, S., Jin, Q., Cao, Q., Sun, S., & Xiao, M. (2019). Role of maize root exudates in promotion of colonization of *Bacillus velezensis* strain S3–1 in rhizosphere soil and root tissue. *Current Microbiology*, *76*(7), 855–862.

Kuiper, I., Bloemberg, G. V., Noreen, S., Thomas-Oates, J. E., & Lugtenberg, B. J. (2001). Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. *Molecular Plant-Microbe Interactions*, *14*(9), 1096–1104.

Li, X., Dong, J., Chu, W., Chen, Y., & Duan, Z. (2018). The relationship between root exudation properties and root morphological traits of cucumber grown under different nitrogen supplies and atmospheric CO2 concentrations. *Plant and Soil*, *425*(1), 415–432.

Mehmeti, V., Fragner, L., & Wienkoop, S. (2013). Medicago truncatula root and shoot metabolomics: protocol for the investigation of the primary carbon and nitrogen metabolism based on GC–MS. In W. Weckwerth & G. Kahl (Eds.), *The handbook of plant metabolomics* (pp. 111–123). Wiley.

Munoz-Ucros, J., Wilhelm, R. C., Buckley, D. H., & Bauerle, T. L. (2022). Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. *Plant and Soil*, *471*(1), 443–461.

Neumann, G., Bott, S., Ohler, M. A., Mock, H. P., Lippmann, R., Grosch, R., & Smalla, K. (2014). Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. *Frontiers in Microbiology*, *5*, 2. https://doi.org/10.3389/fmicb.2014.00002

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50.

Schachtman, D. P., & Goodger, J. Q. (2008). Chemical root to shoot signaling under drought. *Trends in Plant Science*, *13*(6), 281–287.

Schneider, S., Turetschek, R., Wedeking, R., Wimmer, M. A., & Wienkoop, S. (2019). A protein-linger strategy keeps the plant on-hold after rehydration of drought-stressed *Beta vulgaris*. *Frontiers in Plant Science*, *10*, 381. https://doi.org/10.3389/fpls.2019.00381

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environmental Science & Technology*, *48*, 2097–2098.

Tardieu, F., Simonneau, T., & Muller, B. (2018). The physiological basis of drought tolerance in crop plants: A scenario-dependent probabilistic approach. *Annual Review of Plant Biology*, *69*, 733–759.

Trovato, M., Mattioli, R., & Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei*, *19*(4), 325–346.

Tsugawa, H., Ikeda, K., Takahashi, M., Satoh, A., Mori, Y., Uchino, H., Okahashi, N., Yamada, Y., Tada, I., Bonini, P., Higashi, Y., Okazaki, Y., Zhou, Z., Zhu, Z.-J., Koelmel, J., Cajka, T., Fiehn, O., Saito, K., Arita, M., & Arita, M. (2020). A lipidome atlas in MS-DIAL 4. *Nature Biotechnology*, *38*(10), 1159–1163.

Turetschek, R., Desalegn, G., Epple, T., Kaul, H. P., & Wienkoop, S. (2017). Key metabolic traits of Pisum sativum maintain cell vitality during *Didymella pinodes* infection: Cultivar resistance and the microsymbionts' influence. *Journal of Proteomics*, *169*, 189–201.

Ulrich, D. E., Clendinen, C. S., Alongi, F., Mueller, R. C., Chu, R. K., Toyoda, J., Gallegos-Graves, V., Goemann, H. M., Peyton, B., Sevanto, S., & Dunbar, J. (2022). Root exudate composition reflects drought severity gradient in blue grama (*Bouteloua gracilis*). *Scientific Reports*, *12*(1), 1–16.

Vetterlein, D., Lippold, E., Schreiter, S., Phalempin, M., Fahrenkampf, T., Hochholdinger, F., Marcon, C., Tarkka, M., Oburger, E., Ahmed, M., Javaux, M., & Schlüter, S. (2021). Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. *Journal of Plant Nutrition and Soil Science*, *184*(1), 35–50.

Vílchez, J. I., García-Fontana, C., Román-Naranjo, D., González-López, J., & Manzanera, M. (2016). Plant drought tolerance enhancement by trehalose production of desiccation-tolerant microorganisms. *Frontiers in Microbiology*, *7*, 1577. https://doi.org/10.3389/fmicb.2016.01577

Vílchez, S., Molina, L., Ramos, C., & Ramos, J. L. (2000). Proline catabolism by *Pseudomonas putida*: Cloning, characterization, and expression of the put genes in the presence of root exudates. *Journal of Bacteriology*, *182*(1), 91–99.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., & Pérez-Clemente, R. M. (2020). Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports*, *39*(1), 3–17.

Vranova, V., Rejsek, K., Skene, K. R., Janous, D., & Formanek, P. (2013). Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. *Journal of Plant Nutrition and Soil Science*, *176*(2), 175–199.

Wedeking, R., Mahlein, A. K., Steiner, U., Oerke, E. C., Goldbach, H. E., & Wimmer, M. A. (2016). Osmotic adjustment of young sugar beets (*Beta vulgaris*) under progressive drought stress and subsequent rewatering assessed by metabolite analysis and infrared thermography. *Functional Plant Biology*, *44*(1), 119–133.

Weiszmann, J., Clauw, P., Jagoda, J., Reichardt-Gomez, I., Koemeda, S., Jez, J., Nordborg, M., Walther, D., Nägele, T., & Weckwerth, W. (2020). Plasticity of the primary metabolome in 241 cold grown *Arabidopsis thaliana* accessions and its relation to natural habitat temperature. *bioRxiv*, https://doi.org/10.1101/2020.09.24.311092

Wolfinger, R. (1993). Covariance structure selection in general mixed models. *Communications in Statistics-Simulation and Computation*, *22*(4), 1079–1106.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Werner, L. M., Hartwig, R. P., Engel, I., Franzisky, B. L., Wienkoop, S., Brenner, M., Preiner, J., Repper, D., Hartung, J., Zörb, C., & Wimmer, M. A. (2022). Local and systemic metabolic adjustments to drought in maize: Hydraulic redistribution in a split-root system. *Journal of Plant Nutrition and Soil Science*, 1–11. https://doi.org/10.1002/jpln.202200279

TABLE S1. Detected compounds in root and rhizosphere solution after 9 days of drought. Shown are the retransformed relative adjusted means. Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way ANOVA; Fisher's LSD) between the control (well-watered) and the treatments local drought (watered, droughted) and full drought.

	well-watered	Local drought		full drought	well-watered	Local drought		full drought
Compound		**watered**	**drought**			**watered**	**drought**	
Σ amines	0.54	0.51	1.02	0.96	a	a	a	a
Putrescine	0.04	0.07	0.17	0.17	b	ab	a	a
Spermidine	0.47	0.39	0.76	0.71	a	a	a	a
Σ amino acids	5.72	9.75	11.06	9.76	a	a	a	a
Aspargine	0.07	0.11	0.09	0.08	a	a	a	a
Aspartic acid	0.02	0.03	0.04	0.03	a	a	a	a
Glutamic acid	0.25	0.45	0.24	0.15	ab	a	ab	b
Glutamine	0.02	0.03	0.04	0.02	a	a	a	a
Glycine	2.98	1.46	5.57	3.32	a	a	a	a
Isoleucine	0.05	0.03	0.05	0.06	a	a	a	a
Leucine	0.39	1.35	0.55	0.87	a	a	a	a
Lysine	0.20	0.18	0.19	0.16	a	a	a	a
Methionine	0.04	0.15	0.06	0.06	a	a	a	a
Ornithine	0.14	0.19	0.14	0.12	a	a	a	a
Phenylalanine	0.05	0.14	0.03	0.04	ab	a	b	b
Proline	0.33	0.61	1.06	1.79	b	ab	ab	a
Serine	0.19	0.08	0.21	0.20	a	a	a	a
Threonine	0.10	0.40	0.12	0.08	b	a	ab	b
Tryptophan	0.04	0.02	0.01	0.01	a	ab	b	ab
Tyrosine	0.02	0.03	0.01	0.01	a	a	a	a
Valine	0.00	0.00	0.00	0.00	a	a	a	a
Σ organic acids	18.16	21.96	18.15	14.71	a	a	a	a
2-Oxoglutaric acid	0.28	0.65	0.14	0.36	ab	a	b	ab
Citric acid	0.08	0.11	0.10	0.07	a	a	a	a
Fumaric acid	0.07	0.05	0.09	0.12	a	a	a	a

Gluconic acid	0.08	0.08	0.20	0.12	a	a	a	a
Lactic acid	13.60	18.56	15.38	9.43	a	a	a	a
Malic acid	0.11	0.08	0.25	0.04	a	ab	a	b
Oxalacetate	0.00	0.03	0.01	0.00	ab	a	ab	b
Pyruvic acid	0.07	0.13	0.17	0.10	a	a	a	a
Succinic acid	0.43	0.56	0.55	0.59	a	a	a	a
Threonic acid	0.10	0.11	0.14	0.13	a	a	a	a
Σ sugars	72.70	54.86	65.41	70.19	a	a	a	a
Fructose	20.98	13.93	14.91	9.58	a	a	a	a
Glucose	15.33	6.53	10.24	7.67	a	ab	ab	b
Maltose	5.85	5.10	7.86	14.43	b	b	ab	a
Melibiose	9.95	6.40	10.28	9.58	a	a	a	a
Raffinose	0.02	0.08	0.03	0.02	a	a	a	a
Sucrose	8.94	5.34	7.12	8.71	a	a	a	a
Trehalose	3.70	3.30	5.00	9.34	b	b	ab	a
Σ sugar alcohol	1.49	1.69	1.73	1.40	a	a	a	a
Galactinol	0.53	0.45	0.55	0.62	a	a	a	a
myo-inositol	0.72	0.91	0.86	0.66	a	a	a	a
Threitol	0.19	0.21	0.24	0.20	a	a	a	a

TABLE S2. Primers used for qPCR gene expression analysis in roots. Gene expression was performed by qPCR (CFX96 C1000 touch, BioRad) using the iTaq Universal SYBR Green Supermix.

Primer	Function	Forward	Reverse	According to
GADPH	HKG	TTGTTTCCCTTCCTGCTACC	AAACTGCAACCTCACCACAAG	(2)
Actine	HKG	GCCCTGCTGTATGAAATGGA	AAAGGAACCAGCTAAAAGCAAAC	(2)
PIP1.2	GOI	CTATTTTATGCGTTGGGATGT	ACTGAAACCAAGAAAACCCTGA	(1)
PIP2.1	GOI	CGGGTCGCCTTTTTTTTG	CCCTTGAGAGTCACGACATGA	(1)
PIP2.5	GOI	TGTCGTCGTTGGTTGCCT	CACAACAATCACACTAGCTTGGAA	(1)

TABLE S3. Expression of PIP1.2, PIP2.1 and PIP2.5 in roots after 10 days of drought. Each biological replicate was measured in triplicate. Gene expression was calculated according to the $2^{-\Delta\Delta CT}$ (3). Values represent adjusted means and standard errors (n=6). Means with at least one identical letter are non-significant from each other ($p < 0.05$; Fisher's LSD) between the control (well-watered) and the treatments local drought (watered, droughted) and full drought.

Treatment	Adjusted mean	Standard error	
PIP1.2			
WW	1.00		n.s.*
$LD_{watered}$	1.19	0.48	
$LD_{droughted}$	0.80	0.32	
FD	2.10	0.75	
PIP2.1			
WW	1.00		B
$LD_{watered}$	0.92	0.17	AB
$LD_{droughted}$	0.62	0.11	A
FD	0.69	0.10	A
PIP2.5			
WW	1.00		C
$LD_{watered}$	0.48	0.12	AB
$LD_{droughted}$	0.42	0.10	A
FD	0.69	0.14	BC

*Global F test indicated no significant differences between the treatments and the controll with *p*-value > 0.05

(1) Hachez, C., Moshelion M., Zelazny E., Cavez D., Chaumont F. (2006) Localization and Quantification of Plasma Membrane Aquaporin Expression in Maize Primary Root: A Clue to Understanding their Role as Cellular Plumbers. *Plant Mol Biol* **62**, 305–323 doi:10.1007/s11103-006-9022-1

(2) Heinen,R.B., Bienert, G.P., Cohen, D., Chevalier A. S., Uehlein N., Hachez C., Kaldenhoff R., Le Thiec D., Chaumont F. (2014) Expression and characterization of plasma membrane aquaporins in stomatal complexes of *Zea mays* . *Plant Mol Biol* **86**, 335–350 doi:10.1007/s11103-014-0232-7

(3) Schmittgen T.D. and Livak, K.J. (2008) Analyzing real-time PCR data by the comparative CT method. Nature Protocols 3, 1101– 1108 doi:10.1038/nprot.2008.73

Chapter 4

Physico-chemical properties of maize (*Zea mays* L.) mucilage differ with the collection system and corresponding root type and developmental stage of the plant

Werner, L, M., Knott, M., Diehl, D., Ahmed M, A., Banfield, C., Dippold, M., Vetterlein, D., Wimmer, M. A.

Plant Soil (2022)

Published online: 16 August 2022

https://doi.org/10.1007/s11104-022-05633-9

Plant Soil
https://doi.org/10.1007/s11104-022-05633-9

RESEARCH ARTICLE

Physico-chemical properties of maize (*Zea mays* L.) mucilage differ with the collection system and corresponding root type and developmental stage of the plant

Lena M. Werner · Matthilde Knott · Doerte Diehl · Mutez A. Ahmed · Callum Banfield · Michi Dippold · Doris Vetterlein · Monika A. Wimmer

Received: 22 February 2022 / Accepted: 28 July 2022

Abstract
Purpose Mucilage plays crucial roles in root-soil interactions. Collection systems for maize (*Zea mays* L.) use primary and seminal roots of aeroponically-grown seedlings (CS_A), or brace roots of soil-grown plants (CS_B). While each method represents specific plant developmental stages, and root types growing in specific (micro-)environments, these factors are rarely considered. It is unclear whether mucilage exhibits distinct physico-chemical properties related to collection system-inherent factors.
Methods Mucilage of maize genotype B73 was collected from systems CS_A and CS_B. Chemical composition was assessed by pH, nutrient contents, neutral sugar composition, and polysaccharide polymer length. Viscosity, surface tension and contact angle represented physical properties.
Results The share of hexoses among total polysaccharides was 11% higher in CS_B than in CS_A, whereas pentoses were predominant in CS_A, together with higher nutrient concentrations and pH values. Mannose was detected only in CS_B, which also exhibited higher surface tension, viscosity and contact angle compared to CS_A.
Conclusions Physico-chemical differences between the two mucilages are related to root type functions, environmental root growth conditions, and plant developmental state. Higher fractions of pentoses in

Responsible Editor: Eric Paterson.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-022-05633-9.

L. M. Werner · M. A. Wimmer (✉)
Institute of Crop Science, Department Quality of Plant Products, University of Hohenheim, Stuttgart, Germany
e-mail: m.wimmer@uni-hohenheim.de

M. Knott · D. Diehl
iES Institute for Environmental Sciences, Environmental and Soil Chemistry, University Koblenz-Landau, Landau in der Pfalz, Germany

M. A. Ahmed
Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

M. A. Ahmed
Department of Land, Air and Water Resources, University of California Davis, Davis, CA, USA

C. Banfield · M. Dippold
Department of Geosciences, University of Tuebingen, Tuebingen, Germany

D. Vetterlein
Helmholtz-Centre for Environmental Research – UFZ, Halle, Germany

D. Vetterlein
Martin Luther University Halle-Wittenberg, Halle, Germany

Published online: 16 August 2022

CS_A mucilage seem related to semi-sterile system conditions. Higher viscosity of CS_B mucilage might reflect the need for enhanced water holding capacity of brace roots growing in drier conditions. A strong influence of environmental factors on mucilage properties even for a single genotype might play additional roles e.g. in the attraction of microbiomes. These aspects are relevant when assessing the role of mucilage in the rhizosphere, or when developing models of rhizosphere processes.

Keywords Mucilage collection · Root type · Rhizosphere · Mucilage properties

Introduction

Mucilage is a polymeric gel that is primarily secreted by plants from the cap cells of the root tip (Carminati and Vetterlein 2013). It plays a crucial role in chemical and physical root-soil interactions (Ahmed et al. 2015), and facilitates root penetration into as well as root growth through the soil by lubrication of the root-soil interface and maintenance of a tight root-soil contact (Iijima et al. 2003). In addition, mucilage is a substrate boosting microbial activity (Ahmed et al. 2018a, b; Hawes et al. 1998; Knee et al. 2007), is involved in the formation and stabilization of soil aggregates (Watt et al. 1994), can improve soil stability during soil drying (Carminati et al. 2017) and reduce the energy required for root penetration into dry soil (Rosskopf et al. 2021).

Interactions of the chemical and physical properties of mucilage determine its unique and broad functions (Carminati and Vetterlein 2013), and are crucial for hydraulic processes in the rhizosphere (Benard et al. 2019). However, these interactions are complex, and seem to differ between species (Zickenrott et al. 2016) or genotypes (Nazari et al. 2020). The collection of mucilage is difficult, because root tips are usually not readily accessible, and the amount of mucilage produced per plant is species-specific and very limited in aeroponic systems (8-12 µg DW per plant: Guinel and McCully 1986; 56 µg DW per plant: Zickenrott et al. 2016), resulting in a general lack of simultaneous measurements of chemical and physical properties in the same batch of mucilage. Different mucilage collection methods have been established, each having its own advantages and disadvantages. For example, many studies are from hydroponic (Ahmed et al. 2015; Chaboud 1983; Naveed et al. 2017, 2019; Oburger and Jones 2018; Read et al. 2003), or from percolation systems (Mikutta et al. 2006), where mucilage represents only a small fraction of what is collected besides cell debris, low molecular weight compounds and enzymes (Oburger and Jones 2018). To collect mucilage as a less diluted fraction, seedlings are often grown in aeroponic systems (Brax et al. 2020; Holz et al. 2018; Zickenrott et al. 2016) or on filter paper (Read et al. 2003). A benefit of these methods are (semi-)sterile growth conditions, reducing a possible microbial degradation or contamination of mucilage (Chaboud 1983; Morel et al. 1986). Also, the system is efficient since root tips, which produce the mucilage, are dominant. However, aeroponic systems are restricted to very young seedlings of only several days of age, and a major disadvantage is the lack of opportunity to investigate the quality of mucilage cultivated under different abiotic conditions, such as varying nutrient supply or water availability. This is relevant, since environmental conditions can alter quantity and composition of mucilage (Ahmed et al. 2015; Nazari et al. 2020).

To overcome these problems, another collection method has been developed for some plant species including maize, which exude mucilage also from aboveground brace roots (Ahmed et al. 2015; McCulley and Boyer 1997; Morel et al. 1986; Zickenrott et al. 2016). Advantages of this method are the good accessibility of the roots, production of relatively large amounts of mucilage, and sampling of relatively undiluted material. However, a drawback is that this method can only be applied to a limited number of species, and to plants of a certain developmental stage, when brace roots start to develop but do not yet reach the soil. Maybe even more importantly, brace roots represent a very distinct root type that initially develops in mid-air, i.e. in a very dry micro-environment, and not in the usually humid soil as is the case for other root types such as primary or seminal roots.

Even though different collection systems are inherently linked with a specific physiological and developmental stage of the mucilage-producing plants, with different root types and very specific (micro-)environments in which the respective roots are growing, this aspect is rarely considered when discussing physicochemical properties of mucilage and their impact on root-soil interactions. To our knowledge, a possible impact of these factors is also usually not considered when plant-soil interaction models are developed, even

though they have been shown to affect water and nutrient uptake (Ahmed et al. 2016b; Ahmed et al. 2018c; Hetz et al. 1996). It still remains unclear whether mucilages collected by different methods from the same genotype are indeed comparable or not.

Chemically, up to 97% (w/w) of mucilage are carbohydrates (Carminati and Vetterlein 2013), consisting mainly of neutral and acidic polysaccharides but also monomeric sugars. Minor components include amino acids, organic acids, (poly)uronic acids, phenolic acids, minerals, proteins, glycolipids and other phospholipids (Bacic et al. 1986; Brax et al. 2020; Read et al. 2003). The maize mucilage carbohydrate fraction was extensively analyzed and consists of fucose, galactose, glucose, arabinose, xylose, mannose, rhamnose, ribose as well as acidic galacturonic and glucuronic acid (Amicucci et al. 2019; Bacic et al. 1986; Chaboud 1983; Morel et al. 1986; Nazari et al. 2020; Osborn et al. 1999; Watanabe et al. 2008).

The chemical composition has a significant impact on the physical behaviour of mucilage (Carminati and Vetterlein 2013), and significant differences in both, chemical and physical characteristics have been observed between plant species (Nazari et al. 2020; Zickenrott et al. 2016). Mucilage is characterized by a high viscosity, which positively correlates with the amount and molecular weight of the polysaccharide polymers present (Amicucci et al. 2019; Benard et al. 2019; Brax et al. 2020; Naveed et al. 2017; Read and Gregory 1997). Suface tension of mucilage, on the other hand, seems to be reduced by higher levels of phospholipids (Carminati and Vetterlein 2013; Moradi et al. 2012; Read et al. 2003). Cations like calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}) are also present in mucilage (Brax et al. 2020) and can bind to uronic acids, which act as cation exchangers (Mimmo et al. 2005; Morel et al. 1986). The amount of Ca^{2+} is partly determined by the amount of uronic acids in the polysaccharides and probably also by proteins (Brax et al. 2019, 2020). Recent work indicates that Ca^{2+} in the mucilage improved the microstructural stability of soil particles and thus possibly transport, availability and storage of nutrients and water, without correlating with mucilage viscosity (Brax et al. 2020).

Mucilage also affects soil water repellency or wettability, usually measured as the optical contact angle of water droplets on a surface of dried mucilage. For maize, contact angles increased with increasing mucilage concentrations to values above 90°, typical for hydrophobic substances, suggesting that mucilage may lead to a temporarily water repellent rhizosphere especially in drying soils (Ahmed et al. 2016a; Kaltenbach et al. 2018; Moradi et al. 2012). On the other hand, these results suggest that mucilage properties could be altered depending on the wetting/drying history of the mucilage.

In the present study, we address the question whether physico-chemical properties of maize mucilage collected by two different but widely used methods are comparable. We focus on mucilage collection systems from aeroponically-grown seedlings (CS_A), and from aboveground brace roots of soil-grown plants (CS_B). Both methods intrinsically differ in (i) the root type used for collection, (ii) the growth environment in which the root is growing, (iii) the developmental stage of the plant from which mucilage is sampled and (iv) most likely associated microbiome abundance and composition. We hypothesize that mucilages collected with these two systems exhibit distinct physico-chemical properties due to system-inherent differences in and interactions between these factors. Specifically, due to semi-sterile growth conditions, mucilage from the aeroponic system (CS_A) is expected to contain a smaller fraction of microorganism-derived hexoses and more pentoses compared to mucilage form the brace root system (CS_B). On the other hand, due to growth conditions with frequent wetting/drying cycles, CS_B mucilage is expected to exhibit physico-chemical characteristics which enhance its water holding capacity and growth through drier surface soil layers.

Material and methods

Mucilage sampling

Plant material

Mucilage was collected from the maize (*Zea mays* L.) genotype B73. The developmental stage (BBCH) was determined according to Meier et al. (2009).

Primary and seminal root mucilage collection system (CS_A: aeroponic)

Primary and seminal root mucilage was collected from seedlings grown in an aeroponic system (CS_A) as described by Brax et al. (2020). Semi-sterile conditions were obtained by cleaning the system with 10% (v/v) H_2O_2 solution. Seeds were sterilized with 10% H_2O_2 for 10 minutes and subsequently rinsed four times with deionized water. Approximately two hundred seeds were placed on a stainless steel mesh

(mesh size: 2.0 mm; Drahtweberei Pausa GmbH) fixed 22 cm above the bottom of a 52 L PE box, and covered with a lid (37×53×27 cm^3 top). The box was filled with deionized water up to 12 cm, submersing a heater (Eheim Jäger, 25 watts) and two air outlets (Hobby Long Long air outlets; 250 mm×50 mm) connected to an aquarium pump (TetraTec APS 400), thus maintaining 100% relative humidity in the box above the water. The heater was adjusted to 25 °C until the shoots emerged, and the whole system was kept in the dark. As soon as roots started to grow through the mesh (day 3), mucilage drops were collected by vacuum suction from the primary and seminal root tips (Fig. 1a). Mucilage was collected daily until the roots reached the water surface (day 7), immediately shock-frozen and stored at −20 °C. Mucilage from all collection days (d3-d7) was pooled.

Brace root mucilage collection system (CS_B: pot and field)

Mucilage of brace roots was collected from plants grown in a quartz sand mixed with 16.7% haplic phaeozem loam, either in 5 L pots in a temperature-controlled greenhouse ($CS_{B\text{-}pot}$) or in the field ($CS_{B\text{-}field}$). In both pot and field experiment, the identical maize genotype B73 was grown in the identical soil type. The field experiment was conducted in 2019 at the research station Bad Lauchstädt (51°22′0"N, 11°49′60″E; experimental details are described in detail in Vetterlein et al. 2020). Mucilage was collected in the field according to the method described by Nazari et al. (2020). Briefly, brace roots (not touching the soil yet) were cut shortly after full tassel emergence (approximately 2 weeks after reaching BBCH 59) and immediately immersed in water for 12 h. The water was then drained using a 0.2 mm sieve (Atechnik GmbH, Leinburg, Germany), and rehydrated mucilage was collected using a syringe and fine tweezers, shock-frozen and stored at −20 °C. Because the amount of mucilage that could be collected from each plant by this method was somewhat lower than expected, the mucilage from $CS_{B\text{-}field}$ was sufficient only for analysis of neutral sugars and viscosity. Since the time window for mucilage sampling in the field is restricted to only a few days, when brace roots are in the correct developmental stage, it was not possible to collect additional mucilage from the field. Thus, to conduct more physico-chemical analyses, the field experiment was complemented by a second batch of mucilage collected in a pot trial ($CS_{B\text{-}pot}$).

The pot experiment was conducted at the University of Hohenheim (48°42′39.2"N, 9°11′53.0″E). Throughout the experiment, plants were fertilized (Supplementary Table S1) and watered as required without causing water logging in the soil. This was achieved by adding water to the pot saucer in a daily amount that was fully taken up by the plant. Mucilage was non-destructively collected from brace roots, which had not yet touched the soil (according to Ahmed et al. 2015), until the beginning of flowering (1-2 weeks after BBCH 59). Before mucilage collection, roots were immersed overnight in ultrapure water in a 2 mL Eppendorf-style reaction tube. The tube was carefully removed in the morning, and the rehydrated mucilage was collected from the root using a pipet (Fig. 1b). Roots were not cut for mucilage sampling, which allowed us to sample different brace roots from

Fig. 1 Mucilage collection systems. The arrows indicate mucilage drops at the root tip. **a** Semi sterile aeroponic collection system for primary and seminal root mucilage (CS_A). Roots are growing through a stainless steel mesh. **b** Brace root mucilage collection system in the pot experiment ($CS_{B\text{-}pot}$). Mucilage was rehydrated on the root tip over night

the same plant over a period of several days. Brace roots from maize plants do not develop at exactly the same time, and the amount of collected mucilage was significantly increased by sampling roots at a similar stage of development over several days. However, to maintain maximum comparability with the field mucilage, each individual root was collected only once. The mucilage was subsequently frozen and stored at −20 °C. The mucilage from $CS_{B\text{-}pot}$ was used for the determination of pH, surface tension, contact angle, nutrient concentration and size of polymers. Viscosity was additionally analyzed to assess comparability between $CS_{B\text{-}pot}$ and $CS_{B\text{-}field}$.

Mucilage analysis

Mucilage preparation and analysis of pH

Collected CS_A and $CS_{B\text{-}pot}$ mucilage was defrosted, and the pH was measured at room temperature using a pH microelectrode (phenomenal MIC 220; 662-1163; VWR, Germany). Measurements were conducted individually per box (CS_A: three boxes) and per pot ($CS_{B\text{-}pot}$: ten pots), and each pH measurement was repeated two times. Subsequently, mucilage from all boxes / pots was pooled, filtered (100 µm stainless steel; Retch GmbH, Germany), freeze-dried (Christ, Alpha 1-2 LDplus, Osterode, Germany), and weighed. Dried mucilage was re-dissolved in ultrapure water at a concentration of 3 mg mL^{-1} by overhead mixing (48 hrs at 4 °C) for surface tension, viscosity, contact angle, nutrient and size exclusion chromatography measurements.

Surface tension, viscosity and contact angle

The pendent drop method was used to determine the surface tension of CS_A and $CS_{B\text{-}pot}$ mucilage. Briefly, the volume of a liquid drop of redissolved mucilage hanging from the needle (Sterican® 18G / 1,2×40 mm, B. Braun Melsungen AG, Melsungen, Germany) of a disposable 1 mL syringe (Omnifix®-F, B. Braun Melsungen AG, Melsungen, Germany) at 19 °C was increased by 0.01 µL sec^{-1} until the drops fell from the needle, and the pendant drop form was captured by a video-based optical contact angle device (OCA15Pro, DataPhysics, Filderstadt, Germany). The video was then exported as AVI files which were evaluated for the surface tension of each frame by the pendent drop plug-in (Daerr and Mogne 2016) of the ImageJ software (Schneider et al. 2012). The needle diameter was utilized to scale the pixel mm^{-1}. Surface tensions resulting from the last 10 frames that revealed a root mean square fitting distance <0.01 mm before the drop fell were averaged as result. The measurement was repeated for at least five pendant drops per mucilage type.

Viscosity of CS_A, $CS_{B\text{-}pot}$ and $CS_{B\text{-}field}$ mucilage was assessed by flow measurements using an MCR 102 rheometer (Anthon Paar, Ostfildern, Germany) with a truncated cone and plate geometry (CP50-1, d = 50 mm; angle of 1°) at 20 °C. The gap was 0.01 mm for 800 µL sample volume. Viscosity of the redissolved mucilage was measured at a shear rate between 0.001 s^{-1} and 10,000 s^{-1}. Samples were measured in triplicates.

Contact angles of CS_A and $CS_{B\text{-}pot}$ were measured by the sessile drop method with a video-based optical contact angle measuring device (OCA15Pro, DataPhysics, Filderstadt, Germany). Glass slides were first cleaned consecutively in an ultrasonic bath (10 min) with acetone, ethanol, and distilled water. Redissolved mucilage was diluted to a concentration of 1 mg mL^{-1}, and 0.138 mL cm^{-2} were evenly distributed on the glass slides to reach an average mucilage cover of 0.138 mg cm^{-2}. After drying the slides at room temperature in an exsiccator for four days, 3 µL of ultrapure water (18.2 MΩ•cm) were dropped on the dried mucilage. By the SCA20 software (DataPhysics Filderstadt, Germany), shape variation of the water drop and thus the contact angle over drop age was recorded for ~3 minutes with 18 frames per second. For each sample, ten to nineteen replicate drops were measured.

Nutrient concentrations (Ca, K, Mg, Na)

The concentrations of calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) were determined in CS_A and $CS_{B\text{-}pot}$ by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720 Series, Germany). Weighed samples of approximately 1 mL of re-dissolved mucilage were digested in 2 mL of *aqua regia* (a 3:1 mixture of 32% HCl and 65% HNO_3, Carl Roth GmbH, Germany) in a microwave (MarsX-press, CEM GmbH, Germany) using a heating ramp of 15 min followed by constant heating at 200 °C for 40 min. Digests were diluted in 8 mL ultrapure water

and results were expressed on a mucilage dry mass basis. All samples were analyzed in triplicate.

Size exclusion chromatography

Redissolved CS_A and $CS_{B\text{-}pot}$ mucilage was further diluted in ultrapure water to a final concentration of 2.5 mg mL^{-1} and then filtered through a 0.45 µm filter. The polymer size distribution of the samples was measured using LC-ELSD (liquid chromatography coupled with an evaporative light scattering detector) equipped with a guard column (50 ×8 mm, particle size 10 µm, PSS Suprema) and two columns for gel permeation chromatography (300 ×8 mm, particle size 10 µm, PSS Suprema). A sample volume of 80 µL was injected at room temperature at a constant flow of 1 mL min^{-1} of ammonium formate (50 mM) with 60 min measurement time and detected with an ELSD (70 °C, Gain 1, filter 1 s). Dextran standards (from PSS: 80.9 kDa, 312 kDa, 490 kDa; from Sigma: 147.6 kDa, 409.8 kDa, 1.5 mDa) were used to calculate sample molecular sizes. All samples were analyzed in triplicate.

Neutral sugars

Before neutral sugar analysis, the freeze-dried mucilage was homogenized in an agate mortar. Approximately 1 mg of freeze-dried $CS_{B\text{-}field}$ mucilage was weighed into flasks. The analysis was performed according to Banfield et al. (2018) with minor adjustments. Each sample was hydrolyzed with 10 mL of 4 M trifluoroacetic acid (TFA) at 105 °C for four hours. After cooling to room temperature, an internal standard (Allose (D +)) was added to the hydrolysate, which was then filtered with 5 mL ultrapure water through a glass fibre filter (GF6, Whatman GmbH, GE Healthcare, Freiburg, Germany). Samples were subsequently dried in a rotary evaporator (40 °C; 30 mbar). A volume of 0.5 mL ultrapure water was added and evaporated to ensure the complete removal of TFA (two times). Subsequently, samples were resolved in ultrapure water, sonicated for 10 min, mixed well and then transferred to a reaction vessel, dried under pure nitrogen gas and then stored at −20 °C.

Derivatisation to aldononitrile acetates was also performed according to Banfield et al. (2018). A targeted analysis was conducted for the neutral sugars arabinose (D -), fucose (L -), galactose (D +), mannose (D +), rhamnose (L +), ribose (D -), and xylose (D +). Neutral sugars were separated by gas chromatography (Agilent 7820A GC, Agilent Technologies, Waldbronn, Germany) and detected by mass spectrometry (Agilent 5977B, Agilent Waldbronn, Germany). Integration and quantification were performed with the Agilent Mass Hunter Quantitative Data Analysis software (Agilent Technologies, Waldbronn, Germany). Quantification of each peak was performed by linear regression with external standards, which ensured identity and comparable characteristic fragments of each peak. A first internal standard (Allose (D +)) allowed recovery correction, while peak areas were normalized using a second internal standard (methyl tridecanoate). The samples were analyzed in quadruplicates.

Data management and statistical analysis

Calculation of means, standard errors and data analysis were performed with JMP Pro 15 by SAS, using the LS mean model for a one-factor (CS_A and CS_B) analysis. Determination of pH was conducted with ten (pots), and three (aeroponic boxes) replicates. Data were tested for normal distribution by visual inspection of the residual plots and Levene's test. A one-way analysis of variance (ANOVA) was performed at a significance level (α) of 0.05 to test significant differences between the means. Tukey's HSD (Honestly Significant Differences) test was used for the pair-wise comparison of the arithmetic means. Subsequent measurements with the dried mucilage were performed with pooled mucilage in technical replicates as indicated in the methods, and variability is indicated as error bars representing standard deviations in the figures.

Results

Surface tension, contact angle and viscosity

The surface tension of $CS_{B\text{-}pot}$ mucilage was higher compared to that of CS_A, reaching mean values of 74.9 mN m^{-1} ($CS_{B\text{-}pot}$) and 68.7 mN m^{-1} (CS_A), respectively (Fig. 2a). In addition, the sessile water drop contact angle (CA_{sess}) at a drop age of 5 sec was 96.4° for $CS_{B\text{-}pot}$ and 67.9° for CS_A (Fig. 2b),

indicating a higher wettability of dried CS_A mucilage. Similar differences in contact angle were also observed for all other measured time points (Supplementary Fig. S1).

Viscosity of CS_A, $CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$ mucilages decreased with increasing shear rate (Fig. 3), which is classified as shear thinning behaviour. Thereby, polymers are aligned along the shear direction with increasing shear rate, which reduces viscosity. Viscosity of both CS_B batches was always higher than that of CS_A for all applied shear rates (Fig. 3). Despite a significantly higher viscosity of $CS_{B\text{-pot}}$ compared to $CS_{B\text{-field}}$ at the lower shear rates (Fig. 3), the shape of the viscosity flow curves of $CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$ were overall relatively similar with a slow reduction in viscosity at low to medium, followed by a steeper decline at higher shear rates. This curve shape is very different from that of CS_A, which shows a steep decline in viscosity even at the lowest shear rates (Fig. 3).

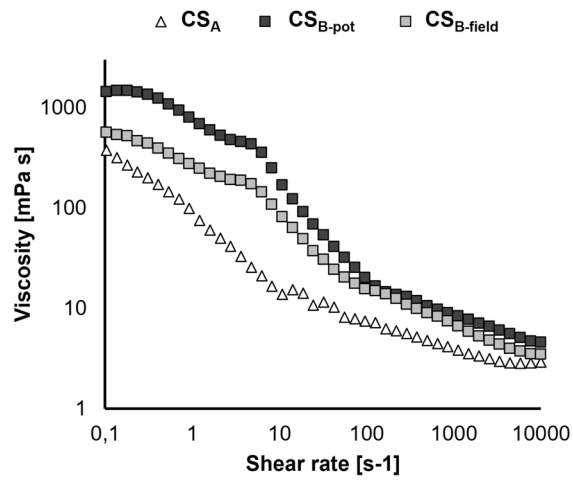


Fig. 3 Viscosity flow curve of aeroponically produced seedling root (CS_A) and brace root mucilage ($CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$) of maize (*Zea mays* L.)

Nutrient (K, Ca, Mg, Na) concentrations and pH values

The concentrations of all measured nutrients were higher in CS_A compared to $CS_{B\text{-pot}}$ (Fig. 4a). The most abundant of the measured nutrients in both mucilage types was consistently K with 127 (CS_A) and 95 ($CS_{B\text{-pot}}$) µmol g^{-1} dry mucilage, followed by Ca with 105 (CS_A) and 47 ($CS_{B\text{-pot}}$), Mg with 29 (CS_A) and 15 ($CS_{B\text{-pot}}$) and Na with 62 (CS_A) and 2 ($CS_{B\text{-pot}}$) µmol g^{-1} dry mucilage. Interestingly, Na was more abundant than Mg in CS_A, while the opposite was observed in $CS_{B\text{-pot}}$ (Fig. 4a). The pH value of CS_A mucilage was higher by half a unit (6.5) compared to $CS_{B\text{-pot}}$ (6.1) (Fig. 4b).

Size exclusion chromatography

The retention time of the smallest dextran standard with a mass of 80 kDa was 19 min, whereas that of the dextran standard with the highest mass of 1500 kDa was 16 min. For mucilage, three peaks at 13 min, 24 min and 51 min were observed for CS_A, and two peaks at 13 min and 51 min for $CS_{B\text{-pot}}$. Even though the calibration covered a broad range of molar masses, no peak

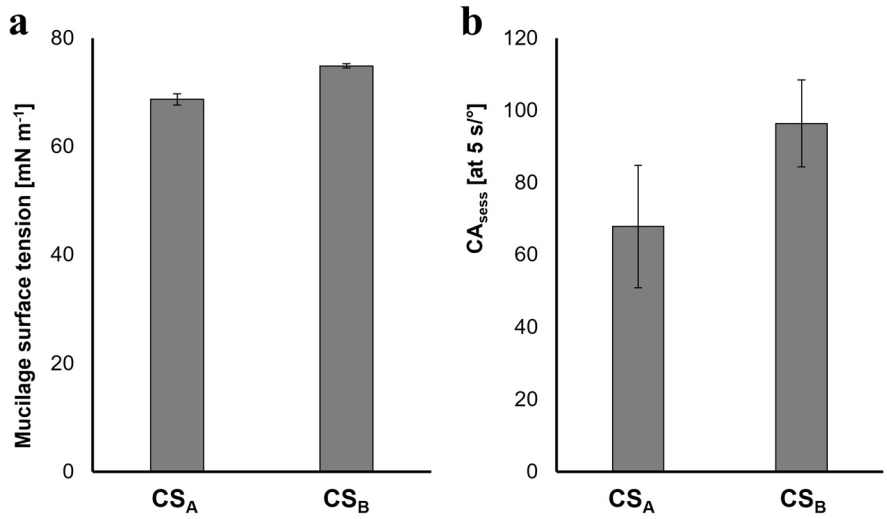


Fig. 2 Surface tension (**a**) and sessile water drop contact angle (CA_{sess}) at a drop age of 5 s on dried mucilage (**b**) of aeroponically produced seedling root (CS_A) and brace root ($CS_{B\text{-pot}}$) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of five (**a**) and ten to nineteen (**b**) technical replicates

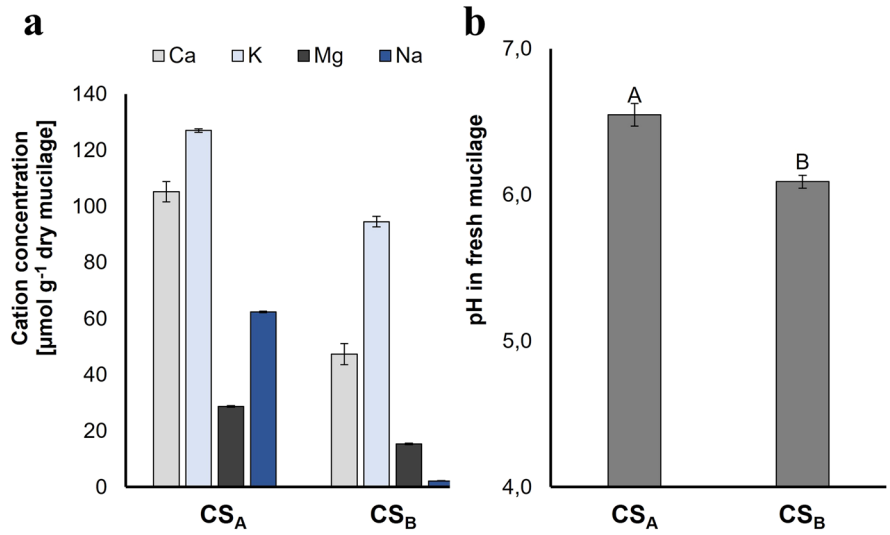


Fig. 4 Concentrations of Ca, K, Mg and Na (**a**) and pH values (**b**) in aeroponically produced seedling root (CS_A) and brace root ($CS_{B\text{-}pot}$) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of three technical replicates (**a**) and standard errors of three (CS_A) and ten ($CS_{B\text{-}pot}$) biological replicates

was in the calibration range. This suggests the presence of very high molar mass compounds larger than 1500 kDa with a retention time of 13 min, and very small molecules with a retention time of 51 min in both mucilages, and additional compounds of intermediate mass smaller than 80 kDa with a retention time of 24 min only in CS_A. The different relative peak areas indicate a higher amount of the very high molecular mass but also of very low molecular weight compounds in $CS_{B\text{-}pot}$ compared to CS_A (Fig. 5).

Neutral sugar composition and pH

The total content of neutral sugars was more than 1.5 times higher in $CS_{B\text{-}field}$ compared to CS_A (Fig. 6a). Galactose was always the most abundant sugar with 43% in $CS_{B\text{-}field}$ and 34% in CS_A, followed by fucose with 21% for both mucilages, arabinose with 13% and 18%, xylose with 11% and 17%, and glucose with 6% and 11% for $CS_{B\text{-}field}$ and CS_A, respectively. Mannose was only detectable in $CS_{B\text{-}field}$ with 7%. While hexoses were more prominent in $CS_{B\text{-}field}$ (76%) than in CS_A (65%) mainly due to a very large ratio of galactose and the presence of mannose in $CS_{B\text{-}field}$, pentoses were higher in CS_A (35%) compared to $CS_{B\text{-}field}$ (24%), and this was observed for both identified pentoses arabinose and xylose (Fig. 6b).

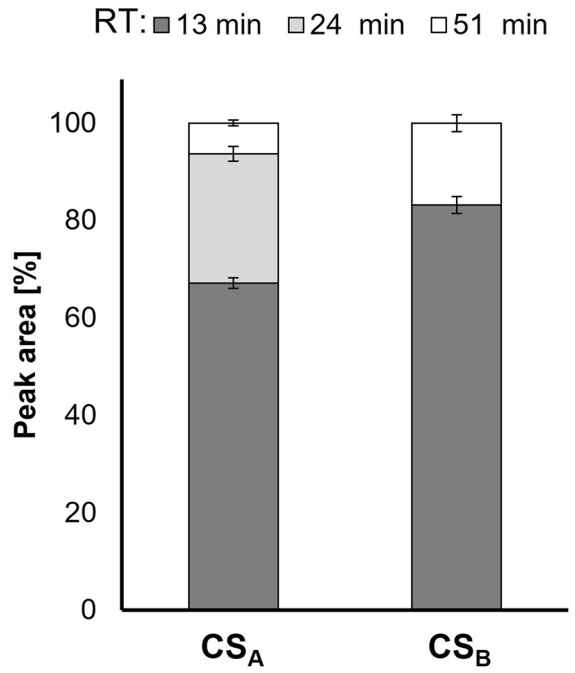


Fig. 5 Relative peak areas corresponding to the retention times (RT) measured of aeroponically produced seedling root (CS_A) and brace root ($CS_{B\text{-}pot}$) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of three technical replicates

Discussion

Physico-chemical properties differ in maize mucilage from two collection systems

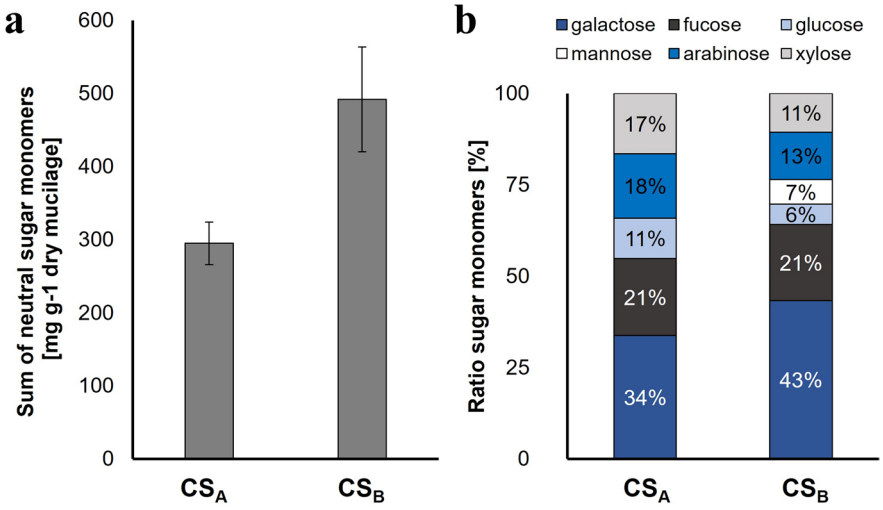


Fig. 6 Total neutral sugar content (**a**) and composition of neutral sugar monomers (**b**) of aeroponically produced seedling root (CS_A) and brace root ($CS_{B\text{-field}}$) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of four technical replicates

Distinct and consistent differences were observed between all measured physico-chemical properties of CS_A and CS_B mucilage.

Both CS_B batches ($CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$) had higher viscosities compared to CS_A (Fig. 3). Despite differences in absolute values of $CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$, the viscosity flow curve shapes of both were similar and significantly different from that of CS_A, suggesting different mechanisms of shear resistance, i.e. against the disentanglement of polymers, between CS_A and CS_B mucilages. In other words, the intermolecular interactions were more easily overcome at lower shear rates in CS_A, while those in CS_B resisted until higher shear rates were applied. For $CS_{B\text{-pot}}$, this is in line with the larger polymer sizes identified by SEC analysis (Fig. 5), as larger polymers increase the strength of the interaction between the polymers and thus the viscosity of a fluid (Mezger 2020). Another factor that could influence mucilage viscosity is its content of divalent cations, especially Ca^{2+}, even though this effect is complex and not yet fully understood (Brax et al. 2020). For example, Ca^{2+} concentrations can either increase or decrease mucilage viscosity, depending on the plant species, and probably other mucilage type specific factors (Brax et al. 2020). On the one hand, Ca^{2+} can form intermolecular associations with non-esterified uronic acids, which would increase friction between the molecules and thus viscosity. On the other hand, Ca^{2+} can contribute to a collapse of the polymer network by reducing the repulsion between negative charges, with the consequence of a reduced molecule expansion and inter-molecular friction, and lower viscosity (Brax et al. 2020; Medina-Torres et al. 2000). This effect is described for different cations, but is strongest for Ca^{2+}, followed by Mg^{2+}, K^+ and Na^+ (Medina-Torres et al. 2000). The higher cation concentrations in CS_A compared to $CS_{B\text{-pot}}$ (Fig. 4a) would thus be in line with the lower viscosity of CS_A mucilage. Together, these results indicate that CS_A contained a lower amount of high molar mass compounds, which in combination with a higher concentration of cations might have contributed to a less viscous behaviour compared to $CS_{B\text{-pot}}$.

A higher viscosity was also observed for $CS_{B\text{-field}}$ compared to CS_A (Fig. 3). Here, a significantly higher content of total sugar monomers (Fig. 6a) and especially of galactose was determined in $CS_{B\text{-field}}$. Galactose makes up the backbone of the heterogeneous mucilage polysaccharides present in maize CS_B mucilage (Amicucci et al. 2019), likely indicating a higher content or a larger size of polysaccharides in $CS_{B\text{-field}}$. This would be fully in line with the larger polymer sizes identified in $CS_{B\text{-pot}}$ (Fig. 5). We would like to point out that $CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$ showed differences in absolute values of viscosities, indicating that likely the chemical composition was not identical. This might possibly be related to the slight sampling differences (destructive vs. non-destructive sampling as described in the Materials and Methods section above). A direct correlation of sugar analysis from $CS_{B\text{-field}}$ and SEC or other measurements from $CS_{B\text{-pot}}$ is thus not possible, and interpretation should be done carefully. However, the results clearly suggest that

differences between CS_A and CS_B are significantly larger than those between the two batches of CS_B (e.g. for viscosity flow curve), and a higher content of larger polysaccharides in CS_B compared to CS_A is suggested independently by different measurements (e.g. sugar analysis and SEC analysis) for both CS_B batches.

To our knowledge, no sugar analysis is yet available for seedling root mucilage collected in an aeroponic system, but the sugar composition of CS_A in our study was reasonably similar to the one determined for seedling root slime (collected from maize seedlings grown under sterile conditions on filter paper, after overnight incubation of root tips in water) with 31% galactose, 19% fucose, 18% glucose, 15% xylose and 13% arabinose (Chaboud 1983). The higher ratio of glucose could be related to the fact that "root slime" also contains low molecular weight root exudates in addition to the mucilage. The abundances of galactose, fucose, arabinose, xylose and mannose in our $CS_{B\text{-field}}$ mucilage (Fig. 6b) are comparable to values reported for brace root mucilage (Amicucci et al. 2019; Nazari et al. 2020), even though some minor differences can be observed. These might be explained either by methodological differences in the polysaccharide analysis, or by genotypic differences in mucilage composition, previously described by Nazari et al. (2020) for brace root mucilage of different maize genotypes from contrasting climatic regions. In addition, the observed differences in viscosity between $CS_{B\text{-pot}}$ and $CS_{B\text{-field}}$ further indicate that minor differences in physico-chemical composition are to be expected even for the same genotypes grown under different environmental conditions.

The molecular size distribution (Fig. 5) suggests the presence of more high molecular weight polymers, but also of very low molecular weight substances in $CS_{B\text{-pot}}$ compared to CS_A. Together with the higher total sugar concentration in $CS_{B\text{-field}}$ (Fig. 6a), this could be an indication of a higher amount of free sugar monomers in CS_B, which would be in line with a higher surface tension (Fig. 2a), given that the latter is thought to be moderately increased by sugar monomers (Shaw 1980, cited in Read et al. 2003). However, it cannot be excluded that the difference in surface tension might be related to higher levels of phospholipids or other lipidic surfactants (not determined in the present study) in CS_A, which have been previously reported to be present in mucilage (Read et al. 2003) and are suggested to reduce surface tension (Naveed et al. 2019; Read and Gregory 1997). It is not yet clear, whether the lipid contents in mucilage are sufficiently high to have a measurable effect on surface tension, and a combined effect of sugar and lipid concentrations might be more feasible.

Collectively, the results indicate that mucilages collected by two different methods from the same maize genotype exhibit distinct physico-chemical differences, but they also suggest that environmental factors may additionally alter mucilage composition collected by the same method.

Differences in physico-chemical properties are explainable by root type and collection system specifics

Even though mucilages collected by different methods deploy some commonalities, such as a high viscosity (higher than water) and the presence of high molecular mass polysaccharides with a galactose-dominated sugar composition (Carminati and Vetterlein 2013), it is striking that reported physico-chemical properties of mucilages even from the same species are rarely identical, and significant differences have been observed between species (Zickenrott et al. 2016) and between genotypes (Nazari et al. 2020). Near to nothing is known about the effect of root type, plant age and micro-climatic conditions on mucilage composition, even though these differ significantly between collection methods. For example, it is not possible to collect mucilage from mature plants using an aeroponic sytem comparable to the systems used for sampling of young seedlings. It is thus hardly possible to distinguish between the impact of each of these effects, even though each can be expected to alter physico-chemical properties. In order to develop better models of root-soil interactions, needed e.g. for the prediction of climate change scenarios, it seems indispensible to gain a better understanding of relevant factors that need to be taken into account. As a first step, we compared properties of two mucilage types collected by two different methods from the same maize genotype, and found distinct differences in all measured physico-chemical properties. These differences might be related to the combination of different physiological functions of the roots providing the mucilages, and the conditions in which they develop.

Brace roots often reach the ground and push through the soil surface (van Deynze et al. 2018), fulfilling two main functions. Firstly, they are relevant for plant lodging resistance by providing mechanical stability for the large maize plants (Hetz et al. 1996), which was shown to significantly improve grain yield during limited water availability and flooding (Hochholdinger and Tuberosa 2009). Secondly, they significantly participate in the water uptake of mature maize (Ahmed et al. 2018c; van Deynze et al. 2018). For both functions, brace roots need to rapidly establish good contact with the soil once they enter the ground. However, brace roots first develop in mid-air, i.e. in an environment exposed to dry conditions during the day and more humid conditions during the night. Thus, brace root mucilage experiences frequent wetting/drying cycles. This is reflected in the fact that brace roots need to be rehydrated for several hours before mucilage can be collected (Ahmed et al. 2015), unless the collection occurs in the early morning when dew is forming, or after a rain event. Upon drying, however, mucilage changes its physicochemical properties and becomes more water repellent (Ahmed et al. 2015, 2016a). In addition, brace roots enter the soil from the top, i.e. they first have to pass through a soil layer which is typically relatively dry and subject to repeated wetting and drying events. A higher viscosity might be an adaptation to these conditions and improve water uptake in this frequently drying environment, since it correlates with a high water holding capacity and with the maintenance of the root-soil connection especially during drying, when it leads to the formation of thin filaments spanning through the soil, and to a continuous propagation of the liquid phase across soil pores (Benard et al. 2019; Carminati et al. 2017). On the other hand, mucilage from the aeroponic system is collected from very young roots, which grow in 100% relative humidity and did not yet face dry conditions. The same is usually true also for soil-grown seedlings of this age (3 d), since maize germination only occurs when a sufficiently high humidity is reached in the soil. Since more metabolic investment is needed for the production of a more viscous mucilage, young roots probably can save energy and carbon investment (e.g. for root and shoot growth) instead of producing a viscous mucilage, because a better capacity to hold water due to higher viscosity would not be an advantage at this point. Instead, an intermediate viscosity of the mucilage would ensure good enough lubrication between soil particles and, thus, allow for rapid growth through the soil. However, we cannot rule out that mucilage from young seedlings grown in soil might show a higher viscosity, especially if dry soil conditions occur. It would thus be relevant to find ways to collect and analyze mucilage from very young but soil-grown seedlings, and to consider this possible difference between aeroponic and soil-grown seedlings when extrapolating results from aeroponic systems e.g. in models of mucilage functions in soils.

Another function of brace root mucilage might be to hold water after a rain event or to capture dew during the night in mucilage drops at the brace root tips, and this would be improved by more and larger polysaccharides forming a stronger mucilage gel with higher viscosity. The higher surface tension of CS_B mucilage might additionally help to reduce the spreading of the CS_B mucilage on the brace root surface, which would reduce the evaporation of the water and thus increase water availability compared to mucilage with a lower surface tension.

Despite an overall similar pattern of sugar composition, differing ratios of monosaccharides between the two mucilage types might also be related to the growth environment of the root. One major difference in our study was the lower content of glucose units in the polysaccharides in $CS_{B\text{-field}}$, and the lack of mannose in CS_A. These results are in line with previous studies reporting glucose to be a minor component of brace root mucilage (Amicucci et al. 2019), while it was highly abundant in root mucilage of three-day-old maize seedlings grown on filter paper (Osborn et al. 1999). Mannose levels similar to our study were also reported for brace root mucilage by Amicucci et al. (2019) and Nazari et al. (2020), while Osborn et al. (1999) did not identify mannose in root mucilage of filter paper-grown maize seedlings. Mannose is not a common sugar in plants and usually only occurs in connection with degradation of storage and reserve polymers, or sometimes of glycoproteins and glycolipids (Herold and Lewis 1977). Since it is usually very rapidly metabolized, its presence in $CS_{B\text{-field}}$ mucilage might indicate a higher rate of cell wall degradation during mucilage production of brace roots (Herold and Lewis 1977). It is noteworthy that the pH of the $CS_{B\text{-pot}}$ mucilage was lower than that of CS_A (Fig. 4b). Mannose and glucuronic acid are reported main constituents of an acidic component of maize

brace root mucilage (Amicucci et al. 2019). The lack of mannose in CS_A would thus be in line with the observed higher pH value of this mucilage type, even though uronic acids were not determined in the present study.

The differing amounts of hexoses and pentoses as well as the lack of mannose in CS_A could also be related to the amount of microorganisms (MO) present, which is another important disparity between both mucilage collection systems used. While the semi-sterile aeroponic system likely represents a lower microbial colonization, brace root mucilage produced in a soil system probably contains diverse bacterial consortia (Estrada et al. 2002; van Deynze et al. 2018). Colonization of roots with MO could have a major effect on mucilage sugar composition, since MO not only consume sugars, but they also mainly synthesize the hexoses galactose, mannose and fucose, and only in minor amounts the pentoses arabinose and xylose (Spielvogel et al. 2016). Pentoses, on the other hand, are mainly produced by plants (Kögel-Knabner 2002). In addition, enzymes for mannose metabolism are frequently observed in bacteria and fungi, but are not common in higher plants (Herold and Lewis 1977). Overall, the higher ratio of pentoses in CS_A and of hexoses and mannose in $CS_{B\text{-field}}$ would agree with a higher microbial colonization of brace roots compared to aeroponically grown seedlings.

The developmental stage of the plant might also affect mucilage properties and especially mucilage nutrient concentrations. Overall, more nutrients (K, Ca, Mg, Na) were present in CS_A compared to $CS_{B\text{-pot}}$ (Fig. 4a). CS_A mucilage is produced from very young plant roots that still fully rely on the nutrient supply from the seed, since a green shoot was not yet developed and roots had not yet touched soil or nutrient solution. At this stage, nutrients are translocated from the seed towards the main growing organ, the root, which needs to be well supplied because of its rapid growth. Since mucilage from primary and lateral roots contains root cap cells and their content (Carminati and Vetterlein 2013), a relatively high nutrient concentration in CS_A mucilage might be expected. CS_B mucilage, on the other hand, is produced from roots of plants with an actively growing shoot, representing a strong sink for available nutrients. The main direction of nutrient movement is acropetally, i.e. from root to shoot, and nutrients provided to brace roots need to be "re-directed" from the main xylem stream into the brace roots. This could be limited by ion uptake systems (transporters, channels), and to our knowledge it is not known, to which extent ion supply to brace roots occurs, before these roots get in contact with the soil. Overall, it seems feasible that nutrient concentrations of CS_B mucilage are lower, at least until the soil contact is established.

The developmental stage also affects the plant photosynthetic activity during mucilage production, and the corresponding allocation of fixed carbon into rhizodeposits (Jones et al. 2009). As a rough estimate, approximately 11% of the total photosynthetic output is deposited in the rhizosphere (Jones et al. 2009), representing a significant carbon expense for the plant. The absence of light and lack of assimilates seems to directly affect root exudation, as indicated by a 3.3% reduction in root-derived C in the rhizosphere of shaded as compared to sun-exposed wheat plants (Kuzyakov and Cheng 2001). During the collection of CS_A mucilage, maize seedlings were kept in the dark and were only exposed very briefly to dim artificial light during the collection process. Therefore, the photosynthetic activity of these plants was probably low. On the other hand, maize plants used for CS_B collection were exposed to a typical day and night light rhythm allowing for regular photosynthetic activity. These differing assimilation rates might be one further explanation for the lower neutral sugar concentration in CS_A mucilage.

Summary / Conclusions

Collectively, all results indicate that despite some general consistent patterns, CS_A and CS_B mucilage types differ in their physico-chemical properties. Most of these differences can at least in part be related to the complex interplay between the different environmental conditions in which the roots grow (e.g. humidity and/or microbial colonization), and the different physiological and developmental state of the plants (e.g. photosynthetically active vs. dark grown; very young seedlings vs. older plants). The distinct pattern of carbohydrate fractions points out the importance of microbial colonization of roots with respect to mucilage composition. In addition, the stronger than expected modifying influence of environmental factors on mucilage properties even from a single genotype

might play additional roles e.g. in the attraction and shaping of corresponding microbiomes, and downstream rhizosphere processes. The higher viscosity of CS_B mucilage seems related to frequent wetting/drying cycles of the mucilage during the growth of brace roots first in air and then in drier soil layers, and might reflect the need for enhanced water holding capacity of brace roots. Whether the observed differences in mucilage properties will also affect plant-soil interactions in the rhizosphere, e.g. wettability of and water movement towards roots during conditions of repeated drought spells, still needs to be assessed. We conclude that it is important to pay more attention to these aspects when developing spatial and temporal models of rhizosphere processes and hydraulic patterns, as well as for discussion of mucilage function and behaviour in soils. It seems that a more systematic investigation of the impact of the environment in combination with management practices (e.g. drought, nutrient supply, sunlight etc.) would only be feasible with mucilage collected form brace roots of field-grown plants. However, this would remain limited to plants at later growth stages rather than young seedlings.

Acknowledgements This work was conducted within the framework of the priority program 2089, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation). Seeds of the maize genotype B73 were kindly provided by Caroline Macron and Frank Hochholdinger (University of Bonn). LMW and MAW gratefully acknowledge excellent technical support by Dagmar Repper and Christiane Beierle, Reinhold Renner and his team for constructing the aeroponic system, and the staff members of the phytotechnikum of the University of Hohenheim for their support during the pot experiment.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [LM Werner], [M Ahmed], [M Knott], [D Diehl] and [C Banfield]. The first draft of the manuscript was written by [LM Werner] and [MA Wimmer] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was conducted within the framework of the priority program 2089, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Grant numbers 403626025 (M.A. Wimmer), 40381423 (D Vetterlein), 403670197 (M Ahmed), 403668613 (D Diehl).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Ahmed MA, Holz M, Woche SK, Bachmann J, Carminati A (2015) Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. J Plant Nutr Soil Sci 178:821–824. https://doi.org/10.1002/jpln.201500177

Ahmed MA, Kroener E, Benard P, Zarebanadkouki M, Kaestner A, Carminati A (2016a) Drying of mucilage causes water repellency in the rhizosphere of maize: measurements and modelling. Plant Soil 407:161–171. https://doi.org/10.1007/s11104-015-2749-1

Ahmed MA, Zarebanadkouki M, Kaestner A, Carminati A (2016b) Measurements of water uptake of maize roots: the key function of lateral roots. Plant Soil 398:59–77. https://doi.org/10.1007/s11104-015-2639-6

Ahmed MA, Banfield CC, Sanaullah M, Gunina A, Dippold MA (2018a) Utilisation of mucilage C by microbial communities under drought. Biol Fertil Soils 54:83–94. https://doi.org/10.1007/s00374-017-1237-6

Ahmed MA, Sanaullah M, Blagodatskaya E, Mason-Jones K, Jawad H, Kuzyakov Y, Dippold MA (2018b) Soil microorganisms exhibit enzymatic and priming response to root mucilage under drought. Soil Biol Biochem 116:410–418. https://doi.org/10.1016/j.soilbio.2017.10.041

Ahmed MA, Zarebanadkouki M, Meunier F, Javaux M, Kaestner A, Carminati A (2018c) Root type matters: measurement of water uptake by seminal, crown, and lateral roots in maize. J Exp Bot 69:1199–1206. https://doi.org/10.1093/jxb/erx439

Amicucci MJ, Galermo AG, Guerrero A, Treves G, Nandita E, Kailemia MJ, Higdon SM, Pozzo T, Labavitch JM, Bennett AB, Lebrilla CB (2019) Strategy for structural elucidation of polysaccharides: elucidation of a maize mucilage that harbors diazotrophic bacteria. Analytical Chem 91:7254–7265. https://doi.org/10.1021/acs.analchem.9b00789

Bacic A, Moody SF, Clarke AE (1986) Structural analysis of secreted root slime from maize (*Zea mays* L.). Plant Physiol 80:771–777

Banfield CC, Pausch J, Kuzyakov Y, Dippold MA (2018) Microbial processing of plant residues in the subsoil – the role of biopores. Soil Biol Biochem 125:309–318. https://doi.org/10.1016/j.soilbio.2018.08.004

Benard P, Zarebanadkouki M, Carminati A (2019) Physics and hydraulics of the rhizosphere network. J Plant Nutr Soil Sci 182:5–8. https://doi.org/10.1002/jpln.201800042

Brax M, Schaumann GE, Diehl D (2019) Gel formation mechanism and gel properties controlled by Ca^{2+} in chia seed mucilage and model substances. J Plant Nutr Soil Sci 182:92–103. https://doi.org/10.1002/jpln.201800430

Brax M, Buchmann C, Kenngott K, Schaumann GE, Diehl D (2020) Influence of the physico-chemical properties of root mucilage and model substances on the microstructural stability of sand. Biogeochemistry 147:35–52. https://doi.org/10.1007/s10533-019-00626-w

Carminati A, Vetterlein D (2013) Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Ann Bot 112:277–290. https://doi.org/10.1093/aob/mcs262

Carminati A, Benard P, Ahmed MA, Zarebanadkouki M (2017) Liquid bridges at the root-soil interface. Plant Soil 417:1–15. https://doi.org/10.1007/s11104-017-3227-8

Chaboud A (1983) Isolation, purification and chemical composition of maize root cap slime. Plant Soil 73:395–402

Daerr A, Mogne A (2016) Pendent_drop: An ImageJ plugin to measure the surface tension from an image of a pendent drop. J Open Res Software 4. https://doi.org/10.5334/jors.97

Estrada P, Mavingui P, Cournoyer B, Fontaine F, Balandreau J, Caballero-Mellado J (2002) A N_2-fixing endophytic Burkholderia sp. associated with maize plants cultivated in Mexico. Can J Microbiol 48:285–294. https://doi.org/10.1139/w02-023

Guinel FC, McCully ME (1986) Some water-related physical properties of maize root-cap mucilage. Plant Cell Environ 9:657–666

Hawes MC, Brigham LA, Wen F, Woo HH, Zhu Y (1998) Function of root border cells in plant health: pioneers in the rhizosphere. Annu Rev Phytopathol 36:311–327

Herold A, Lewis DH (1977) Mannose and green plants: occurence, physiology, and metabolism, and use as a tool to study the role of orthophosphate. New Phytol 79:1–40. https://doi.org/10.1111/j.1469-8137.1977.tb02178.x

Hetz W, Hochholdinger F, Schwall M, Feix G (1996) Isolation and characterization of rtcs, a maize mutant deficient in the formation of nodal roots. Plant J 10:845–857

Hochholdinger F, Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. Curr Opin Plant Biol 12:172–177. https://doi.org/10.1016/j.pbi.2008.12.002

Holz M, Leue M, Ahmed MA, Benard P, Gerke HH, Carminati A (2018) Spatial distribution of mucilage in the rhizosphere measured with infrared spectroscopy. Front Environ Sci 6:1. https://doi.org/10.3389/fenvs.2018.00087

Iijima M, Higuchi T, Barlow PW, Bengough AG (2003) Root cap removal increases root penetration resistance in maize (*Zea mays* L). J Exp Bot 54:2105–2109. https://doi.org/10.1093/jxb/erg226

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil 321:5–33

Kaltenbach R, Diehl D, Schaumann GE (2018) Links between nanoscale and macroscale surface properties of natural root mucilage studied by atomic force microscopy and contact angle. J Colloid Interf Sci 516:446–455. https://doi.org/10.1016/j.jcis.2018.01.079

Knee EM, Gong F-C, Gao M, Teplitski M, Jones AR, Foxworthy A, Mort AJ, Bauer WD (2007) Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. Mol Plant Microbe In 14:775–784

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162. https://doi.org/10.1016/S0038-0717(01)00158-4

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1925. https://doi.org/10.1016/S0038-0717(01)00117-1

McCulley ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. Physiol Plant 99:169–177

Medina-Torres L, Brito-De La Fuente E, Torrestiana-Sanchez B, Katthain R (2000) Rheological properties of the mucilage gum (Opuntia ficus indica). Food Hydrocoll 14:417–424. https://doi.org/10.1016/S0268-005X(00)00015-1

Meier U, Bleiholder H, Buhr L, Feller C, Hacks H, Hess M, Lancashire PD, Schnock U, Stauss R, Boom T v d, Weber E, Zwerger P (2009) The BBCH system to coding the phenological growth stages of plants - history and publications. J Kult 61:41–52

Mezger T (2020) The rheology handbook. For users of rotational and oscillatory rheometers, 5th edn. Vincentz Network, Hannover

Mikutta C, Neumann G, Lang F (2006) Phosphate desorption from goethite in the presence of galacturonate, polygalacturonate, and maize mucigel (*Zea mays* L.). Soil Sci Soc Am J 70(1731). https://doi.org/10.2136/sssaj2005.0414

Mimmo T, Marzadori C, Montecchio D, Gessa C (2005) Characterisation of Ca- and Al-pectate gels by thermal analysis and FT-IR spectroscopy. Carbohydr Res 340:2510–2519

Moradi AB, Carminati A, Lamparter A, Woche SK, Bachmann J, Vetterlein D, Vogel H-J, Oswald SE (2012) Is the rhizosphere temporarily water repellent? Vadose Zone J 11:vzj2011.0120. https://doi.org/10.2136/vzj2011.0120

Morel JL, Mench M, Guckert A (1986) Measurement of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates from maize (*Zea mays* L.) roots. Biol Fertil Soils 2:29–34

Naveed M, Brown LK, Raffan AC, George TS, Bengough AG, Roose T, Sinclair I, Koebernick N, Cooper L, Hackett CA, Hallett PD (2017) Plant exudates may stabilize or weaken soil depending on species, origin and time. Eur J Soil Sci 68:806–816. https://doi.org/10.1111/ejss.12487

Naveed M, Ahmed MA, Benard P, Brown LK, George TS, Bengough AG, Roose T, Koebernick N, Hallett PD (2019) Surface tension, rheology and hydrophobicity of rhizodeposits and seed mucilage influence soil water retention and hysteresis. Plant Soil 437:65–81. https://doi.org/10.1007/s11104-019-03939-9

Nazari M, Riebeling S, Banfield CC, Akale A, Crosta M, Mason-Jones K, Dippold MA, Ahmed MA (2020) Mucilage polysaccharide composition and exudation in maize from contrasting climatic regions. Front Plant Sci 11:587610. https://doi.org/10.3389/fpls.2020.587610

Oburger E, Jones DL (2018) Sampling root exudates – Mission impossible? Rhizosphere 6:116–133. https://doi.org/10.1016/j.rhisph.2018.06.004

Osborn HM, Lochey F, Mosley L, Read D (1999) Analysis of polysaccharides and monosaccharides in the root mucilage of maize (*Zea mays* L.) by gas chromatography. J Chromatography A 831:267–276. https://doi.org/10.1016/S0021-9673(98)00935-2

Read DB, Gregory PJ (1997) Surface tension and viscosity of axenic maize and lupin root mucilages. New Phytol 137:623–628. https://doi.org/10.1046/j.1469-8137.1997.00859.x

Read DB, Bengough AG, Gregory PJ, Crawford JW, Robinson D, Scrimgeour CM, Young IM, Zhang K, Zhang X (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytol 157:315–326. https://doi.org/10.1046/j.1469-8137.2003.00665.x

Rosskopf U, Uteau D, Peth S (2021) Effects of mucilage concentration at different water contents on mechanical stability and elasticity in a loamy and a sandy soil. Eur J Soil Sci 1-14. https://doi.org/10.1111/ejss.13189

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Shaw DJ (1980) Introduction to colloid and surface chemistry, 3rd edn. Butterworth-Heinemann, Oxford

Spielvogel S, Prietzel J, Kögel-Knabner I (2016) Stand scale variability of topsoil organic matter composition in a high-elevation Norway spruce forest ecosystem. Geoderma 267:112–122. https://doi.org/10.1016/j.geoderma.2015.12.001

van Deynze A, Zamora P, Delaux P-M, Heitmann C, Jayaraman D, Rajasekar S, Graham D, Maeda J, Gibson D, Schwartz KD, Berry AM, Bhatnagar S, Jospin G, Darling A, Jeannotte R, Lopez J, Weimer BC, Eisen JA, Shapiro H-Y et al (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLoS Biol 16:e2006352. https://doi.org/10.1371/journal.pbio.2006352

Vetterlein D, Lippold E, Schreiter S, Phalempin M, Fahrenkampf T, Hochholdinger F, Marcon C, Tarkka M, Oburger E, Ahmed M, Javaux M, Schlüter S (2020) Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere-laboratory and field scale. J Plant Nutr Soil Sci 35:121. https://doi.org/10.1002/jpln.202000079

Watanabe T, Misawa S, Hiradate S, Osaki M (2008) Characterization of root mucilage from Melastoma malabathricum, with emphasis on its roles in aluminum accumulation. New Phytol 178:581–589. https://doi.org/10.1111/j.1469-8137.2008.02397.x

Watt M, McCully ME, Canny MJ (1994) Formation and stabilization of rhizosheaths of *Zea mays* L. - effect of soil water content. Plant Physiol 106:179–186. https://doi.org/10.1104/pp.106.1.179

Zickenrott I-M, Woche SK, Bachmann J, Ahmed MA, Vetterlein D (2016) An efficient method for the collection of root mucilage from different plant species-A case study on the effect of mucilage on soil water repellency. J Plant Nutr Soil Sci 179:294–302. https://doi.org/10.1002/jpln.201500511

Supplements

Physico-chemical properties of maize (Zea mays L.) mucilage differ with the collection system

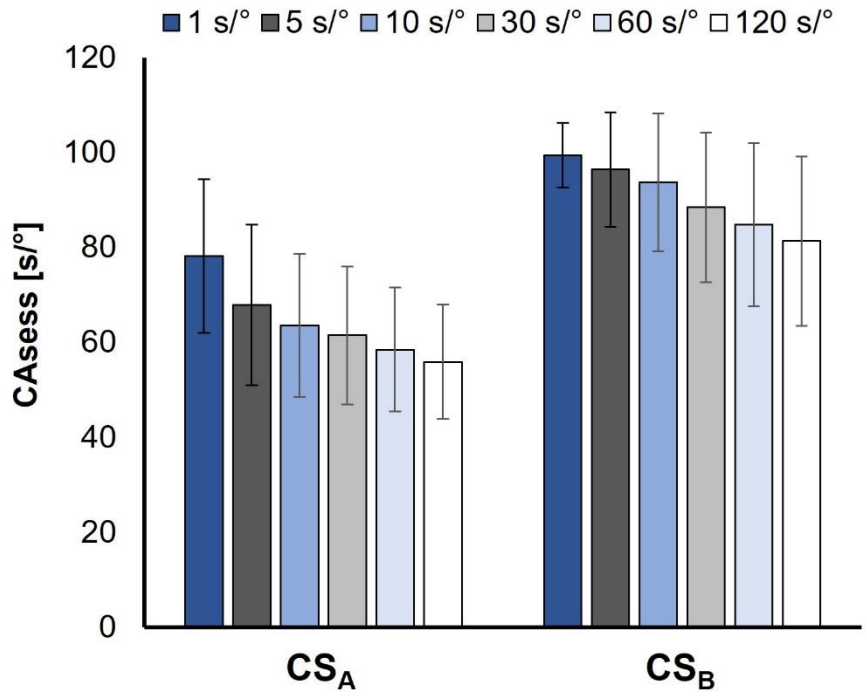
Plant and Soil

Lena M. Werner[1]; Matthilde Knott[2]; Dörthe Diehl[2]; Mutez Ahmed[3]; Callum Banfield[4]; Michi Dippold[4]; Doris Vetterlein[5,6]; Monika A. Wimmer[1*]

[1]University of Hohenheim, Dpt. Quality of Plant Products, Stuttgart, Germany

[*]corresponding author: m.wimmer@uni-hohenheim.de

Supplementary Figure S1



Supplementary Fig. S1 Sessile water drop contact angle (CA_{sess}) at a drop age of 1, 5, 10, 60 and 120 sec on dried mucilage, of CS_A and $CS_{B\text{-}pot}$ of maize (Zea mays L.). Error bars indicate standard deviations of ten to nineteen technical replicates

Physico-chemical properties of maize (Zea mays L.) mucilage differ with the collection system Plant and Soil

Lena M. Werner[1]; Matthilde Knott[2]; Dörthe Diehl[2]; Mutez Ahmed[3]; Callum Banfield[4]; Michi Dippold[4]; Doris Vetterlein[5,6]; Monika A. Wimmer[1*]

[1]University of Hohenheim, Dpt. Quality of Plant Products, Stuttgart, Germany

[*]corresponding author: m.wimmer@uni-hohenheim.de

Supplementary Table S1

Time of application	**Amount per 7 kg soil [g]**	**Fertilizer**	**Ingredients**
Inital	5.6	Hakaphos© Blau	4% NO_3^-; 11% NH_4^+; 10% P_2O_5; 15% K_2O; 2% MgO; 0.01% B; 0.02% Cu; 0.05% Fe; 0.05% Mn; 0.001% Mo; 0.02% Zn
Inital	0.4	Fertiloncombi©	3,3% MgO; 0.5% B; 1.5% Cu; 4.0% Fe; 4.0% Mn; 0.1% Mo; 1.5% Zn
Inital	0.7	Fe EDTA	Fe EDTA
10 days after sowing	1.5	Plantaaktiv®	10% NO_3^-; 8% NH_4^+; 12% P_2O_5; 18% K_2O 2% MgO; 0.02% B; 0.04% Cu; 0.1% Fe; 0.05 Mn; 0.01% Mo; 0.01 Zn
12 days	2.0		$(NH_4)SO_4$
13 days after sowing	0.7	Fe EDTA	Fe EDTA
14 days after sowing	1.0	$Ca(NO_3)_2$	$Ca(NO_3)_2$
20 days after sowing	1.5	Plantaaktiv®	10% NO_3^-; 8% NH_4^+; 12% P_2O_5; 18% K_2O 2% MgO; 0.02% B; 0.04% Cu; 0.1% Fe; 0.05 Mn; 0.01% Mo; 0.01 Zn
30 days after sowing, weekly application	1.0	NH_4NO_3	NH_4NO_3

Supplementary Table S1 Fertilization per pot (7 kg soil) in the pot experiment for $CS_{B\text{-}pot}$ mucilage collection

Chapter 5

General discussion

5. General discussion

Processes in the rhizosphere under drought conditions have been thoroughly discussed (Ahmed et al., 2014; Vetterlein et al., 2020; Vetterlein et al., 2022; Vives-Peris et al., 2020; Zia et al., 2021) and key factors in these processes are root hairs, exudates, and mucilage (Ahmed et al., 2014; Gilroy & Jones, 2000; Vetterlein et al., 2020; Vives-Peris et al., 2020). However, linking these diverse and highly dynamic processes remains a challenge (Vetterlein et al., 2020). This thesis contributes to elucidate these questions regarding rhizosphere processes by discussing the plasticity of root hairs, local and systemic responses under drought, and the influences of the collection system on the physico-chemical properties of mucilage. In detail, this thesis provides a study of the functions, behavior, and development of root hairs, considering nutrient and water availability and uptake (Chapter 2). Chapter 3 discussed hydraulic redistribution, as well as local and systemic osmotic adjustment of roots and their exudates under local drought. Chapter 4 compared two common methods for maize mucilage sampling and discussed the possible influence of the surrounding environment, plant age, and root type (depending on the sampling system) on mucilage properties. Furthermore, the role of mucilage from different collection systems when interpreting the role of mucilage in rhizosphere processes was discussed.

5.1. The expected benefit of root hairs under drought stress

To determine whether root hairs are relevant for water uptake and what role they might play under drought, results from published studies considering root hairs were summarized (Chapter 2). A review of these studies revealed the high plasticity of root hairs in response to nutrient and water availability. Nutrient deficiency can increase root-hair density (B, Mg, Fe, Mn, P, S, Zn), elongation (B, K, Mg, Mn, NH_4^+, NO_3^-, P), growth at non-hair cell positions (Mn, P), and shortening (Ca), and it can change root-hair morphology (Fe, Mn) (summarized in Chapter 2, Fig. 1). Nutrient toxicity can also increase root-hair density (Ca. Cu, Ni, NO_3^-), elongation (Ca, Ni), and shortening (Cl, Cu, K, Mg, Mn, Na, Ni, NH_4^+), and it also changes the morphology (Cu, NH_4^+, Zn) (summarized in Chapter 2, Fig. 1). Reported root-hair responses under water deficiency (drought) included root-hair elongation and changed morphology, as well as root-hair shortening and reduced density (summarized in Chapter 2, Fig. 1).

Whether root hairs contribute to water uptake has been highly discussed in published literature (Chapter 2). An increased surface area between plant roots and soil is advantageous for water acquisition (Gilroy & Jones, 2000). Under drought, crop growth activity depends on (besides assimilates) nutrient uptake and allocation (Dietz et al., 2021), and root hairs have a role in nutrient uptake and resource exchange (Robertson-Albertyn et al., 2017; Vetterlein et al., 2022).

Therefore, better nutrient uptake could be advantageous, especially when the water supply is restored (Rongsawat et al., 2021). However, in experiments with highly fertile and humid soils, the benefit of root hairs was not apparent (Carminati et al., 2017b; Wen & Schnable, 1994).

The role of root hairs in water uptake is under discussion, due to varying conclusions between different studies and plant species. The correlation between water uptake and root hair density and length was measured for several plant species (Marzec et al., 2015). Experiments with root-hairless mutants showed a lower water uptake in *Arabidopsis* and oat mutants (Cailloux, 1972; Tanaka et al., 2014) but no difference in barley and rice mutants (Dodd & Diatloff, 2016; Suzuki et al., 2003). Under drought, however, a beneficial effect of root hairs has been described for barley and maize (Brown et al., 2012; Klamer et al., 2019; Marin et al., 2021); the shoot growth of maize lacking root hairs (*rth3*) was reduced under drought compared to that of the wild-type (WT) (Klamer et al., 2019).

Besides a role of root hairs in nutrient and water uptake, other functions are described, which again are assigned to be drought beneficial. For example, root hairs release mucilage (Werker & Kislev, 1978) and influence the rhizosphere microbiota community (Gebauer et al., 2021; Robertson-Albertyn et al., 2017). The role of mucilage in water uptake, especially under drought, has been highly discussed (Ahmed et al., 2014; Ahmed et al., 2015; Benard et al., 2019). It seems that mucilage facilitates root water uptake during soil desiccation by keeping the soil close to the root wet and hydraulically conductive (Ahmed et al., 2014). Furthermore, root exudates can form the rhizosphere's microbiota, for example, under stress, an altered exudate pattern can increase beneficial microorganisms (Rolfe et al., 2019; Vives-Peris et al., 2020). Robertson-Albertyn et al. (2017) measured a reduced complexity of the microbiota community in the rhizosphere of barley root hair-less mutants compared to the rhizosphere of the WT. Such shifts in the microorganism community can be formed by root exudates (Rolfe et al., 2019; Vives-Peris et al., 2020). Mucilage exudation happens mainly at root tips (Carminati & Vetterlein, 2013). Werker and Kislev (1978) examined "small drops of mucilaginous character near the tip of root hairs" (page 809) for sorghum, meaning mucilage is also released from root hair tips. Therefore, a consequence of lacking root hairs might be a lower release of mucilage in the soil and, thus, a loss of the described beneficial effects on drought robustness. This theory is supported by an experiment by Holz et al. (2018b) with barley, where the WT exuded three times more carbon than the corresponding root hairless mutant (*brb*). Additionally, in a corresponding experiment to the study described in Chapter 4, maize mucilage was also collected by an aeroponic system from the root-hairless maize mutant *rth3*. In this corresponding experiment, the mucilage dry weight per plant of *rth3* was lower

than the WT (data not shown), indicating a similar conclusion to Holz et al. (2018b). Oburger and Santangeli (2022; personal communication) compared the exudation rate of the same maize genotypes in field, hydroponic, and pot experiments, and measured a higher exudation rate per root surface area for the *rth3* than the WT in all experimental setups. Though, in separate experiments in sand and loam soil (data not published), a lower root biomass was measured for the *rth3* maize mutant. Thus, even if the *rth3* mutant exudes more per root surface area, the total amount of released exudates in the soil could be lower. Therefore, this is not in direct contradiction with the observations described above. Furthermore, root exudation depends on species, genotype, and age (Oburger & Jones, 2018). As a result, the role of root hairs in releasing exudates in the rhizosphere and thus influencing microorganisms should be topics of further investigation.

To conclude, the influences of root hairs, such as improving microbiota and releasing exudates and mucilage in the rhizosphere, seem promising. Published literature revealed the high plasticity of root hairs under differing water and nutrient availabilities, and it seems that overall, root hairs improve drought resilience.

5.2. Local and systemic adjustment under local drought in maize (*Zea mays*)

Several studies have considered the adaption and resilience of plants to drought (Dietz et al., 2021; Tardieu et al., 2018; Vives-Peris et al., 2020). Most studies have investigated the entire plant under drought and subsequently described responses considering the entire plant (summarized in Tardieu et al., 2018). However, questions about the interaction and communication of plant parts during drought remain unanswered for many mechanisms. To understand rhizosphere processes under drought in more detail, whether crop responses to drought can be triggered locally must be determined. For this purpose, split root experiments have been utilized to investigate the role of roots and the communication between roots and shoot (Koebernick et al., 2015; Marino et al., 2007). In these experimental setups, individual parts of the root system can be treated differently (Koebernick et al., 2015) to investigate the role and behavior of the individual parts of the root system. Drought split root experiments have shown that plants can redistribute water to drier roots (Caldwell et al., 1998; Hafner et al., 2017; Liste & White, 2008), thereby increasing water use efficiency (Schachtman & Goodger, 2008). However, less is known about root exudation in drought split root setups. Thus far, when the entire root system suffers soil desiccation, the root exudation has been found to change qualitatively and quantitatively (Vives-Peris et al., 2020; Zia et al., 2021).

To investigate distinguishable local and systemic physiological and metabolic responses of shoot, root, and root exudates, a split root experiment was performed in soil-filled rhizoboxes

(Chapter 3). Since, most of the reported drought split root experiments have been performed by not limiting the water demand on the watered root compartment (Blackman & Davies, 1985; Iqbal et al., 2019), in this experimental setup (Chapter 3), one root half and its corresponding water demand were excluded from watering. Consequently, the other root half was watered as one half of the well-watered control.

Excluding one-half of the root system and its corresponding water supply triggered a drought stress response, which was measurable in reduced shoot biomass, length, and relative water content, as well as increased osmolality (chapter 3, Fig. 3A, 2A, 4A, 4B). Accumulation of osmolytes (Tardieu et al., 2018) likely enabled a high (93%) but decreasing relative water content in the shoot. However, the local drought did not significantly increase proline in the shoot (Chapter 3, Fig. 4C).

Root growth was assessed by root biomass (dry weight) of each root compartment. Root dry weight did not differ between the differently treated compartments of the local drought (Chapter 3, Fig. 3B). Furthermore, the root dry weight did not differ significantly from the well-watered control. However, the root-shoot ratio of the local drought increased. Meaning, shoot growth was reduced, but root growth was still equally maintained between the differently treated root compartments. Poorter et al. (2012) describe a more rapid growth reduction in the shoot than in the roots under moderate drought. To access water in deeper layers of the soil, root growth can even be enhanced under moderate drought (Dietz et al., 2021). In summary, the local drought after 10 days did probably not (yet) trigger compensatory root growth.

However, gravimetric water content (Chapter 3, Fig. 3D), proline concentration in roots (Chapter 3, Fig. 4D) and changes in root exudates (Chapter 3, Fig. 5) indicate a response to local drought. Lower gravimetric water content in the watered root compartment compared to the well-watered control indicated a compensatory water uptake. The slightly higher gravimetric water content in the drought-stressed root compartment compared to full drought may have occurred due to hydraulic redistribution (the redistribution of water within roots; Hafner et al., 2017) or a lower uptake in the locally drought-stressed root compartment, or both. Poorter et al. (2012) suggest that a too quickly changed allocation of the root system risks suboptimal plant growth in case of a restored water supply. Hence, at this point of local drought stress, the crop responded at least partially systemically by hydraulic redistribution instead of local compensatory root growth.

Under drought, crops accumulate osmotically active substances to maintain their water potential (Dietz et al., 2021). Such osmotic adjustment occurred in roots and exudates of the local drought and likely contributed to the above-described hydraulic redistribution. Osmotic

adjustment is indicated by increased osmolality in the shoot, as well as in the drought stresses root compartment by an increased proline concentration in roots and slight changes in exudates. The amino acid proline increases under drought to function as an osmolyte and protect the cell membranes (Ilahi & Dorffling, 1982; Trovato et al., 2008). Such increase of proline occurred in the drought-stressed roots, however, neither in the shoot nor in the roots of the watered compartment. This finding indicated a local response, and slight changes in exudate composition further supported this theory. For instance, a trend was observed that proline, maltose, putrescine, and trehalose tended to increase by drought intensity. Through by this osmolyte accumulation, the water potential would be lowered in the drought-stressed root compartment, leading to a steeper water potential gradient between the root, rhizosphere, and bulk soil and, therefore, improved water movement to the root.

Changes in exudate composition were more prominent in full drought than in local drought. However, drought intensity is reported to influence exudate changes; nevertheless, under extreme drought, exudation decreases (Gargallo-Garriga et al., 2018; Preece & Peñuelas, 2016). Besides a potential role in hydraulic redistribution, the exudate compounds proline, putrescine, trehalose, and maltose have been reported as enhancing beneficial microorganisms under drought (Jin et al., 2019; Kuiper et al., 2001; Vílchez et al., 2016; Vílchez et al., 2000). Therefore, a function in attracting beneficial microorganisms should be considered.

In summary, local drought triggered local and systemic responses. These responses enabled the plant to perform hydraulic redistribution. Furthermore, the local responses indicated that rhizosphere processes under drought are partially influenced and locally triggerable by the root and its exudates. Data from this experiment indicate that further experiments, which consider microorganism populations, different local drought intensities, and continuous monitoring of soil water content, would be useful to understand rhizosphere processes in more detail.

5.3. Physico-chemical properties of *Zea mays* mucilage differs between collection systems

Maize mucilage is typically collected in two ways: from seedlings (beginning at BBCH 06), growing in humid air (aeroponic system = CS_A) (Holz et al., 2018a; Zickenrott et al., 2016) or from aerial brace roots (beginning at about BBCH 33 = CS_B) from maize growing in soil (Ahmed et al., 2015). To evaluate the reliability and possible impact of the mucilage collection system when interpreting the role of mucilage in rhizosphere processes, the physico-chemical properties of these two systems were compared. The analyzed physical properties included surface tension, contact angle, and viscosity, while the chemical properties were the polysaccharide polymer length, neutral sugar composition, pH, and nutrient content.

These physico-chemical properties of mucilage differed between the two collection systems. Differences can be explained (up to a certain point) by differing root type, plant age, and collection environment. In terms of root type the different physiological functions of seminal/primary (CS_A) and brace roots (CS_B) should be considered (Ahmed et al., 2018). For instance, brace roots can take up more water than seminal or primary roots and enable better mechanical stability for the plant (Ahmed et al., 2018; Hetz et al., 1996). Both functions demand quick and proper contact between root and soil as soon as brace roots enter the soil. Mucilage plays an important role in this process by inter alia functioning as a lubricant during root penetration, having a role in rhizosphere hydraulics and root water uptake, and enabling soil-aggregate stabilization (Ahmed et al., 2014; Carminati et al., 2010; Iijima et al., 2003; Morel et al., 1991). Therefore, the demand for CS_B mucilage properties to enable these functions is particularly high, reflected, for example, in more viscous CS_B mucilage (Chapter 4, Fig. 3). The functions of root types are related to plant age. Brace roots, suitable for CS_B mucilage collection, grow from older plants (about BBCH 33), while CS_A mucilage is collected from seedlings with seminal/primary roots in aeroponics. These differing developmental stages also mean different physiological stages, for instance, in nutrient translocation. The seed performs nutrient supply at seedling stage (CS_A). Nutrients are translocated to the main organs (roots) for rapid growth. While at the developmental stage, when brace roots emerge, nutrients are translocated from root to shoot; thus, a "re-direction" to the brace roots would be necessary. Furthermore, roots in an aeroponic system depend completely on the nutrient supply provided by the seed because the roots do not contact soil or a nutrient solution. In contrast, the plants used for CS_B mucilage collection obtained nutrients from the soil. Therefore, higher Ca, K, Mg, and Na concentrations in CS_A seem feasible (Chapter 4, Fig. 4a).

The third difference between the two collection systems is the environment, as the systems differ in terms of light availability, sterility, and air humidity. The CS_B mucilage was collected from photosynthetic active plants exposed to a typical light rhythm (day, night). In contrast, seedlings for CS_A mucilage collection were kept in the dark during the entire experiment (7 days). Thus, the photosynthetic activity was probably low during CS_A mucilage production. Lack of light and thus assimilates results in a reduced root-derived carbon release in the rhizosphere (Kuzyakov & Cheng, 2001). Therefore, lower neutral sugar contents in CS_A mucilage might be due to lower assimilation rates. A further environmental difference was sterility. Aeroponics are kept semi-sterile, whereas no sterile conditions were intended during the collection of CS_B mucilage. A diverse consortium of microorganisms in CS_B mucilage has been suggested (Estrada et al., 2002; van Deynze et al., 2018). These microorganisms

synthesize mainly hexoses (galactose, mannose, fucose), and synthesize pentoses (arabinose, xylose) only in minor amounts (Spielvogel et al., 2016). The activity of microorganisms during CS_B mucilage production/collection might explain higher shares of hexoses in CS_B mucilage (Chapter 4, Fig. 6b). Furthermore, the mannose a hexose was not detected, and the share of pentoses was higher in CS_A mucilage, which was produced/collected in semi-sterile conditions and therefore probably under a lower pressure by microorganisms. Thirdly, humidity surrounding the roots differs in the two systems. While aeroponic systems remain in highly humid air the whole time, mucilage on airborne brace roots faces wetting/drying cycles by changing air humidity and rain. Physico-chemical properties of mucilage change by drying, and water repellency of mucilage increases (Ahmed et al., 2015; Ahmed et al., 2016); furthermore, sugars influence the water binding capacity (Carminati & Vetterlein, 2013). Therefore, a higher neutral sugar concentration (Chapter 4, Fig. 6a) and viscosity of CS_B mucilage might be an adaption to wetting/drying cycles and to increase the water binding capacity. Amicucci et al. (2019) state that "the mucilage contains a single heterogeneous polysaccharide composed of a highly fucosylated and xylosylated galactose backbone with arabinan and mannoglucuronan branches," (page 7254) and its structure is unique. Galactose, the backbone of the mucilage polysaccharide (Amicucci et al., 2019), was particularly higher in CS_B (Chapter 4, Fig. 6b). This is probably reflected in higher amounts of compounds with high molecular mass (size exclusion chromatography) (Chapter 4, Fig. 5) and higher viscosity.

In summary, analysis of the physico-chemical properties showed vast differences between the mucilage. These differences are highly relevant when calculating models and interpreting the role and influence of mucilage in rhizosphere processes. For instance, Carminati et al. (2017a) assigned mucilage a shaping function of the rhizosphere's hydraulic properties. In detail, by absorbing water and influencing the soil solution by increasing its viscosity and decreasing its surface tension (Carminati et al., 2017a). Therefore, soil solution properties and rhizosphere water dynamics are influenced by mucilage (Benard et al., 2019). Physico-chemical properties of mucilage facilitate these functions, for instance, in soil solution, the extensional viscosity is increased by long mucilage polymers (Benard et al., 2019). When interpreting the role of mucilage on extensional viscosity, it should be considered that CS_B mucilage is probably a bigger polymer due to higher amounts of compounds with high molecular mass and galactose, and therefore, CS_B would increase extensional viscosity more than CS_A mucilage (considering Benard et al., 2019). A further influence by high mucilage viscosity is a decreased hydraulic conductivity in the rhizosphere (Kroener et al., 2014). Consequently, CS_B mucilage would decrease hydraulic conductivity more than CS_A mucilage. Furthermore, mucilage compounds,

such as phospholipids or sugars, influence water binding capacity and the physical properties of the mucilage (Carminati & Vetterlein, 2013). The higher sugar-concentrated CS_B mucilage might have a better water holding capacity (considering Carminati & Vetterlein, 2013), which again would be relevant when interpreting the role of mucilage in rhizosphere processes. In summary, the role of mucilage, for instance, under soil drying, would be assessed slightly stronger by CS_B than by CS_A mucilage. According to Vetterlein et al. (2020), "the main knowledge gaps in rhizosphere research are related to the difficulty in mechanistically linking the physical, chemical, and biological processes […]." (Page 2) The results of this experiment indicate that the collection system highly influences mucilage properties and must be considered when interpreting the role of mucilage in these processes. In order to assign the role of root type, plant age, and environment in more detail, new experimental setups are needed, which can take the individual claims into account.

5.4. Conclusion

This thesis contributes to linking, understanding, and interpreting rhizosphere processes by considering root hairs, local and systemic drought responses, and the influence of the collection system on the physico-chemical properties of mucilage. First, the literature reported a beneficial effect of root hairs under drought (Brown et al., 2012; Klamer et al., 2019; Marin et al., 2021). Second, local drought in maize resulted in hydraulic redistribution enabled by systemic and local metabolic responses. These responses included changes in the root exudation pattern, proline accumulation in locally drought stressed roots, and increased osmolality in the shoot. Third, a comparison between two maize mucilage collection systems revealed the influence of the collection system on the physico-chemical properties of mucilage. Differing physico-chemical properties were related to differences in the crops' developmental stage, root type functions, and the growth environment. These results emphasized the role of the mucilage collection system when interpreting the role of mucilage in rhizosphere processes.

References

Ahmed, M. A., Kroener, E., Benard, P., Zarebanadkouki, M., Kaestner, A., Carminati, A. (2016). Drying of mucilage causes water repellency in the rhizosphere of maize: measurements and modelling. Plant Soil 407:161–171. doi.org/ 10. 1007/ s11104- 015-2749-1

Ahmed, M. A., Holz, M., Woche, S.K., Bachmann, J., Carminati, A. (2015). Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. Journal of Plant Nutrition and Soil Science 178:821–824. doi:10.1002/jpln.201500177.

Ahmed, M. A., Kroener, E., Holz, M., Zarebanadkouki, M., Carminati, A. (2014). Mucilage exudation facilitates root water uptake in dry soils. Functional plant biology : FPB 41:1129–1137. doi:10.1071/FP13330.

Ahmed, M. A., Zarebanadkouki, M., Meunier, F., Javaux, M., Kaestner, A., Carminati, A. (2018). Root type matters: measurement of water uptake by seminal, crown, and lateral roots in maize. Journal of experimental botany 69:1199–1206. doi:10.1093/jxb/erx439.

Amicucci, M. J., Galermo, A. G., Guerrero, A., Treves, G., Nandita, E., Kailemia, M. J., Higdon, S. M., Pozzo, T., Labavitch, J. M., Bennett, A. B., Lebrilla, C. B. (2019). Strategy for Structural Elucidation of Polysaccharides: Elucidation of a Maize Mucilage that Harbors Diazotrophic Bacteria. Analytical chemistry 91:7254–7265. doi:10.1021/acs.analchem.9b00789.

Benard, P., Zarebanadkouki M., Carminati, A. (2019). Physics and hydraulics of the rhizosphere network. Journal of Plant Nutrition and Soil Science 182:5–8. doi:10.1002/jpln.201800042.

Blackman, P. G., Davies, W. J. (1985). Root to Shoot Communication in Maize Plants of the Effects of Soil Drying. Journal of Experimental Botany 36:39–48. doi:10.1093/jxb/36.1.39.

Brown, L. K., George, T. S., Thompson, J. A., Wright, G., Lyon, J., Dupuy, L., Hubbard, S. F., White, P. J. (2012). What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (Hordeum vulgare)? Annals of botany 110:319–328. doi:10.1093/aob/mcs085.

Cailloux, M. (1972). Metabolism and the absorption of water by root hairs. Canadian Journal of Botany 50:557–573. doi:10.1139/b72-069.

Caldwell, M. M., Dawson, T. E., Richards, J. H. (1998). Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113:151–161. doi:10.1007/s004420050363.

Carminati, A., Benard, P., Ahmed, M. A., Zarebanadkouki, M. (2017a). Liquid bridges at the root-soil interface. Plant and Soil 417:1–15. doi:10.1007/s11104-017-3227-8.

Carminati, A., Moradi, A. B., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H.-J., Oswald, S. E. (2010). Dynamics of soil water content in the rhizosphere. Plant and Soil 332:163–176. doi:10.1007/s11104-010-0283-8.

Carminati, A., Passioura, J. B., Zarebanadkouki, M., Ahmed, M.A., Ryan, P. R., Watt, M., Delhaize, E. (2017b). Root hairs enable high transpiration rates in drying soils. New Phytologist 216:771–781. doi:10.1111/nph.14715.

Carminati, A., Vetterlein, D. (2013). Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Annals of botany 112:277–290. doi:10.1093/aob/mcs262.

Dietz, K.-J., Zörb, C., Geilfus, C.-M. (2021). Drought and crop yield. Plant biology 23:881–893. doi:10.1111/plb.13304.

Dodd, I. C., Diatloff, E. (2016). Enhanced root growth of the brb (bald root barley) mutant in drying soil allows similar shoot physiological responses to soil water deficit as wild-type plants. Functional plant biology : FPB 43:199–206. doi:10.1071/FP15303.

Estrada, P., Mavingui, P., Cournoyer, B., Fontaine, F., Balandreau, J., Caballero-Mellado, J. (2002). A N2-fixing endophytic Burkholderia sp. associated with maize plants cultivated in Mexico. Canadian journal of microbiology 48:285–294. doi:10.1139/w02-023.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., Peñuelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. Scientific reports 8:12696. doi:10.1038/s41598-018-30150-0.

Gebauer, L., Bouffaud, M.-L., Ganther, M., Yim, B., Vetterlein, D., Smalla, K., Buscot, F., Heintz-Buschart, A., Tarkka, M. T. (2021). Soil Texture, Sampling Depth and Root Hairs Shape the Structure of ACC Deaminase Bacterial Community Composition in Maize Rhizosphere. Frontiers in microbiology 12:616828. doi:10.3389/fmicb.2021.616828.

Gilroy, S., Jones, D. L. (2000). Through form to function: root hair development and nutrient uptake. Trends in plant science 5:56–60. doi:10.1016/S1360-1385(99)01551-4.

Hafner, B. D., Tomasella, M., Häberle, K.-H., Goebel, M., Matyssek, R., Grams, T. E. E. (2017). Hydraulic redistribution under moderate drought among English oak, European

beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment. Tree physiology 37:950–960. doi:10.1093/treephys/tpx050.

Hetz, W., Hochholdinger, F., Schwall, M., Feix, G. (1996). Isolation and characterization of rtcs, a maize mutant deficient in the formation of nodal roots. The Plant Journal 10:845–857. doi:10.1046/j.1365-313X.1996.10050845.x.

Holz, M., Leue, M., Ahmed, M. A., Benard, P., Gerke, H. H., Carminati, A. (2018a). Spatial Distribution of Mucilage in the Rhizosphere Measured With Infrared Spectroscopy. Frontiers in Environmental Science 6. doi:10.3389/fenvs.2018.00087.

Holz, M., Zarebanadkouki, M., Kuzyakov, Y., Pausch, J., Carminati, A. (2018b). Root hairs increase rhizosphere extension and carbon input to soil. Annals of botany 121:61–69. doi:10.1093/aob/mcx127.

Iijima, M., Higuchi, T., Barlow, PW., Bengough, A.G. (2003). Root cap removal increases root penetration resistance in maize (Zea mays L). Journal of experimental botany 54:2105–2109. doi:10.1093/jxb/erg226.

Ilahi, I., Dorffling, K. (1982). Changes in abscisic acid and proline levels in maize varieties of different drought resistance. Physiologia plantarum 55:129–135. doi:10.1111/j.1399-3054.1982.tb02275.x.

Iqbal, N., Hussain, S., Raza, M. A., Yang, C.-Q., Safdar, M. E., Brestic, M., Aziz, A., Hayyat, M. S., Asghar, M. A., Wang, X. C., Zhang, J., Yang, W., Liu, J. (2019). Drought Tolerance of Soybean (Glycine max L. Merr.) by Improved Photosynthetic Characteristics and an Efficient Antioxidant Enzyme Activities Under a Split-Root System. Frontiers in physiology 10:786. doi:10.3389/fphys.2019.00786.

Jin, Y., Zhu, H., Luo, S., Yang ,W., Zhang, L., Li, S., Jin, Q., Cao, Q., Sun, S., Xiao, M. (2019). Role of Maize Root Exudates in Promotion of Colonization of Bacillus velezensis Strain S3-1 in Rhizosphere Soil and Root Tissue. Current microbiology 76:855–862. doi:10.1007/s00284-019-01699-4.

Klamer, F., Vogel, F., Li, X., Bremer, H., Neumann, G., Neuhäuser, B., Hochholdinger, F., Ludewig, U. (2019). Estimating the importance of maize root hairs in low phosphorus conditions and under drought. Annals of Botany 124:961–968. doi:10.1093/aob/mcz011.

Koebernick, N., Huber, K., Kerkhofs, E., Vanderborght, J., Javaux, M., Vereecken, H., Vetterlein, D. (2015). Unraveling the hydrodynamics of split root water uptake

experiments using CT scanned root architectures and three dimensional flow simulations. Frontiers in plant science 6:370. doi:10.3389/fpls.2015.00370.

Kroener, E., Zarebanadkouki, M., Kaestner, A., Carminati, A. (2014). Nonequilibrium water dynamics in the rhizosphere: How mucilage affects water flow in soils. Water Resources Research 50:6479–6495. doi:10.1002/2013WR014756.

Kuiper, I., Bloemberg, G. V., Noreen, S., Thomas-Oates, J. E., Lugtenberg, B. J. (2001). Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by Pseudomonas fluorescens strain WCS365. Molecular plant-microbe interactions: MPMI 14:1096–1104. doi:10.1094/MPMI.2001.14.9.1096.

Kuzyakov, Y., Cheng, W. (2001). Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1925. doi. org/ 10. 1016/S0038-0717(01) 00117-1

Liste, H.-H., White, J. C. (2008). Plant hydraulic lift of soil water – implications for crop production and land restoration. Plant and Soil 313:1–17. doi:10.1007/s11104-008-9696-z.

Marin, M., Feeney, D. S., Brown, L. K., Naveed, M., Ruiz, S., Koebernick, N., Bengough, A. G., Hallett, P. D,, Roose, T,, Puértolas, J., Dodd, I. C., George, T. S. (2021). Significance of root hairs for plant performance under contrasting field conditions and water deficit. Annals of botany 128:1–16. doi:10.1093/aob/mcaa181.

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C., González, E. M. (2007). Nitrogen fixation control under drought stress. Localized or systemic? Plant physiology 143:1968–1974. doi:10.1104/pp.106.097139.

Marzec, M., Melzer, M., Szarejko, I. (2015). Root hair development in the grasses: what we already know and what we still need to know. Plant Physiology 168:407–414. doi:10.1104/pp.15.00158.

Morel, J. L., Habib, L., Plantureux, S., Guckert, A. (1991). Influence of maize root mucilage on soil aggregate stability. Plant and Soil 136:111–119. doi:10.1007/BF02465226.

Oburger, E., Jones, D. L. (2018). Sampling root exudates – Mission impossible? Rhizosphere 6:116–133. doi:10.1016/j.rhisph.2018.06.004.

Oburger, E., Santangeli, M. (2022). Personal communication via E-mail with Michael Santangeli on 03.-08.06.2022. Data was unpublished at the time of submission of this Thesis

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. The New phytologist 193:30–50. doi:10.1111/j.1469-8137.2011.03952.x.

Preece, C., Peñuelas, J. (2016). Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. Plant and Soil 409:1–17. doi:10.1007/s11104-016-3090-z.

Robertson-Albertyn, S., Alegria Terrazas, R., Balbirnie, K., Blank, M., Janiak, A., Szarejko, I., Chmielewska, B., Karcz, J., Morris, J., Hedley, P. E., George, T. S., Bulgarelli, D. (2017). Root Hair Mutations Displace the Barley Rhizosphere Microbiota. Frontiers in plant science 8:1094. doi:10.3389/fpls.2017.01094.

Rolfe, S. A., Griffiths, J., Ton, J. (2019). Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current opinion in microbiology 49:73–82. doi:10.1016/j.mib.2019.10.003.

Rongsawat, T., Peltier, J.-B., Boyer, J.-C., Véry, A.-A., Sentenac, H. (2021). Looking for Root Hairs to Overcome Poor Soils. Trends in plant science 26:83–94. doi:10.1016/j.tplants.2020.09.001.

Schachtman, D. P., Goodger, J. Q. D. (2008). Chemical root to shoot signaling under drought. Trends in plant science 13:281–287. doi:10.1016/j.tplants.2008.04.003.

Spielvogel, S., Prietzel, J., Kögel-Knabner, I. (2016). Stand scale variability of topsoil organic matter composition in a high-elevation Norway spruce forest ecosystem. Geoderma 267:112–122. doi:10.1016/j.geoderma.2015.12.001.

Suzuki, N., Taketa, S., Ichii, M. (2003). Morphological and physiological characteristics of a root-hairless mutant in rice (Oryza sativa L.). Plant and Soil 255:9–17. doi:10.1023/A:1026180318923.

Tanaka, N., Kato, M., Tomioka, R., Kurata, R., Fukao, Y., Aoyama, T., Maeshima, M. (2014). Characteristics of a root hair-less line of Arabidopsis thaliana under physiological stresses. Journal of Experimental Botany 65:1497–1512. doi:10.1093/jxb/eru014.

Tardieu, F., Simonneau, T., Muller, B. (2018). The Physiological Basis of Drought Tolerance in Crop Plants: A Scenario-Dependent Probabilistic Approach. Annual review of plant biology 69:733–759. doi:10.1146/annurev-arplant-042817-040218.

Trovato, M., Mattioli, R., Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei 19:325–346. doi:10.1007/s12210-008-0022-8.

van Deynze, A., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., Schwartz, K. D., Berry, A. M., Bhatnagar, S., Jospin, G., Darling, A., Jeannotte, R., Lopez, J., Weimer, B. C., Eisen, J. A., Shapiro, H.-Y,, Ané, J.-M., Bennett, A. B. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLoS biology 16:e2006352. doi:10.1371/journal.pbio.2006352.

Vetterlein, D., Carminati, A., Kögel-Knabner, I., Bienert, G. P., Smalla, K., Oburger, E., Schnepf, A., Banitz, T., Tarkka, M. T., Schlüter S (2020). Rhizosphere Spatiotemporal Organization–A Key to Rhizosphere Functions. Frontiers in Agronomy 2. doi:10.3389/fagro.2020.00008.

Vetterlein D, Lippold E, Schreiter S, Phalempin M, Fahrenkampf T, Hochholdinger F, Marcon C, Tarkka M, Oburger E, Ahmed M, Javaux M, Schlüter S (2021). Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. Journal of Plant Nutrition and Soil Science 184:35–50. doi:10.1002/jpln.202000079.

Vetterlein, D., Phalempin, M., Lippold, E., Schlüter, S., Schreiter, S., Ahmed, M. A., Carminati, A., Duddek, P., Jorda, H., Bienert, G. P., Bienert, M. D., Tarkka, M., Ganther, M., Oburger, E., Santangeli, M., Javaux, M., Vanderborght, J. (2022). Root hairs matter at field scale for maize shoot growth and nutrient uptake, but root trait plasticity is primarily triggered by texture and drought. Plant and Soil. doi:10.1007/s11104-022-05434-0.

Vílchez, J.I., García-Fontana, C., Román-Naranjo, D., González-López, J., Manzanera, M. (2016). Plant Drought Tolerance Enhancement by Trehalose Production of Desiccation-Tolerant Microorganisms. Frontiers in microbiology 7:1577. doi:10.3389/fmicb.2016.01577.

Vílchez, S., Molina, L., Ramos, C., Ramos, J. L. (2000). Proline catabolism by Pseudomonas putida: cloning, characterization, and expression of the put genes in the presence of root exudates. Journal of bacteriology 182:91–99. doi:10.1128/JB.182.1.91-99.2000.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R. M. (2020). Root exudates: from plant to rhizosphere and beyond. Plant cell reports 39:3–17. doi:10.1007/s00299-019-02447-5.

Wen, T.-J., Schnable, P. S. (1994). Analyses of Mutants of Three Genes that Influence Root Hair Development in Zea mays (Gramineae) Suggest that Root Hairs are Dispensable. American Journal of Botany 81:833. doi:10.2307/2445764.

Werker, E., Kislev, M. (1978). Mucilage on the Root Surface and Root Hairs of Sorghum: Heterogeneity in Structure, Manner of Production and Site of Accumulation. Annals of Botany 42:809–816. doi:10.1093/oxfordjournals.aob.a085520.

Zia, R., Nawaz, M. S., Siddique, M. J., Hakim, S., Imran, A. (2021). Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiological research 242:126626. doi:10.1016/j.micres.2020.126626.

Zickenrott, I.-M., Woche, S. K., Bachmann, J., Ahmed, M. A., Vetterlein, D. (2016). An efficient method for the collection of root mucilage from different plant species—A case study on the effect of mucilage on soil water repellency. Journal of Plant Nutrition and Soil Science 179:294–302. doi:10.1002/jpln.201500511.

Chapter 6

Summary

Summary

Drought events are increasing due to climate change, resulting in significant yield losses. Many breeding strategies focus on drought resistance to avoid these yield losses or complete crop failure. Additionally, to improve drought resistance under soil desiccation, the soil and particularly rhizosphere processes are more and more in the focus of research. Specifically, linkages between the diverse and highly dynamic interactions of soil, plant, and microorganism community must be understood. This thesis thus aims to answer the following research questions: *i*) Are root hairs relevant for water uptake, and what role do they play under drought? *ii*) Does local drought in *Zea mays* result in distinguishable systemic and local metabolic and physiological responses, as well as compensatory water uptake? *iii*) Do the physico-chemical properties of *Zea mays* mucilage differ between two common collection systems?

In the first part, published studies considering root hairs in nutrient and water uptake were summarized, and show a high plasticity of root hairs under different nutrient and water availability states. This plasticity was apparent through changes in root hair morphology and development. Furthermore, the role of root hairs in water uptake is under discussion due to variable results from different studies and crop species. Nevertheless, it seems that overall root hairs improve drought resilience. Furthermore, a better nutrient uptake and mucilage exudation by root hairs and thus an increased drought stability is discussed. This suggests a beneficial role of root hairs for drought stress robustness.

In the second part, local and systemic drought responses of maize and their effect on rhizosphere processes were assessed in a split-root experiment. The root system of maize was separated into two differently watered (watered, drought stressed) rhizobox chambers. The local drought treatment was performed for 10 days. Under these conditions, the local drought led to a local and systemic response through osmotic adjustment. Osmolarity increased in the shoot, while increased proline concentrations and slight changes in root exudates indicated a local response in the drought stressed root compartment. This metabolic adjustment contributed to a hydraulic redistribution of water between the root halves and enhanced water availability.

Comparing the physico-chemical properties of maize mucilage collected by two common collection systems emphasized the impact of mucilage collection when interpreting the role of mucilage in rhizosphere processes. The mucilage differed in terms of physico-chemical properties, which included contact angle, viscosity, surface tension (physical) and nutrient content, pH, polysaccharide polymer length, and neutral sugar composition (chemical). The mucilage was collected in two ways: 1) from primary and seminal roots of seedlings

growing in a semi-sterile aeroponic system and 2) from airborne brace roots of maize growing on sandy soil. The two collection systems differed in terms of plant age, environment (sterility, light availability, air humidity), and root type. The higher viscosity of the brace root mucilage may have reflected the drier air humidity surrounding the root and therefore the need to enhance water holding capacity. Non-sterile conditions during brace root mucilage collection probably resulted in higher shares of hexoses, while semi-sterile conditions may explain the lack of mannose in the aeroponic mucilage. Brace root mucilage may therefore have a greater relevance during soil desiccation than aeroponic mucilage.

In summary, this work helps to fill knowledge gaps in understanding and linking rhizosphere processes by *i*) providing a state-of-the-art summary of root hair plasticity related to nutrient and water availability and concluding a beneficial role of root hairs in drought robustness, *ii*) showing local and systemic osmotic adjustment and hydraulic redistribution under local drought, and *iii*) emphasizing the role of the mucilage collection systems when interpreting the role of mucilage in rhizosphere processes

Chapter 7

Zusammenfassung

Zusammenfassung

Trockenstressereignisse häufen sich bedingt durch den Klimawandel und führen zu Ertragsverlusten. Deshalb sind zahlreiche Züchtungsprogramme auf Trockenstressresistenz ausgelegt, um so Ertragsverluste oder Komplettausfälle zu verhindern. Zusätzlich rücken der Boden und im Besonderen die Rhizosphären-Prozesse in den Forschungsvordergrund, um so die Trockenstressresistenz unter Bodenaustrocknung zu verbessern. Es ist dabei notwendig die Zusammenhänge zwischen den zahlreichen und sehr dynamischen Interaktionen von Boden, Pflanze und Mikroorganismenpopulationen zu verstehen. Folglich zielt diese Arbeit darauf ab, folgende Forschungsfragen zu beantworten: *i*) Sind Wurzelhaare relevant in der Wasseraufnahme und welche Rolle haben sie unter Trockenheit? *ii*) Führt lokaler Trockenstress bei *Zea mays* zu systemisch- und lokal unterscheidbaren metabolischen und physiologischen Reaktionen sowie zur Kompensation in der Wasseraufnahme? *iii*) Unterscheiden sich die physikalisch-chemischen Eigenschaften der Mucilage von *Zea mays* aus zwei unterschiedlichen Sammelsystemen?

Im ersten Teil wurden publizierte Studien zusammengefasst, welche Wurzelhaare im Zusammenhang mit Nährstoff- und Wasseraufnahme berücksichtigen. Diese zeigen eine hohe Plastizität von Wurzelhaaren unter verschiedenen Nährstoff- und Wasserverfügbarkeiten. Diese Plastizität ist erkennbar durch Veränderungen bei der Wurzelhaar-Morphologie und - Entwicklung. Die Rolle von Wurzelhaaren bei der Wasseraufnahme wird kontrovers diskutiert, da sich die Ergebnisse von verschiedenen Studien und Spezies unterscheiden. Allerdings scheint es, dass insgesamt Wurzelhaare die Trockenstressresilienz verbessern. Des Weiteren wird eine bessere Nährstoffaufnahme und Mucilage-Exsudation durch Wurzelhaare und dadurch eine erhöhte Trockenstress-Stabilität diskutiert. Dies lässt eine fördernde Rolle von Wurzelhaaren zur Trockenstressstabilität vermuten.

Im zweiten Teil wurden lokale und systemische Antworten von Mais und deren Effekte auf Rhizosphären- Prozessen in einem Split-Root-Experiment beurteilt. Dafür wurde das Wurzelsystem von Mais in zwei Rhizoboxkammern aufgeteilt und unterschiedlich bewässert (bewässert, trockengestresst). Der lokale Trockenstress dauerte 10 Tage. Unter diesen Bedingungen führte die lokale Trockenheit zu einer lokalen und systemischen osmotischen Anpassung. Die Osmolalität stieg im Spross an, während erhöhte Prolin-Konzentrationen und leichte Veränderungen bei den Wurzelexsudaten eine lokale Antwort der trockengestressten Wurzelseite zeigen. Diese metabolischen Anpassungen trugen zu einer hydraulischen

Umverteilung von Wasser zwischen den Wurzelhälften bei und verbesserten die Wasserverfügbarkeit.

Ein Vergleich der physikalisch-chemischen Eigenschaften von Mais-Mucilagen, die aus zwei üblichen Sammelsystemen stammen, zeigt die Relevanz des Sammelsystems, wenn die Rolle der Mucilage in Rhizosphären-Prozessen interpretiert werden soll. Die Mucilagen unterschieden sich in ihren physikalisch-chemischen Eigenschaften, welche Kontaktwinkel, Viskosität, Oberflächenspannung (physikalisch) und Nährstoffkonzentrationen, pH-Wert, Länge der Polysaccharid-Polymere und Zusammensetzung der neutralen Zucker (chemisch) umfassen. Die Mucilage wurde auf zwei Arten gewonnen: 1) Absammeln von primären und seminalen Wurzeln von Maiskeimlinge, die in einem semi-sterilen aeroponischen System angezogen wurden und 2) von Luftwurzeln oberhalb des Bodens von Mais, der in Sandboden angezogen wurde. Diese zwei Sammelsysteme unterschieden sich in Pflanzenalter, Umwelt (Sterilität, Lichtverfügbarkeit, Luftfeuchtigkeit) und Wurzeltyp. Die höhere Viskosität der Luftwurzelmucilage reflektiert möglicherweise die trockenere Luftfeuchte um die Wurzel und der daraus resultierenden Notwendigkeit die Wasserhaltekapazität zu verbessern. Nicht-sterile Bedingungen bei dem Absammeln von Luftwurzelmucilage führte vermutlich zu höheren Anteilen an Hexosen, während semi-sterile Bedingungen das Fehlen von Mannose in aeroponisch gesammelter Mucilage erklären könnte. Luftwurzelmucilage hätte daher vermutlich größere Effekte auf Rhizosphären-Prozesse während Bodenaustrocknung als aeroponisch gesammelte Mucilage.

Zusammenfassend trägt diese Arbeit dazu bei, die Wissenslücken zu Prozessen in der Rhizosphäre zu füllen, um diese zu verstehen und miteinander zu verbinden, indem gezeigt wurde, dass *i*) die publizierte Literatur beschreibt, dass Wurzelhaare in Bezug auf Nährstoff- und Wasserverfügbarkeit plastisch reagieren und eine vorteilhafte Rolle unter Trockenheit haben können, *ii*) eine lokale und systemische osmotische Anpassung sowie hydraulische Umverteilung stattfindet und *iii*) die Rolle des Mucilage-Sammelsystems hervorzuheben ist, wenn die Rolle der Mucilage in Rhizosphären-Prozessen interpretiert werden soll.

Danksagung

Mein herzlicher Dank gilt meinem Doktorvater Prof. Dr. Christian Zörb für seine tatkräftige Unterstützung meiner wissenschaftlichen Arbeit, sowie seine stets motivierenden Worte möchte ich Ihm danken.

Ganz besonders möchte ich mich bei Dr. Monika Wimmer, für Ihre tatkräftige Unterstützung bei den Projektkonzeptionen, Durchführung und der Erstellung wissenschaftlicher Beiträge bedanken. Des Weiteren bin ich für das stets offene Ohr, den Austausch und der sehr aktiven Unterstützung in der Durchführung dankbar.

Ich danke meinen zahlreichen Kooperationspartnern die mich tatkräftig bei der Versuchsdurchführung, sowie Anfertigung wissenschaftlicher Arbeiten unterstützt haben. Hier möchte ich mich bei Dr. Desiree Bienert und Prof. Dr. Gerd Patrick Bienert bedanken, die immer ansprechbar waren und stets gute Ideen und Lösungsansätzen parat hatten. Zudem möchte ich mich bei Dr. Mutez Ahmed, Prof. Dr. Michi Dippold, Dr. Callum Banfield, Dr. Doerte Diehl und Dr. Mathilde Knott für die konstruktive Zusammenarbeit und den regen Austausch bedanken. Ebenfalls möchte ich mich bei Prof Dr. Stefanie Wienkoop, Martin Brenner, Julian Preiner und Dr. Jens Hartung für Ihre Unterstützung und erfolgreiche Kooperation bedanken.

Mein Dank gilt auch Prof. Dr. Uwe Ludewig, Prof. Dr. Günter Neumann und Melissa Mang für Ihre großartige Unterstützung und Austausch über Rhizoboxen und Wurzelproben. Ebenfalls möchte ich Dr. Evi Oburger und Michael Santangeli für Ihr Hilfsbereitschaft und Ideen danken. Prof. Dr. Doris Vetterlein, Dr. Susanne Schreiter sowie Eva Lippold möchte ich für Ihre Organisation, Ideen und stets schnelle Rückmeldung danken. Des Weiterem Danke ich Johannes Günther und Lydia Foitzik für ihre Motivation und Geduld bei den NMR-Messungen.

Für ihre tatkräftige Unterstützung, Geduld und nahezu unendliche Kreativität möchte ich der TZ Werkstatt Hohenheim unter der Leitung von Reinhold Renner danken. Ebenso danke ich den Gärtnern der Serviceeinheit der Hohenheimer Gewächshäuser. Vielen Dank Stefan Rühle, Kerstin Maier, Helmut Fuchs, Fabian Gaiser, Matthias Bader, Bärbel Rassow, Erika Schmidt-Guilliard für eure kompetente und großartige Unterstützung.

Mein herzlicher Dank an Isabelle Engel, Roman Hartwig, Lilith Weissflog und Christian Platz für Ihre großartigen Abschlussarbeiten im Rahmen dieses Projektes.

Ich danke dem „Team 340e“ Dr. Nikolaus Merkt, Lilit Manukyan, Dr. Markus Dier, Azin Rekowski, Dr. Xudong Zhang, Alex Kröper, Johanna Beeck, Susanne Rohrbach und Patrick

Lehr für die gemeinsame Zeit. Vielen Dank Dr. Maria Romo Pérez, Melissa Kleb und Dr. Bastian Leander Franzisky für die großartige Zeit in unserem Büro. Bei Christiane Beierle, Gyöngyi Bardos und Dagmar Repper will ich mich für ihre großartige Unterstützung und Wissen im Labor bedanken.

Großer Dank gilt meiner Familie und Freunden, Christiane, Klaus-Jürgen, Roman und besonders Katharina und Christoph. Vielen Dank für eure unablässige Unterstützung und Verständnis.