



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

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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

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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 (Roszkopf 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 above-ground 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 physico-chemical 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). Surface 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 from 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 ($37 \times 53 \times 27 \text{ cm}^3$ 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; $250 \text{ mm} \times 50 \text{ mm}$) connected to an aquarium pump (TetraTec APS 400), thus maintaining 100% relative humidity in the box above the water. The heater was adjusted to $25 \text{ }^\circ\text{C}$ 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 $-20 \text{ }^\circ\text{C}$. Mucilage from all collection days (d3-d7) was pooled.

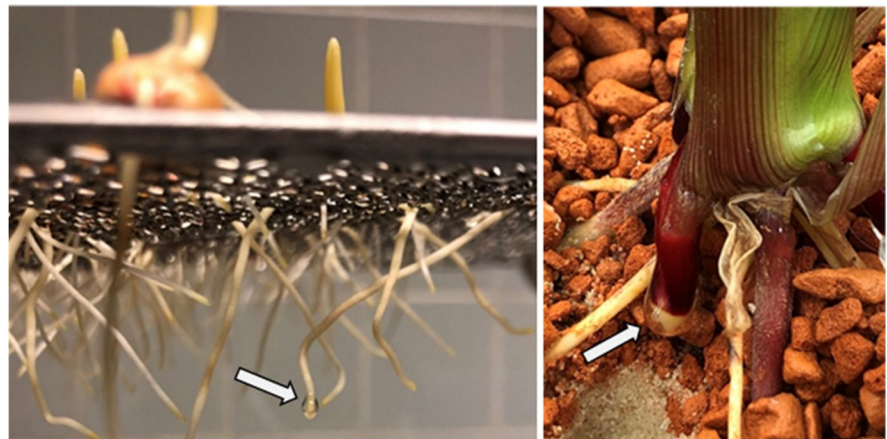
Brace root mucilage collection system (CS_B : pot and field)

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-pot}) or in the field ($CS_{B-field}$). 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 ($51^\circ 22' 0''\text{N}$, $11^\circ 49' 60''\text{E}$; 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 $-20 \text{ }^\circ\text{C}$. Because the amount of mucilage that could be collected from each plant by this method was somewhat lower than expected, the mucilage from $CS_{B-field}$ 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-pot}).

The pot experiment was conducted at the University of Hohenheim ($48^\circ 42' 39.2''\text{N}$, $9^\circ 11' 53.0''\text{E}$). 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-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 $-20\text{ }^{\circ}\text{C}$. The mucilage from $\text{CS}_{\text{B-pot}}$ 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 $\text{CS}_{\text{B-pot}}$ and $\text{CS}_{\text{B-field}}$.

Mucilage analysis

Mucilage preparation and analysis of pH

Collected CS_{A} and $\text{CS}_{\text{B-pot}}$ 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 ($\text{CS}_{\text{B-pot}}$: 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 $4\text{ }^{\circ}\text{C}$) 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 $\text{CS}_{\text{B-pot}}$ 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 $19\text{ }^{\circ}\text{C}$ was increased by $0.01\text{ }\mu\text{L sec}^{-1}$ 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 $<0.01\text{ mm}$ 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} , $\text{CS}_{\text{B-pot}}$ and $\text{CS}_{\text{B-field}}$ mucilage was assessed by flow measurements using an MCR 102 rheometer (Anthon Paar, Ostfildern, Germany) with a truncated cone and plate geometry (CP50-1, $d=50\text{ mm}$; angle of 1°) at $20\text{ }^{\circ}\text{C}$. The gap was 0.01 mm for $800\text{ }\mu\text{L}$ sample volume. Viscosity of the redissolved mucilage was measured at a shear rate between 0.001 s^{-1} and $10,000\text{ s}^{-1}$. Samples were measured in triplicates.

Contact angles of CS_{A} and $\text{CS}_{\text{B-pot}}$ 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, $3\text{ }\mu\text{L}$ of ultrapure water ($18.2\text{ M}\Omega\cdot\text{cm}$) 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 $\text{CS}_{\text{B-pot}}$ 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 (MarsXpress, CEM GmbH, Germany) using a heating ramp of 15 min followed by constant heating at $200\text{ }^{\circ}\text{C}$ 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-pot} mucilage was further diluted in ultrapure water to a final concentration of 2.5 mg mL^{-1} and then filtered through a $0.45 \mu\text{m}$ 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 ($50 \times 8 \text{ mm}$, particle size $10 \mu\text{m}$, PSS Suprema) and two columns for gel permeation chromatography ($300 \times 8 \text{ mm}$, particle size $10 \mu\text{m}$, PSS Suprema). A sample volume of $80 \mu\text{L}$ 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 ($70 \text{ }^\circ\text{C}$, 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 $CS_{B-field}$ 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 $105 \text{ }^\circ\text{C}$ 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 ($40 \text{ }^\circ\text{C}$; 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 $-20 \text{ }^\circ\text{C}$.

Derivatisation to aldonitrile 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 (aerobic 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-pot} mucilage was higher compared to that of CS_A , reaching mean values of 74.9 mN m^{-1} (CS_{B-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-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-pot} and $CS_{B-field}$ 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-pot} compared to $CS_{B-field}$ at the lower shear rates (Fig. 3), the shape of the viscosity flow curves of CS_{B-pot} and $CS_{B-field}$ 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).

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

The concentrations of all measured nutrients were higher in CS_A compared to CS_{B-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-pot}) $\mu\text{mol g}^{-1}$ dry mucilage, followed by Ca with 105 (CS_A) and 47 (CS_{B-pot}), Mg with 29 (CS_A) and 15 (CS_{B-pot}) and Na with 62 (CS_A) and 2 (CS_{B-pot}) $\mu\text{mol g}^{-1}$ dry mucilage. Interestingly, Na was more abundant than Mg in CS_A , while the opposite was observed in CS_{B-pot}

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-pot}) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of five (a) and ten to nineteen (b) technical replicates

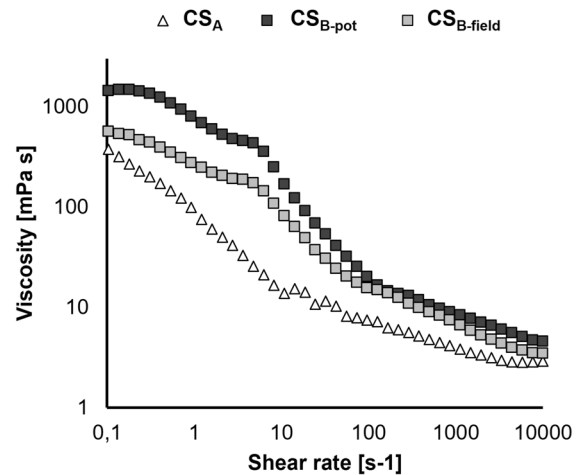
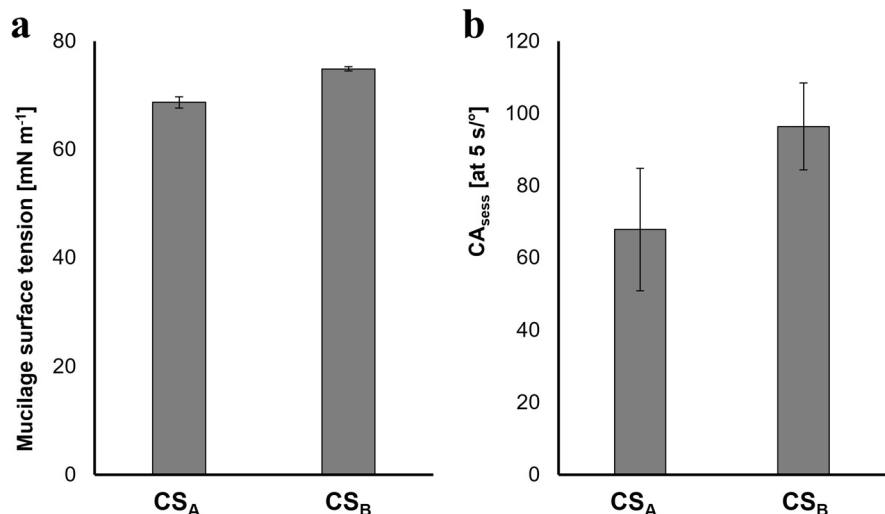


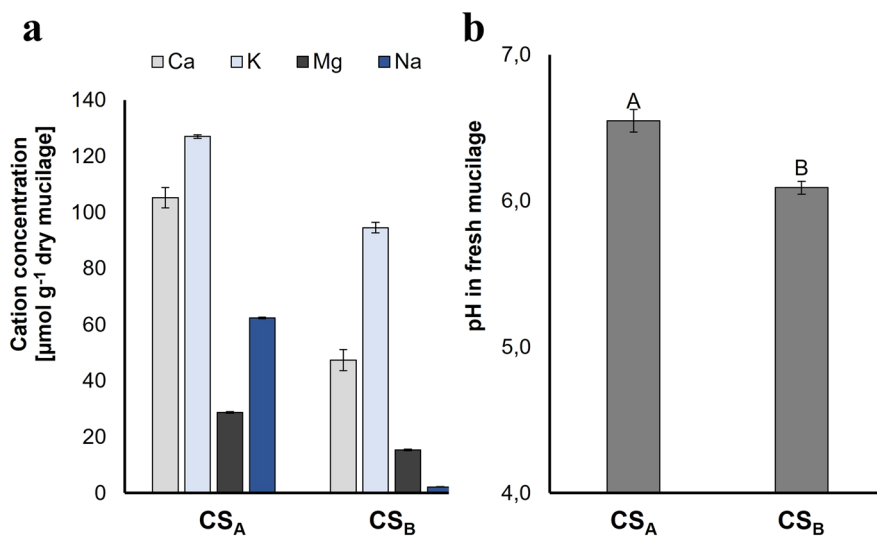
Fig. 3 Viscosity flow curve of aeroponically produced seedling root (CS_A) and brace root mucilage (CS_{B-pot} and $CS_{B-field}$) of maize (*Zea mays* L.)

(Fig. 4a). The pH value of CS_A mucilage was higher by half a unit (6.5) compared to CS_{B-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-pot} . Even though the calibration covered a broad range of molar masses, no peak

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-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-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-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 $CS_{B-field}$ compared to CS_A (Fig. 6a). Galactose was always the most abundant sugar with 43% in $CS_{B-field}$ 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 $CS_{B-field}$ and CS_A , respectively. Mannose was only detectable in $CS_{B-field}$ with 7%. While hexoses were more prominent in $CS_{B-field}$ (76%) than in CS_A (65%) mainly due to a very large ratio of galactose and the presence of mannose in $CS_{B-field}$, pentoses were higher in CS_A (35%) compared to $CS_{B-field}$ (24%), and this was observed for both identified pentoses arabinose and xylose (Fig. 6b).

RT: ■ 13 min □ 24 min □ 51 min

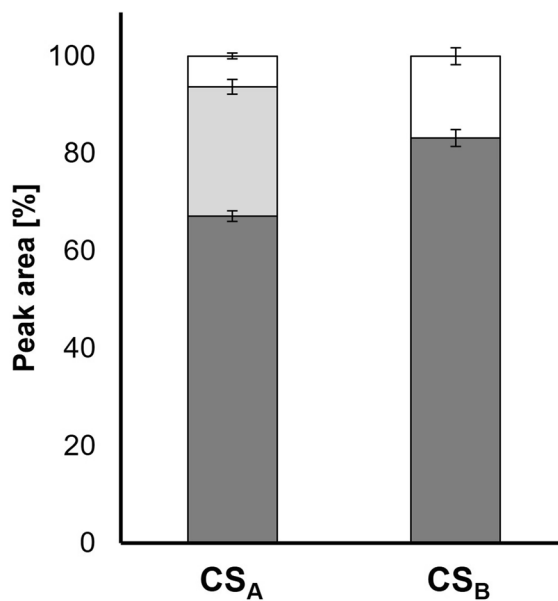
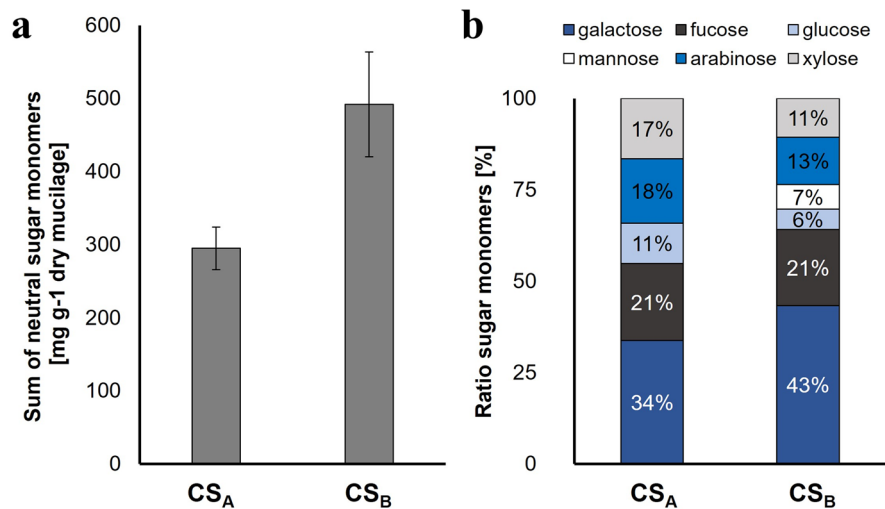


Fig. 5 Relative peak areas corresponding to the retention times (RT) measured of aeroponically produced seedling root (CS_A) and brace root (CS_{B-pot}) mucilage of maize (*Zea mays* L.). Error bars indicate standard deviations of three technical replicates

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 aerobically produced seedling root (CS_A) and brace root ($CS_{B-field}$) 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-pot} and $CS_{B-field}$) had higher viscosities compared to CS_A (Fig. 3). Despite differences in absolute values of CS_{B-pot} and $CS_{B-field}$, 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-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-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-pot} .

A higher viscosity was also observed for $CS_{B-field}$ compared to CS_A (Fig. 3). Here, a significantly higher content of total sugar monomers (Fig. 6a) and especially of galactose was determined in $CS_{B-field}$. 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 $CS_{B-field}$. This would be fully in line with the larger polymer sizes identified in CS_{B-pot} (Fig. 5). We would like to point out that CS_{B-pot} and $CS_{B-field}$ 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 $CS_{B-field}$ and SEC or other measurements from CS_{B-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 $CS_{B-field}$ 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-pot} and $CS_{B-field}$ 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-pot} compared to CS_A . Together with the higher total sugar concentration in $CS_{B-field}$ (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 system 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 indispensable 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 physico-chemical 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 $CS_{B-field}$, 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 $CS_{B-field}$ 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-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 $CS_{B-field}$ 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-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 from brace roots of field-grown plants. However, this would remain limited to plants at later growth stages rather than young seedlings.

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Declarations

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

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