Effect of changing chemical environment on physical properties of maize root mucilage

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Abstract

Aims High viscosity, low surface tension and hydrophobicity are specific properties of maize root mucilage which contribute to modulate the spatial configuration of the liquid phase in soil pores. Several processes in the rhizosphere, in particularly nutrient absorption, root exudation and microbial activity, may cause strong temporal variations in the chemistry of the soil solution of the rhizosphere. Although the physical properties of maize root mucilage have been repeatedly measured in the last years, their variation upon a changing chemical environment and understanding of the chemical mechanisms governing these properties remain unexplored.

Methods We investigated how flow and surface properties of maize root mucilage varied by changes in pH, calcium chloride \((\text{CaCl}_2)\) and lecithin concentrations.

Results The physical properties of mucilage can strongly vary depending on the environmental conditions. Low surface tension of maize root mucilage at \(\text{pH}7\) was increased by addition of calcium. Upon pH change and lecithin addition, hydrophobic mucilage turned hydrophilic. High Ca concentration above 0.83 mmol Ca (g dry mucilage)\(^{-1}\), the addition of 167 μg lecithin (g dry mucilage)\(^{-1}\) and a pH rise to 9 decreased the viscosity of mucilage.

Conclusion Such variations strongly suggest that the role of mucilage in hydraulic processes in the rhizosphere depends on changes of solutes concentration and composition, which themselves vary according to plant growth and soil water content. It seems that mucilage can best serve as a hydraulic bridge only under certain chemical environments, whose spatio-temporal occurrence in the changing rhizosphere remains to be defined.

Keywords Maize mucilage · Wettability · Surface tension · Viscosity · Microstructure

Introduction

In the last years, proof has been repeatedly made that mucilage exuded by plant roots plays an important role in processes at the root-soil interface. Due to its specific properties, it modulates hydraulic and
structural properties of the rhizosphere in comparison to bulk soil (Ahmed et al. 2017; Kroener et al. 2018; Naveed et al. 2019, 2017). Gel and surface properties of hydrated mucilage, like viscosity and surface tension, as well as of dried mucilage, like surface roughness and contact angle, are key properties affecting rhizosphere hydraulic processes.

Surface tension determines the spatial configuration of the capillary bridge formed by water between two soil particles and the work needed to stretch the gas–liquid interface (Benard et al. 2021). Surface tension opposes to the stretching of the liquid bridge, which shrinks during drying and eventually breaks up as drying progresses (Benard et al. 2019). Viscosity opposes to stretching of the bridge and can prevent its break-up. Shear-thinning viscosity means that the polymers disentangle with increasing shear rate (Mezger 2014). Plant mucilage is generally shear-thinning (Brax et al. 2020; Naveed et al. 2017). Physical models predict that the high viscosity and low surface tension of mucilage support the maintenance of the connectivity of the liquid phase in the rhizosphere under dry conditions, so that mucilage could serve as a hydraulic connector for the plant (Benard et al. 2019). The contact angle determines the ability of a solid surface to repel a liquid. Characteristics such as surface roughness or surface chemistry affect the wettability. Substrates with a contact angle over 90° are considered as water-repellent. A high contact angle of dried mucilage indicates that this mucilage may render the dried rhizosphere hydrophobic (Benard et al. 2018; Kaltenbach et al. 2018) and delay its rewetting (Carminati et al. 2010), which can be considered as a strategy for regulating plant water supply (Carminati and Vetterlein 2013).

Physical processes taking place at the root interface are increasingly understood. However, the effect of changes in the chemical environment of mucilage on its physical properties remains to be investigated. Understanding these effects is necessary to get a general understanding of root-soil-interactions. The chemical environment of roots can vary considerably. By diffusion through mucilage or by rehydrating dry mucilage, the soil solution becomes an inherent part of it. As a weak polyelectrolyte and physical gel, root mucilage (RM) is expected to vary greatly in its properties depending on the environmental conditions. A reduction in viscosity with decreasing pH was measured for chia and flax seed mucilage (SM) and expresses a partial suppression of electrostatic repulsion between the polymer chains. In contrast, an increase in viscosity with increasing pH was related to an increasing net electrostatic repulsion between the deprotonated carboxylic groups (with an acidic dissociation constant, pKa, ~2) on the polymer chains (Capitani et al. 2015; Chen et al. 2006). Multivalent cations can strongly increase the viscosity of an ionic gel due to the formation of junction zones between the polymer strands resulting in an increased strength of the polymeric network. Used as model RM (Albalasmeh and Ghezzehei 2014; Barré and Hallett 2009), polygalacturonic acid (100% wt free uronic acid) and low-methoxy pectin (>50% wt free uronic acids) build ionic gels governed by strong interactions between calcium (Ca) and deprotonated uronic acid. In contrast, multivalent cations can also lead to a drop of the viscosity such as for chia SM also used as a model for RM (Benard et al. 2018; Zarebanadkouki et al. 2019), or maize RM (Brax et al. 2020; Koocheki et al. 2013). The gelling mechanism of chia SM is governed by non-ionic electrostatic interactions between entangled polymer chains due to its low uronic acid contents (<30 wt% free uronic acid) (Brax et al. 2017). Nazari et al. (2020) also measured very low amounts of uronic acid (below 5% of all monosaccharides) in maize RM, suggesting that its gelling mechanism is similar to chia SM. In this case, intermolecular charge-charge repulsion in negatively charged polyelectrolyte molecules leads to molecule expansion and entanglement of the polymers, and is partly responsible for their high viscosity. It is not clear if the negative charges from the polyelectrolyte come from deprotonated uronic acids or other components. The addition of positive ions suppresses these repulsion forces and such charge screening leads to the coiling of the polymer chains and a drop of the viscosity, as they do not interact strongly together anymore. Recent results reported a drop of the viscosity of maize root mucilage upon calcium addition (Brax et al. 2020). A similar drop of mucilage’s viscosity in a calcium-rich soil solution could modify the way mucilage affects rhizosphere hydraulic processes.

Observations that mucilage turns hydrophobic upon drying (Ahmed et al. 2015) suggest that surface-active substances accumulate at the solid-air interface during drying (Naveed et al. 2019; Read et al. 2003). Their very low concentration makes
their quantification challenging (Read et al. 2003) but Naveed et al. (2017) achieved a measure with gas chromatography and mass spectrometer (GC–MS) and comparison of their results with an international database revealed that hydroponic maize root mucilage contains around 120 μg fatty acids (g dry mucilage) −1. Also dissolved organic matter in soil, which varies between 15 and 50 mg L−1 for arable soils contains between 30 to 60% little soluble substances such as plant debris (lignocellulose and lignin) and small surface-active humic substances (Blume et al. 2002). Read et al. (2003) took lecithin as model for surface-active substances in mucilage and measured similar effects of lecithin as wheat RM on surface tension in the concentration range 0–1.0 mg mL−1. Lipids are further supposed to decrease the surface tension of mucilage at the gas–liquid interface, which may help roots to extract more water from the surrounding soil (Read et al. 2003; Benard et al. 2019). Additionally, rhizosphere microorganisms such as bacteria (Goswami and Deka, 2019) or endophytic fungi (Adnan et al. 2018) release biosurfactants in the form of glycolipids or fatty acids. Depending on their chemical structure, mucilage polymers and lipids can interact through ionic, hydrophobic and hydrogen-bonding mechanisms (Alvarez-Lorenzo and Concheiro 2003) and lead to modified viscoelastic and surface properties of the gels. Generally, addition of surfactants to polysaccharides decreases the surface tension until a plateau value at the critical micelle concentration (CAC), at which surfactants aggregate in the form of micelles on polymer segments (Goddard 1993; Rütering et al. 2018).

Therefore, we expect that changing environmental conditions in the rhizosphere, e.g., in water content, pH, presence of mono- and divalent cations, and surface-active substances, lead to structural modifications in mucilage and therefore to a variation of its properties. The objective of this study was to reach a mechanistic understanding about the role of various solutes on structural modifications and resulting properties in mucilage. For this, the effect of changes in environmental conditions (pH change, addition of calcium chloride and of lecithin) was studied on purified and reswollen mucilage with measurements of the viscosity, the contact angle and the surface tension of maize RM. The pH and calcium concentration range were chosen in a range of soil solutions of arable soils (Blume et al. 2002) and lecithin concentration in the range of fatty acids estimated in mucilage (Naveed et al. 2017). Purification of mucilage by ethanol (EtOH) precipitation enabled a clear distinction on the effect of the solutes on physico-chemical properties of mucilage. Effect of EtOH precipitation on physico-chemical composition of maize RM is also investigated to clearly assess the effect of solutes on purified mucilage. The following hypotheses were formulated to reach this goal.

**Hypothesis 1**: The removal of small and polar solutes by EtOH precipitation, such as organic acids and phospholipids, increases viscosity due to accumulation of high molecular weight (HMW) substances. Maize RM is hypothesized to behave as a weak polyelectrolyte whose gelling mechanism relies on electrostatic interactions between the polymers. Therefore, we expect a decrease of the viscosity upon Ca addition. Further, higher protonation at low pH should decrease charge-charge interactions and repulsion between polymers and thus the viscosity, which should in contrary increase upon deprotonation at high pH due to stronger repulsion between deprotonated side groups of polymer chains. Addition of low amounts of lecithin below the CAC should not affect viscosity.

**Hypothesis 2**: We hypothesize that the removal of surface-active molecules by EtOH precipitation increases surface tension. Further, we expect that as a strong electrolyte, addition of CaCl₂ increases the surface tension of maize RM. Protonation and deprotonation of acidic functional groups at low and high pH should little affect the surface tension due to the low amount of uronic acid groups previously measured in maize RM. As surface-active molecule, lecithin should decrease the surface tension due to disruption of attractive forces between the polymers at the surface.

**Hypothesis 3**: We hypothesize that EtOH reduces the contact angle due to the removal of surface-active substances that hydrophobized raw mucilage surface after drying. We also expect that hydrophilic polymer segments orientate inward upon drying and hypothesize that addition of a strong electrolyte such as CaCl₂ does not affect its wet-tability, as it will interact with hydrophilic parts of the polymer. Therefore, we hypothesize that protonation and deprotonation at low and high pH of
acidic functional groups orientated inward should not affect strongly the wettability of dried muci-
lage. We also suspect a decrease of wettability due to an outward orientation of the hydrophobic seg-
ments of the lecithin molecules at the mucilage-air interface.

To test these hypotheses and clear how various chemical conditions affect polymer interactions in mucilage, maize RM was purified from amino acids, small peptides or nucleotides and non-polymeric sub-
stances such as organic acids with EtOH precipita-
tion. EtOH acts as an antisolvent which disrupts the screening of charges by water and leads to the precipi-
tation of electrically attracted groups with opposite charges. Crude and purified maize RM were charac-
terized by measuring cations content, by quantifying amount and size of molecular weight material with size exclusion chromatography and by imaging mucilage’s microstructure with atomic force microscopy. Purified mucilage was rehydrated at a concentration of 3 mg mL$^{-1}$. The effects of pH change, Ca ions concentration and lecithin concentration were sepa-
rately tested. The pH was varied between 4,7 and 9. Calcium chloride (CaCl$_2$) and lecithin were added to concentrations between 0.17, 0.83 and 1.7 mmol Ca (g dry mucilage)$^{-1}$ and between 33 and 167 μg leci-
thin (g dry mucilage)$^{-1}$, respectively. Viscosity, sur-
face tension and contact angle were measured for all treatments.

Material and methods

Treatments

Collection of maize root mucilage Maize RM from Zea mays was collected and stored as described in details by Brax et al. (2020) using an aeroponic method to grow the seeds and freeze-drying to dry and store the mucilage. Mucilage solutions of 3 mg mL$^{-1}$ were prepared out of freeze-dried mucilage in ultrapure water (18.2 MΩ·cm) and is further referred as NT (non-treated).

Purification of mucilage Maize RM (10 mL, 3 mg mL$^{-1}$) was mixed with EtOH (40 mL 99.8%) in an ice bath on a shaking bank for 2 h and centrifuged (20 min, 5000 RPM, Hettich Centrifuge, Universal 320, Tuttingen, Germany). The supernatant was carefully removed and stored separately. The mucilage pellet was washed two times with EtOH (5 mL, 99.8%). Each time, it was incubated 1 h in an ice bath on the shaking bank and centrifuged (15 min, 5000 RPM). The EtOH supernatant of the two washing steps was discarded. After washing, the pellet was dissolved in ultrapure water (20 mL), dried in the freeze-dryer and weighted.

The dielectric constant of EtOH is with 24.3 much smaller than the one of water with 78.4 at 298 K, which means that the electrostatic forces between charged particles are lower in water than in EtOH. Thus, EtOH disrupts the screening of charges by water and the electrical attraction between oppositely charged groups becomes strong enough to form stable ionic bonds, which precipitates the polymers.

Treatments of purified maize RM Dry EtOH pre-
cipitated mucilage was redissolved in different solutions. The sample referred to as EtOH was redissolved in ultrapure water. All other samples were redissolved in a solution adjusted to a total activity of 15 mM containing only sodium chloride (NaCl) (pH7), or NaCl in combination with i) hydrogen chloride (HCl) or sodium hydroxide (NaOH) to achieve pH4 or pH9, ii) 0.5, 2.5 and 5 mM CaCl$_2$ corresponding to 0.17, 0.83 and 1.7 mmol Ca (g dry mucilage)$^{-1}$ and iii) 0.1 and 0.5 mg L$^{-1}$ lecithin (L-α-Phosphatidylcholine, ≥ 99% Sigma-Aldrich) corresponding to 33 and 167 μg lecithin (g dry mucilage)$^{-1}$.

Cation composition and molecular size

Cation analysis Dissolved samples of mucilage (1 mL) were weighted and digested in aqua regia (2 mL) in a microwave by a 15 min heating ramp fol-
lowed by constant heating at 200 °C for 40 min. The digests were subsequently analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720 Series, Germany) for the metals Ca, magnesium (Mg), sodium (Na) and potassium (K). Results were related to the dry mass of digested mucilage and samples measured in triplicates.

Size exclusion chromatography Dextran is com-
monly used as a standard to assess the molecular size of polysaccharides (Condezo-Hoyos et al. 2015). Freeze-dried crude and purified mucilage and dextran standards (Weight averaged molecular weight (Mw) 80.9 kDa, 312 kDa, 490 kDa from PSS Suprema;
Mw 147.6 kDa, 409.8 kDa, 1.5 mDa/667.8 kDa from Sigma) were dissolved in ultrapure water (2.4 mg mL\(^{-1}\)) by overhead mixing at 4 °C overnight and then filtered through 0.45 µm filter. The size distribution of the samples was measured with liquid chromatography coupled with an evaporative light scattering detector (LC-ELSD) (Thermo Scientific Accela LC system, Thermo Fisher Scientific, CA USA, coupled with a Sedex 90LT, Sedere, France) based on the methods of Xia et al. (2020) and of Muñoz-Almagro et al. (2018). The device was equipped with a guard column (50×8 mm, particle size 10 µm, PSS Suprema) and two following columns for gel permeation chromatography columns (300×8 mm, particle size 10 µm, PSS Suprema). A sample volume of 80 µL was injected at room temperature under a constant flow of 1 mL/min of ammonium formate (50 mM) with 60 min measurement time and measured with an ELSD detector (70 °C, Gain 1, filter 1 s). The samples were analysed in triplicates.

Microstructure characterization with atomic force microscopy (AFM)

**AFM measurement** Untreated and EtOH precipitated maize RM (30 µL, 0.1 mg mL\(^{-1}\)) was spread on a freshly cleaved mica disc (highest grade V1, 12 mm diameter, NanoAndMore GMBH, Germany) with a dried mucilage surface area concentration of 3 µg cm\(^{-2}\). The samples were air dried for 1 h. Topography and adhesion forces of untreated and EtOH purified maize RM were studied with AFM (Dimension Icon, Bruker) in PeakForce Quantitative Nanomechanical Mapping (PFQNM) (Pittenger et al. 2010). For this, AFM measurements were performed with PeakForce Tapping mode (Nanoscope, Bruker) using silicon nitride probes with v-shaped cantilevers (SNL10-A probes with a nominal spring constant of 0.35 N m\(^{-1}\) and a nominal tip radius of 6 nm, Bruker) at a scan rate of 0.5 Hz and a modulation frequency (PeakForce frequency) of 2 kHz at 24 ± 1 °C. For every measuring point, topography and nanomechanical properties calculated online from the force-distance curves were simultaneously mapped in separate image channels. To control reproducibility and reliability of measurements, the tip radius of the AFM probe was frequently measured with the absolute method (Pittenger et al. 2010). Regions of interest for AFM imaging were chosen visually with the help of the optical camera of the AFM system in order to avoid the regions with high agglomerated material and to identify the smooth coated surfaces. For each sample, three regions of interest were investigated by scanning three different areas with scan size of 1 and 3 µm. Each image consisted of 512 samples per lines.

**Pore size and coverage analysis** Coverage percentage and hole size distribution of dried maize RM network were calculated on AFM pictures with a voxel-based code (Geodict, 2021/44873). Holes and solid areas of the 2D AFM pictures were segmented using the Otsu threshold method, which automatically performed clustering-based image thresholding and reduced a grey-scale image to a binary image (“ImportGeo-Vol User Guide,” 2021). The segmented image was then further analysed as a voxelized geometry for quantitative analysis. The coverage percentage is defined by dividing the number of solid voxels to the total number of voxels. Hole radius was determined by fitting circles into the hole areas and hole size distribution was computed.

**Physical properties**

**Viscosity** Flow measurements were conducted using a 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. Gap was 0.101 mm for 600 µL sample volume measured between 0.001 and 1000 s\(^{-1}\) in 7 logarithmic steps. Samples were measured in triplicates. At very low shear rates, the viscosity tends to a plateau value for some samples, the zero-shear-rate viscosity \(\eta_0\). We were not able to measure reliable plateau value at very low shear rates (< 0.1 s\(^{-1}\)) for all samples: low viscous gels such as mucilage can be subjected to artefacts at very low shear rates due to non-equilibration (Laun et al. 2014). Other methods such as rotational viscometry with concentric cylinders are better adapted to measure the viscosity of low viscous gels at such shear rates (Mezger 2014). In this study, the significance of the differently treated maize RM was assessed for viscosity values at a shear rate of 1 s\(^{-1}\), as mucilage’s viscosity for soil processes such as water flow is most relevant at low shear rates.
Contact angle  Contact angles were measured by the sessile drop method using a video-based optical contact angle measuring device (OCA15Pro, Data-Physics, Filderstadt, Germany). The same maize RM preparations as for the viscosity were spread on a glass slide to a dry concentration of 0.138 mg cm$^{-2}$ and dried at ambient temperature in a desiccator over silica gel for 4 days. The glass slide surface needs to be super hydrophilic to achieve a homogeneous coverage of the surface with mucilage and a complete wetting of the substrate. The glass slides used as substrate showed stable values around 58°, which is unfavourable (Kaltenbach et al. 2018). Therefore, the glass slides were previously dipped for 10 min in an ultrasonic bath consecutively in acetone, isopropanol and distilled water. After cleaning, the contact angle of the glass slides was below 10°. After mucilage was dried, a drop of double distilled water (3 µL) was pipetted carefully on the sample. Variation of the shape of the drop and thus of the contact angle over time was recorded for 25 s using the SCA20 software (DataPhysics Filderstadt, Germany). The contact angle at a drop age of 5 s is reported in this paper. Five to six replicates were measured for each sample.

Surface tension  The surface tension was measured with the pendent drop method, which consists in the analysis of a liquid drop of a maximum volume hanging on a dosing needle. For this, a disposable 1 mL glass syringe (Omnifix®-F) equipped with a canula (Sterican® 18G / 1,2×40 mm, B. Braun Melsungen AG, Melsungen, Germany) was filled with the sample (3 mg mL$^{-1}$) and fixed in the video-based optical contact angle device (OCA15Pro, DataPhysics, Filderstadt, Germany). A hanging drop of 10 – 20 µL was dosed at the needle end by the dosing unit of the device and captured as picture. The drop volume was increased in 0.1 µL steps and captured at each step after an equilibration time of 10 s, until the drop fell down within the equilibration time. The procedure was repeated for five replicate drops. The last picture with the highest drop volume was then evaluated for its surface tension with the pendant drop plugin (Daerr and Mogne 2016) of the ImageJ software (Schneider et al. 2012) using the needle diameter of each picture to set the correct scale of pixel per mm.

Statistical analysis  Significant effects of the three treatments, which are calcium addition, pH change and lecithin addition on surface properties were tested with ANOVA using R (R Core Team 2020) and the rstatix package (Kassambara 2020). Post-hoc testing of pairwise comparisons for the boxplots was performed either by Tukey test in case Shapiro–Wilk normality test and Levene test were not significant (p > 0.05) or by a Games-Howell test. Raw data of all measurements are reported on Mendeley Data (Knott 2021).

Results

Effect of purification on cation composition

Untreated maize RM had a pH value of 7.2 and an electrical conductivity (EC) of 413 µS cm$^{-1}$, while pH increased slightly to 7.9 and EC decreased to 123 µS cm$^{-1}$ upon EtOH precipitation. The cation concentrations of the treated samples (EtOH, SN) are related to the respective used amount of untreated mucilage. Untreated maize RM contained a similar content of K and Ca of 0.52 mmol g$^{-1}$, which was five times more than Na and two times more than Mg contents (Fig. 1). EtOH precipitation induced a stronger depletion of monovalent ions as 80% of K and 72% of Na were removed in contrast to only 10% of Ca and 21% of Mg (Fig. 1). Recovery of K and Na were respectively 53% and 70% in contrast to 112% and 96% for Ca and Mg. Cations were analysed from the supernatant of the first EtOH precipitation. K and Na were probably extracted in high amounts in the second and third EtOH precipitation, explaining the low recovery.

![Fig. 1 Cation content in untreated (NT) maize RM, after EtOH precipitation (EtOH) and in its supernatant (SN) in mmol cation (g dry mucilage)$^{-1}$. Error bars show standard deviation](image)
Effect of purification on molecular size distribution

Although the dextran calibration curve extended for molecular size between 1500 kDa (RT 16 min) and 80 kDa (RT 19 min) (Knott 2021), all mucilage peaks came either before or after the biggest or smallest dextran standard. Thus, their dextran equivalent molar mass is an estimation based on the calculated calibration curve. Untreated Maize RM revealed to be composed to 90% of HMW material, itself fractioned in three distinct polymer populations. One has a very high molar mass with an apex retention time of 14.4 min which corresponds to a molar mass of dextran of 2980 kDa (Fig. 2A). The two further components of middle molecular weight have retention times of 23.4 and 25.5 min corresponding to a molar mass of 2.4 kDa and 850 Da of dextran. A very small component appears at a retention time of 51 min equivalent to a molar mass of a 0.03 Da dextran molecule (Fig. 2A). The distribution of the peaks and the peak areas calculated from the scaled intensity reveal that EtOH precipitation suppressed the low molecular weight components (RT 51 min, light grey) and enriched mucilage in very high molecular weight component (RT 14.4 min, dark) (Fig. 2B). The components with middle molecular weights (RT 23.4 and 25.5 min) could not be clearly separated by EtOH precipitation (Fig. 2B). That these middle-sized components didn’t precipitate upon the drastic reduction of the dielectric constant suggests that they have interacted poorly and may be mostly composed of neutral moieties.

Nanoscale structure of untreated and purified mucilage

The AFM image of untreated maize RM (Fig. 3A) is characterized by a continuous coating of the dried film punctuated by holes and shows therefore similar topographic features to the ones of diluted maize RM published by Kaltenbach et al. (2018). In contrast, the holes in dried EtOH purified mucilage are more numerous but their size is smaller than in untreated maize RM (Fig. 3B-C). The cumulative geometric hole diameter distribution (Fig. 3C) in dried mucilage shows the discrepancy between the hole diameters (D) with a 50th percentile ($D_{50}$) of 97±9 nm in the untreated and a roughly half as large $D_{50}$ of 52±4 nm in the purified maize RM. Despite various holes diameter and number, mucilage covered the glass slides similarly. Mucilage coverage was with 61±2% in the untreated sample slightly lower than of the purified sample with 68±2%.

Viscosity of crude, purified and chemically treated mucilage

As expected, maize RM was shear-thinning (Fig. 4) and whatever treatment applied, treated mucilage had a higher viscosity than untreated one due to the enrichment of mucilage in HMW material shown in Fig. 2 (Fig. 5). Generally, the differences in the viscosity of maize RM subjected to the various treatments are significant until a shear rate of 100 s$^{-1}$, above which the curves merge (Fig. 4, A-D). At higher shear forces, the interactions between the
polymers are overcome and the viscosity approaches that of water of 1 mPa\cdot s.

The viscosity of untreated maize RM (8.4 ± 1.8 mPa\cdot s) increased strongly after EtOH precipitation.

**Fig. 3** Examples of AFM height images for diluted untreated (NT) (A) and EtOH precipitated (B) maize RM (3 μg cm.−2) and corresponding cumulative geometric hole pore distribution of the holes in the dried mucilage film (C) represented for three replicates for each treatment.

**Fig. 4** Viscosity curves of maize RM (3 mg mL−1) nontreated and after EtOH purification in MQ water and at ionic strength 15 mM NaCl (pH 7) (A), after addition of 0.17–1.7 mmol Ca (g dry mucilage)−1 (B), upon pH change (C) and after addition of 33 and 167 μg lecithin (g dry mucilage)−1 (D). Error bars show standard deviation.

**Fig. 5** Viscosity of maize RM (3 mg mL−1) after various treatments at shear rate 1 s.−1. Error bars show standard deviation and significant differences tested with Tukey HSD test are indicated by different letters for p < 0.05.
(87 ± 13.5 mPa·s). Adjustment of the ionic strength to 15 mM with NaCl at pH 7 further increased the viscosity significantly to 477 ± 139 mPa·s (Fig. 5). As the ionic strength of the further samples was also adjusted to 15 mM, their viscosity is compared to the pH 7 sample. Adjustment to a low concentration of 0.17 mmol Ca (g dry mucilage)⁻¹ increased the viscosity, whereas a low lecithin concentration of 33 μg (g dry mucilage)⁻¹ and a low pH of 4 did not affect significantly the viscosity in comparison to pH 7 sample (Fig. 5). In contrast, adjustment to 0.83 and 1.7 mmol Ca (g dry mucilage)⁻¹ decreased strongly the viscosity at 1 s⁻¹ to values below 100 mPa·s and an increase of the pH to 9 also decreased the viscosity to 133 ± 68 mPa·s (Fig. 5). However, none of the further treatments after EtOH precipitation were able to reduce the viscosity to the level of the nontreated sample.

Surface tension of crude, purified and chemically treated mucilage

The EtOH purification slightly increased the low surface tension of untreated maize RM 52.5 ± 4.8 mN m⁻¹ (Fig. 6A). Adjustment of the ionic strength to 15 mM with NaCl had no significant effect on the surface tension, but addition and increase of CaCl₂ concentration increased the surface tension strongly (Fig. 6B). pH change didn’t affect the surface tension (Fig. 6C). Whereas low lecithin concentration of 33 μg (g dry mucilage)⁻¹ did not vary the surface tension significantly and even tended to increase it, further increase of lecithin concentration to 167 μg (g dry mucilage)⁻¹ led to a strong drop of the surface tension (Fig. 6D).

Wettability of crude, purified and chemically treated mucilage

EtOH precipitation rendered untreated maize RM, which had a sessile drop contact angle of 65 ± 12° after 5 s (CA₅₅) hydrophobic with CA₅₅ of 98 ± 6° (Fig. 7A). Maize RM remained hydrophobic upon adjustment of the ionic strength to 15 mM (Fig. 7A). Addition of CaCl₂ had no significant effect on the contact angle, although the adjustment to low concentration of 0.17 mmol Ca (g dry mucilage)⁻¹ increased the median to 120° with high distribution of the replicate values, whereas the adjustment to high concentration >0.83 mmol Ca (g dry mucilage)⁻¹ notably decreased the distribution width of the contact angle values (Fig. 7B). Adjustment to pH 4 and pH 9

![Fig. 6](image-url)
strongly decreased the contact angle turning maize RM hydrophilic and tightened the distribution of the replicate values (Fig. 7C). The same overwhelming effect was caused by the addition of lecithin and a further increase of its concentration (Fig. 7D) strongly reduced the contact angle below 50° with a very tight distribution width.

Discussion

Effect of treatment on viscosity

The viscosity of maize RM at 4.6 mg mL$^{-1}$ at 1 s$^{-1}$ measured by Naveed et al. (2019) is in the range of 1 to 10 mPa s$^{-1}$ and fits to our measurement of untreated maize RM. The drop of K and Na concentration by the EtOH purification of mucilage indicates that most K and Na interacted poorly with mucilage polymers. In comparison, divalent cations, whose removal upon purification was less marked, may play a cross-linking role between the polymer strands and precipitate out of the solution upon reduction of the dielectric constant.

To our current knowledge, size distribution of maize RM polymers was measured for the first time in this study. The strong increase of the HMW fraction and of the viscosity upon EtOH treatment suggests that this fraction is mostly responsible for the high viscosity of maize RM. The accumulation of HMW polymers increases their possibility for an entanglement which increases their internal frictions and thus confirms the part of hypothesis 1 suggesting an increase of viscosity due to higher amount of HMW substances. The changed nanoscale microstructure also outlines changes in the polymeric network structure upon purification. Increase of the entanglement as in purified mucilage leads to the formation of a strong connecting structure in the swollen state, which does not allow the formation of large holes during drying but forms thick strands and small holes. In contrast, the fewer and less entangled large polymers in the untreated mucilage have less junction zones leading to larger holes and thinner strands in the dried structure.

Viscosity surprisingly increased upon NaCl addition, although an increase of the ionic strength is generally expected to decrease the viscosity of...
polyelectrolytes (Medina-Torres et al. 2000). Swelling of polyelectrolytes is determined by a balance between the osmotic pressure of free ions acting to swell the gel and the elasticity of the gel that restricts swelling (Rubinstein et al. 1996). In the ion depleted mucilage, the polymer chains have probably not swollen to their full extent due to lack of favourable interactions between charged polymer moieties and lacking charges in the solvent. By an increase of the ionic strength and thus of the osmotic pressure, the polymer chains may have unfolded and swollen, and their subsequent entanglement increased the viscosity. As ionic strength was kept at 15 mM for Ca, lecithin and pH treatments, the results are comparable to the pH 7 treatment.

In order to exclude the effect of ionic strength and thus of the osmotic pressure, the pure effect of Ca on the viscosity can be assessed by comparison of the pH 7 treatment with the Ca treatments as for all these treatments, ionic strength was kept at 15 mM. The added 0.17 mmol Ca (g dry mucilage)$^{-1}$ is $\sim 2$ times higher than the amount of divalent cation (Ca and Mg) lost upon EtOH precipitation. The slight increase of the viscosity of purified maize RM suggests that some cross-linking sites in maize RM freed upon purification were occupied again upon addition of low contents of Ca. In contrast, the drop of the viscosity upon the addition of Ca over 0.83 mmol Ca (g dry mucilage)$^{-1}$ corresponds to the results of Brax et al. (2020) and expresses an oversaturation of RM with Ca. The intermolecular charge-charge repulsion between the stretched-out polymers, which is partly responsible for high viscosity of polyelectrolyte gels, decreases strongly due to their contraction and coiling around divalent Ca ions (Koocheki et al. 2013). In comparison, the viscosity of polygalacturonate acid (PGA) and low methoxy pectin, whose gelation mechanism relies on Ca-crosslinks with uronic acids, increases upon addition of CaCl$_2$ (de Kerchove and Elimelech 2007). This partial collapse of the gel structure upon Ca addition confirms again that the gelling mechanism of maize RM does not only rely on Ca-crosslinks with uronic acids (Brax et al. 2019) as formerly supposed for RM (Albalasmeh and Ghezzehei 2014; Gessa et al. 2005), but rather on electrostatic interactions as supposed in hypothesis 1. This also applies for chia seed mucilage and mucilage gum of Opuntia ficus indica as their viscosity also decreases upon calcium addition (Capitani et al. 2015; Medina-Torres et al. 2000). The concentration dependent effect of Ca on the viscosity of maize RM has also been reported for flax seed mucilage (Chen et al. 2006). The authors explained it similarly by arguing that low Ca concentration facilitates the formation of 3D networks by Ca cross-linking, whereas high Ca concentration decreases the zeta potential and leads to a contraction of the polymers.

In contrast to hypothesis 1, the decrease of the viscosity measured at pH 9 suggests that the pH driven viscosity changes in maize RM is dominated by hydrogen bonding, as was also measured in a neutral polysaccharide extracted from maca roots (Lee et al. 2020). Hydrogen bonding in polysaccharides is based on hemiacetal oxygen, hydroxyl or methyl groups of the sugar residues (Tako 2015). As deprotonation of hydroxyl groups occurs at higher pH (> 12 for glucose), deprotonation of water in water bridges could partly disrupt the hydrogen bonds network and thus some entanglements and junction zones of the polymers, leading to a decrease of the viscosity. Still, the destabilization of the network at pH 9 is small with respect to the stabilizing effects of purification (increase of entanglements).

Decrease of viscosity upon addition of low amount of lecithin was surprising and contradicts hypothesis 1. Viscosity and surface tension variations upon lecithin addition are discussed together in the next subchapter considering a supposed alteration of mucilage network affecting both properties similarly.

Effect of treatment on surface properties: surface tension

The surface tension of the untreated maize RM of the present study was similar to those measured by Read and Gregory (1997) and by Naveed et al. (2019) for maize RM in comparable concentrations. Surface tension of untreated mucilage did not increase significantly upon EtOH precipitation as expected in hypothesis 2, which suggests that the substances controlling the surface tension were not effectively removed. Probably, polymeric mucilage substances play a larger role in lowering the surface tension with respect to that of pure water. This contrasts with the wide spread expectation that surfactants such as phospholipids present in mucilage are responsible for its low surface tension (Naveed et al. 2019; Read et al. 2003). Polysaccharides can actually also decrease surface tension, as 0.2 wt% of gellan
gum, i-carrageenan and agar show values lower than 60 mN m\(^{-1}\) (De Fenoyl et al. 2018). Reasons for a decrease of surface tension driven by polysaccharides are various. The chemical mechanism explaining the drop of surface tension by increasing concentration of PGA, a highly hydrophilic and negatively charged polymer exhibiting typical surfactant properties, is the repulsion between the negatively charged groups of the polymers (Chen and Arye 2016). Repulsion between these polymers implies that they are not pulled inward the water drop very strongly to occupy minimum surface area and therefore exhibit a reduced surface tension in contrast to water. Another possible explanation for the reduced surface tension in purified mucilage despite the removal of surface-active molecules is that hydrophobic microdomains possibly present in polysaccharides can accumulate at the liquid–air interface and thus decrease the surface tension (Henni et al. 2005). Both mechanisms are probably at play in mucilage.

Among the surface-active molecules, EtOH precipitation may also have removed amino acids, small peptides or nucleotides, which are probably present in RM according to the small C/N ratio of 11 measured in hydroponic maize RM (Brax et al. 2020) and the ~6 wt% amino acids identified in maize RM (Naveed et al. 2017). Chemical screening of the supernatant for surface-active molecules together with studies of its surface activity would help to clear the role of small surface-active molecules on mucilage properties. Around 90% of the proteins identified in maize RM of seedlings grown axenically were close homologues to the ones identified in pea, Arabidopsis and rapeseed mucilage (Ma et al. 2010). This suggests common biotic functions such as protection from fungal infections (Ma et al. 2010; Nazari et al. 2020) and abiotic functions such as water binding by arabinoxylan proteins (Fincher et al. 1983; Knee et al. 2001) also identified in maize RM (Bacic et al. 1986). Still, the question remains non-answered: despite their low concentration in mucilage, how do proteins affect mucilage physical properties? Proteins can lower the surface tension of carbohydrates solutions (Adhikari et al. 2007), which could explain the low surface tension of mucilage.

Adjustment of the ionic strength at 15 mM at pH 7 to compensate the ion depletion after EtOH precipitation did not vary significantly the surface properties of dry and wet mucilage. Also, surface tension did not increase significantly from pH 7 upon pH modification to pH 4 or pH 9. By increasing strongly the surface tension with a narrow distribution of the replicate values with increasing Ca content, the addition of CaCl\(_2\) and Ca-induced changes of the network dominate the effect of mucilage polymers. Ca-driven contraction of the polymers has already been shown to contribute to surface tension increase (Tsaih and Chen 1997). Especially the strong electrolyte character of CaCl\(_2\) leads to an increase of the surface tension with increasing CaCl\(_2\) concentration as the surface layer is depleted of salt. This negative adsorption is explained by the Debye theory of interionic attractions, which states that interionic forces draw the ions away from the surface and that these interionic forces become more effective at higher concentrations (Jones and Ray 1941).

Phospholipids could not be quantified in maize RM due to their extremely low concentration despite using the mass spectrometer in total ion detection mode (Read et al. 2003). The adjustment of lecithin concentration of mucilage to 33 and 167 μg lecithin (g dry mucilage)\(^{-1}\) corresponds to 0.1 and 0.5 mg L\(^{-1}\). Studies on the effect of surfactants on polymers generally focus on much higher concentrations in the range of mg mL\(^{-1}\) around the critical micelle concentration (CMC) which is in the order of 1 mg mL\(^{-1}\) for soy lecithin (Urum and Pekdemir 2004). Although we added an extremely low lecithin amount in comparison to the concentration at which micelles form, we could already observe variations in physical properties of maize RM mucilage. Interestingly, lecithin had a similar concentration dependent effect on both, viscosity and surface tension. Reduction of surfactant efficiency by polymers has already been measured for PGA mixtures, as very low lecithin concentrations in PGA solution had similar surface tension as pure PGA (Chen and Arye 2016). It seems that maize RM also reduces the surface activity of lecithin at very low concentration. Upon further increase of lecithin concentration, the polymers are “overloaded” and lecithin migrates at the surface, thus reducing the surface tension, and disrupts further electrostatic polymer interactions leading to a drop of the viscosity.

Effect of treatment on wettability

The CA of 65±12° from the aeroponically produced untreated maize RM of this study is in good agreement to the CA of around 35° from maize root
mucilage collected from seedlings grown aeroponically and diluted to a concentration of 0.06 mg cm$^{-2}$ (Zickenrott et al. 2016). In contrast, Ahmed et al. (2015) found higher contact angle values $\sim 95^\circ$ after 5 s for a concentration of 0.11 mg/cm$^2$ of brace roots of five-week-old maize plant. Incomplete removal of phospholipids upon EtOH precipitation (Price et al. 2020) does not explain the increase of the contact angle after purification, which contradicts hypothesis 3. One chemical explanation for the appearance of hydrophobic regions in dry mucilage in general suggests an amphiphilic nature of the polymers and a reorientation of amphiphilic molecules such as proteins, or flexible hydrophilic and hydrophobic moieties. In a hydrated state, polar polymer moieties of mucilage interact with water while hydrophobic polymer moieties may interact with other hydrophobic moieties in intra- and intermolecular associations, which form hydrophobic micro domains by the exclusion of water (Henni et al. 2005; Wesslén and Wesslén 2002). With the reduction of hydration water during drying, polar groups interact with each other, coiling into the inside of the mucilage associations, thus exhibiting the hydrophobic moieties towards the outside.

An additional reason for the hydrophobicity of EtOH purified mucilage might be the creation of stronger and more numerous junction zones between polymer aggregates, illustrated by the decrease of the hole diameters in the AFM images of the drying pattern. These junction zones reduce the flexibility of polymer chains so that their re-orientation towards the drop of water needs more time. Variable wettability of soil organic matter surface in soil depending on various cation-water molecules bridges was similarly explained by Schaumann et al. (2013). The stronger the cation-water molecule bridges are, the more difficult it is for water to wet the surface.

The outstanding stability of the contact angle values against changes in Ca$^{2+}$ concentration keeping maize RM hydrophobic confirm hypothesis 3. An explanation for the hydrophobicity is that through coiling of the polymers around the Ca ions, the hydrophilic moieties of the polymers orientate in the inside, whereas the hydrophobic moieties turn towards the outside. For low concentration of 0.17 mmol Ca (g dry mucilage)$^{-1}$, the orientation of the polymers in the dried network follows this trend but not as homogeneously as for the higher concentrations, explaining the wider distribution of the values. The increased wettability and tight distribution of the replicate values upon addition of acid or base contradicts hypothesis 3. The reason may be that NaOH and even more HCl build hygroscopic nano or micro domains upon drying at room temperature. These domains are quicker rehydrated than the dried polymers. Again, in contrast to hypothesis 3, maize RM turned strongly and increasingly hydrophilic with increasing lecithin concentration. This shows that the effect of lecithin dominates the one of mucilage polymers on wettability. We explained decrease in wettability after EtOH purification and removal of small surface-active substances by the inflexibility of the remaining large polymers. Addition of small amphiphilic substances, which are more flexible and thus reorientate faster than large polymers as soon as water is available like lecithin explain the increase of mucilage’s wettability upon lecithin addition.

Can variation of mucilage properties upon environmental changes affect its role in the rhizosphere?

The chemical environment in the rhizosphere differs from the bulk soil due to constant root exudation and exchange with soil solution (McGahan et al. 2014). Ca concentrations in soil solution of arable soils (pH 5–8) vary between 1–4 mmol L$^{-1}$ (Blume et al. 2002) and are in the range of those investigated in this study. Furthermore, a significant decrease of pH in the rhizosphere upon plant growth up to 1–2 units in comparison to the bulk soil solution, an accumulation of Ca and Mg ions and a depletion of K ions as well as an increase of the osmotic potential in rhizosphere soil solution were measured upon time (Nye 1981; Lorenz et al. 1994; Vetterlein and Jahn 2004). Therefore, our experiments cover a potentially possible scenario in the rhizosphere.

Several processes in the rhizosphere depend on mucilage properties. Viscosity of mucilage does not only control the mobility of mucilage itself to spread within the pore space but also the capability of water to flow within the polymeric network and thus the hydraulic conductivity of the rhizosphere, which is roughly proportional to viscosity. E.g., a sandy soil prepared with chia mucilage at concentrations of 3–5 mg dry mucilage per g dry soil had a saturated conductivity that was 100 times reduced compared to
the one of untreated soil (Kroener et al. 2014). Thus, a drop of mucilage’s viscosity, e.g., due to Ca enrichment, would increase the water flow under saturated conditions supporting the water uptake by the plants. Our results further suggest that in highly weathered Ca poor soils maize mucilage would keep its high viscosity during rehydration in a soil solution enriched with monovalent cations. This might reduce the water flow and thus make the water uptake by the plants more difficult, but also reduce the loss of water from the rhizosphere to the bulk soil.

In the unsaturated case, the viscosity of mucilage strongly controls the spatial configuration and formation of liquid bridges across the pore space. According to Carminati et al. (2017), mucilage’s high viscosity and low surface tension also allow long liquid filaments to persist even at very negative water potential (Carminati et al. 2017). The Ohnesorge number (Ohnesorge 1936) defines the break-up force point of liquid filaments in the drying rhizosphere and is inversely proportional to the square root of surface tension and proportional to the viscosity. Accordingly, an increase of its surface tension and drop of viscosity would reduce the formation of liquid bridges under unsaturated conditions. This again is affecting not only hydraulic processes like water retention and unsaturated hydraulic conductivity but probably also solute transport and mobility of microbial communities. Furthermore, it suggests that maize mucilage in tropical soils with low bivalent cation contents may better mitigate the low water availability. By the formation of more stable liquid bridges than possible under conditions with high bivalent cations, the water connectivity between roots and soil can be maintained even at very low water contents.

The wide range of contact angles of dry mucilage from 50° to 120° achieved by chemical treatments in this study, shows that the environmental rhizosphere conditions may control whether dry rhizosphere will turn water repellent or whether it can easily be rewetted after irrigation following a drought period. For hydrophobic dry rhizosphere, Carminati et al. (2010) showed that it may take up to 2–3 days following irrigation to recover and to finally arrive at bulk water content. pH change or phospholipid presence (through bacterial turnover, for example) could decrease the hydrophobicity of the dry rhizosphere and accelerate water absorption.

Variations in the effect of mucilage on rhizosphere processes caused by changes in the chemistry of the environment can be related to rhizosphere functions supporting water availability and thus plant growth. The viscosity of mucilage decreases and its surface tension increases upon high bivalent ion concentrations, which rather appear at low water contents (Vetterlein and Jahn 2004). Thus, the initial ability of mucilage to be a hydraulic bridge between the bulk soil and the roots due to its high viscosity and low surface tension decreases with decreasing soil moisture. In the same time, the polymer concentration of mucilage increases during drying, which strongly increases the viscosity and thus the ability of mucilage to be a hydraulic bridge between the bulk soil and the roots. However, during rehydration after a dry period in which cations are enriched especially in the rhizosphere, mucilage’s properties may be governed by the type of cations. The more bivalent cations are available, the lower is the viscosity of the hydrated mucilage and the better water can flow through the rhizosphere.

Considering that such dry conditions rather appear in mid-summer, the plant may have built a root network deep and wide enough to come to water without help of mucilage as a hydraulic bridge. In contrast, chemical environment in the rhizosphere of the young plant, which possesses small roots which may not be able to get water at low matric potential, could support the role of mucilage by creating a continuity with bulk soil through its high viscosity and low surface tension. This functional shift of the role of mucilage is in accordance with the model of Carminati and Vetterlein (2013), who suggest that the contrasting properties of mucilage are an expression of the rhizosphere’s plasticity and its time dynamics. The authors distinguish between a “young” rhizosphere with mucilage keeping a “good” hydraulic contact between soil and roots. In this young rhizosphere, mucilage would still have a high viscosity and low surface tension. In an older rhizosphere, connection between roots and soil is “loose” due to gaps or hydrophobic mucilage. In this case, mucilage would have a lower viscosity and higher surface tension than in the young rhizosphere and dries more rapidly under drying conditions, not building filaments. In this case, the hydrophobicity of dried mucilage protects roots from losing water to the low moisture soil.
Still, care is needed for the interpretation of the results as changes in rhizosphere soil solution depends on soil. For example, a decrease in Ca and Mg concentrations were measured in the rhizosphere of tropical acid soils (Calba et al. 2004). Fertilization also changes strongly chemical composition of soil solution. A limit of this study for the interpretation of the results on rhizosphere processes is obviously the use of aeroponically produced purified mucilage, whose properties very probably vary from mucilage exuded by grown below-ground roots. Mucilage’s chemical composition was also changed artificially and does not reflect the composition of rhizosphere soil solution. Moreover, it is not clear how long mucilage exuded at one specific time and location keeps its properties before degradation or how far it diffuses in the rhizosphere. Also, only one specific mucilage was investigated. Gel and surface properties of other RM such as from barley and wheat differ from the ones of maize RM (Brax et al. 2020; Naveed et al. 2019). Barley RM was even suggested to behave as a surfactant in the rhizosphere in contrast to maize RM supposed to behave as a hydrogel (Naveed et al. 2019). Therefore, it remains to determine how strong the role of mucilage in the rhizosphere varies with the plant species. Despite these limits, this study gives some keys to assess the role of mucilage in the rhizosphere and suggests that taking into account the variation of mucilage physical properties with chemical environment is highly important when developing physical models taking into account the role of mucilage.

**Conclusion**

Our results outline how closely linked chemical composition, structural arrangement and physical properties of mucilage are. Chemical composition and variation of mucilage microstructure could explain its physical properties, as smaller holes and thicker strands in the microstructure of purified maize RM corresponded to a higher fraction of HMW material and a strong increase of its viscosity. Changes in pH, Ca and dissolved organic matter concentration in the mucilage solution led to structural modifications of mucilage polymers, which modified mucilage physical properties. Low surface tension was increased by the addition of calcium. From hydrophobic, mucilage turned hydrophilic upon pH change and lecithin addition. Viscosity of mucilage decreased upon the higher concentrations of added Ca and lecithin and at high pH. A mechanistic understanding of these underlying processes is indeed necessary, as such variations of flow and surface properties of mucilage may reduce or cancel its effect on hydraulic processes in the rhizosphere depending on rhizosphere soil solution. This study not only finally sheds light on the direct interactions between maize mucilage and its chemical environment, it also gives strong indications for mucilage’s ability for functional shifts mitigating spatio-temporal changes in the environmental conditions in the rhizosphere.

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