

Effect of environmental conditions on physical properties of maize root mucilage.

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Abstract

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. Being a hotspot for nutrient absorption, root exudation and microbial activity, the rhizosphere soil solution is suspected to chemically vary strongly upon time. 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. Therefore, we investigated how flow and surface properties of maize root mucilage varied by changes in pH, CaCl_2 and lecithin concentrations.

Results reveal that the physical properties of mucilage can strongly vary depending on the environmental conditions. Low surface tension of maize root mucilage at pH7 was increased by addition of calcium. Upon pH change and lecithin addition, hydrophobic mucilage turned hydrophilic. Viscosity of mucilage decreased with increasing Ca concentration above 2.5 mM, the addition of 0.5 mg/L lecithin and a pH rise to 9. 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.

Introduction

In the last years, proof has been repeatedly made that mucilage exuded by plant roots plays an important role in the processes at the root-soil interface. Due to its specific properties, it modulates the 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). The gel and surface properties of hydrated mucilage, like viscosity and surface tension, as well as of dried mucilage, like nano-scale pattern and contact angle, are key properties affecting rhizosphere hydraulic processes. 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 water reservoir for the plant (Benard et al., 2019). On the other side, the high contact angle of dried 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 global understanding of the role of mucilage in the rhizosphere. The chemical environment, in which the roots grow, 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 susceptible to vary greatly in its properties

depending on the environmental conditions. Hydrogen bonding, as one main stabilization mechanism of such gels (Brax et al., 2017; Rees and Welsh, 1977), should vary by changes of the pH value. Multivalent cations can increase strongly the viscosity of an ionic gel due to the formation of egg-box-like junction zones or in contrast lead to a drop of the viscosity by charge screening and coiling of the polymer chains such as for maize RM (Brax et al., 2020; Koocheki et al., 2013). 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.

Lipids found in root exudates are principally phosphatidylcholines, also called lecithin, (Read et al., 2003) and are supposed to strongly affect surface properties of mucilage. Lecithin possesses a head charged with ammonium and phosphate groups and a tail which can be a variety of fatty acids. Observations that mucilage turns hydrophobic upon drying (Ahmed et al., 2015) suggest that these surface-active substances accumulate at the solid-air interface during drying (Naveed et al., 2019; Read et al., 2003), although their concentration could not be measured until now (Read et al., 2003). Phospholipids 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 (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 phospholipids 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 (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 environmental conditions (pH change, addition of CaCl_2 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. Purification of mucilage by EtOH precipitation enabled a clear distinction on the effect of the solutes on physico-chemical properties of mucilage. 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. 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 critical aggregation concentration (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 CaCl_2 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 do 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 in the inward upon drying and hypothesize that addition of a strong electrolyte such as CaCl_2 does not affect its wettability, as it will interact with hydrophilic parts of the polymer. For the same reason, protonation and deprotonation of acidic functional groups at low and high pH should not affect strongly the wettability of dried mucilage. We also suspect a decrease of wettability due to orientation of hydrophobic segments of lecithin molecules at mucilage-air interface.

To test these hypotheses and clear how various chemical conditions affect polymer interactions in mucilage, maize RM was purified from non-polymeric substances such as organic acids with ethanol (EtOH) precipitation. EtOH acts as an antisolvent which disrupts the screening of charges by water and leads to the precipitation of electrically attracted groups with opposite charges. Crude and purified maize RM were characterized 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 in a solution with an ionic strength of 15 mM set with NaCl according to the ionic strength of the other additives. The pH was varied between 4,7 and 9. CaCl_2 and lecithin were added to concentrations between 0.5, 2.5 and 5 mM and between 0.1 and 0.5 mg/L, respectively. Viscosity, surface tension and contact angle were measured for all treatments.

Material And Methods

Treatments

Collection of maize root mucilage. Maize RM from *Zea Mais* 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 were prepared out of freeze-dried mucilage in ultrapure water (18.2 M Ω •cm) and is further referred as NT (non-treated).

Purification of mucilage. Maize RM (10 mL, 3 mg/mL) was mixed with EtOH (40 mL 99.8%) in an ice bath on a shaking bank for 2 h and centrifuged (20min, 5000 RPM, Hettich Centrifuge, Universal 320, Tuttlingen, 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.

Treatments of purified maize RM: Dry EtOH precipitated 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 with an activity of 15 mM containing only NaCl (pH7), or NaCl in combination with i) HCl or NaOH to achieve pH4 or pH9, ii) 0.5, 2.5 and 5 mM CaCl₂ and iii) 0.1 and 0.5 mg/L lecithin (L- α -Phosphatidylcholine, Sigma-Aldrich).

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 followed 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, Mg, Na and K. Results were related to the dry mass of digested mucilage and samples measured in triplicates.

Size exclusion chromatography: Freeze-dried crude and purified mucilage and dextran standards (Mw 80.9 kDa, 312 kDa, 490 kDa from PSS; Mw 147.6 kDa, 409.8 kDa, 1.5 mDa/667.8 kDa from Sigma) were dissolved in ultrapure water (2.4 mg/mL) by overhead mixing at 4°C overnight and then filtered through 0.45 μ m filter. The size distribution of the samples was measured with LC-ELSD (liquid chromatography coupled with an evaporative light scattering detector) (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 Munoz-Almagro et al. (2018). The device was equipped with a guard column (50×8 mm, particle size 10 μ m, PSS Suprema) and two following columns for gel permeation chromatography columns (300×8 mm, particle size 10 μ m, PSS Suprema) A sample volume of 80 μ L 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 1s). The samples were analysed in triplicates.

Microstructure characterization with AFM

AFM measurement: Untreated and EtOH precipitated maize RM (30 μL , 0.1 mg/mL) was spread on a freshly cleaved mica disc (highest grade V1, 12 mm diameter, NanoAndMore GMBH, Germany). The samples were air dried for 1 h. The calculated dried mucilage per surface area was 3 $\mu\text{g}/\text{cm}^2$. 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 gray-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 (Anton 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 η_0 . We were not able to measure reliable plateau value at very low shear rates ($\ll 0.1$ 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, T. G., 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, DataPhysics, 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 ($3 \mu\text{L}$) 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, German). 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 x 40 mm, B. Braun Melsungen AG, Melsungen, Germany) was filled with the sample (3 mg/mL) and fixed in the video-based optical contact angle device (OCA15Pro, DataPhysics, Filderstadt, Germany). A hanging drop of $10 - 20 \mu\text{L}$ was dosed at the needle end by the dosing unit of the device and captured as picture. The drop volume was increased in $0.1 \mu\text{L}$ 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 plug-in (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 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. The magnitude of the significative difference is indicated with the numbers of stars, which indicate how many zero are between the comma and the first decimal different from zero of the p-value. 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 $\mu\text{S}/\text{cm}$, while pH increased slightly to 7.9 and EC decreased to 123 $\mu\text{S}/\text{cm}$ 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, 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).

Fig. 1 Cation content in untreated (NT) maize RM, after EtOH precipitation (EtOH) and in its supernatant (SN) in mmol cation per g dry mucilage. Error bars show standard deviation.

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 51min, 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.

Fig. 2 Size exclusion chromatograms (A) and corresponding peak areas according to the peak retention times (RT) (B) of maize root mucilage untreated (NT), ethanol precipitated (EtOH) and its ethanol supernatant (SN). Error bars show standard deviation.

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 show 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. Coverage of mucilage of the untreated sample was with $61\pm 2\%$ slightly lower than of the purified sample with $68\pm 2\%$.

Fig. 3 Examples of AFM height images for diluted untreated (NT) (A) and EtOH precipitated (B) maize RM ($3 \mu\text{g}/\text{cm}^2$) and corresponding cumulative geometric hole pore distribution of the holes in the dried mucilage film (C) represented for three replicates for each treatment.

Viscosity of crude, purified and chemically treated mucilage

As its viscosity decreases under shear strain (Fig. 4), maize RM is shear-thinning, which means that the polymers disentangle with increasing shear rate (Mezger, T. G., 2014). 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 $1 \text{ mPa}\cdot\text{s}$.

Fig. 4 Viscosity curves of maize RM ($3 \text{ mg}/\text{mL}$) nontreated and after EtOH purification in MQ water and at ionic strength 15 mM NaCl (pH7) (A), after calcium addition of $0.5 - 5 \text{ mM CaCl}_2$ (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L^{-1} (D). Error bars show standard deviation.

The viscosity of maize RM at $4.6 \text{ mg}/\text{mL}$ at 1 s^{-1} measured by Naveed et al. (2019) is in the range of 1 to $10 \text{ mPa}\cdot\text{s}$ and fits to our measurement of untreated maize RM. The viscosity of untreated maize RM ($8.4 \pm 1.8 \text{ mPa}\cdot\text{s}$) increased strongly after EtOH precipitation ($87 \pm 13.5 \text{ mPa}\cdot\text{s}$) due to the enrichment of mucilage in HMW material shown in Fig. 2 (Fig. 5). Adjustment of the ionic strength to 15 mM with NaCl at pH7 further increased the viscosity significantly to $477 \pm 139 \text{ mPa}\cdot\text{s}$ (Fig. 5). As the ionic strength of the further samples was also adjusted to 15 mM , their viscosity is compared to the pH7 sample. Adjustment to a low CaCl_2 concentration of 0.5 mM increased the viscosity, whereas a low lecithin concentration of $0.1 \text{ mg}/\text{L}$ and a low pH of 4 did not affect significantly the viscosity in comparison to pH7 sample (Fig. 5). In contrast, adjustment to 2.5 and 5 mM CaCl_2 decreased strongly the viscosity at 1 s^{-1} to values below $100 \text{ mPa}\cdot\text{s}$ and an increase of the pH to 9 also decreased the viscosity to $133 \mp 68 \text{ mPa}\cdot\text{s}$ (Fig. 5).

Fig. 5 Viscosity of maize RM ($3 \text{ mg}/\text{mL}$) after various treatments at shear rate 1 s^{-1} . Error bars show standard deviation and significant differences are indicated by different letters.

Surface tension of crude, purified and chemically treated mucilage

The surface tension of the untreated maize RM of the present study was similar to those measured by Read et al. (1997) and by Naveed et al (2019) for maize RM in comparable concentrations. The EtOH purification slightly increased the low surface tension of untreated maize RM $52.5 \mp 4.8 \text{ mN}/\text{m}$ (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 CaCl_2 concentration increased the surface tension strongly (Fig. 6B). pH change didn't affect the surface tension (Fig. 6C). Whereas low lecithin concentration of $0.1 \text{ mg}/\text{L}$ did not vary the

surface tension significantly and even tended to increase it, further increase of lecithin concentration to 0.5 mg/L led to a drop of the surface tension to values similar to those of untreated mucilage (Fig. 6D).

Fig. 6 Surface tension in mN/m measured at a concentration of 3 mg/mL for maize RM nontreated and after EtOH purification in MQ water and at ionic strength 15 mM (pH7) (A), after calcium addition of 0.5 – 5 mM CaCl₂ (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L⁻¹ (D). Multiple pairwise comparison was done with post-hoc Tukey for A and with Games-Howell test for B-D.

Wettability of crude, purified and chemically treated mucilage

The contact angle 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/cm² (Zickenrott et al., 2016). In contrast, Ahmed et al (2015) found higher contact angle values ~95° after 5 s for a concentration of 0.11 mg/cm² of brace roots of five-week-old maize plant. EtOH precipitation rendered untreated maize RM, which had a sessile drop contact angle of 65±12° after 5 s (CA_{5s}) hydrophobic with CA_{5s} 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 CaCl₂ concentration of 0.5 mM increased the median to 120° with high distribution of the replicate values, whereas the adjustment to high CaCl₂ concentration of >2.5 mM notably decreased the distribution width of the contact angle values (Fig. 7B). Adjustment to pH4 and pH9 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.

Fig. 7 Contact angle 5 s after drop application for 0.138 mg/cm² dry mucilage per area for maize RM (3 mg/mL) nontreated and after EtOH purification in MQ water and at ionic strength 15 mM (pH7) (A), after calcium addition of 0.5 – 5 mM CaCl₂ (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L⁻¹ (D). Multiple pairwise comparison with post-hoc Tukey tests were done for A, B and D and with Games-Howell test for C.

Discussion

Effect of treatment on viscosity

The drop of K and Na concentration by the EtOH purification of mucilage supposes 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 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.

The 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. Further increase of the ionic strength would lead to a drop of the viscosity as polyelectrolytes change their conformation under high ionic strength to a random coil conformation, which is explained by the suppression of intermolecular charge-charge repulsion and enhanced interactions between the polymers (*Roulard et al., 2016*). As ionic strength was kept at 15 mM for Ca, lecithin and low and high pH treatments, the results are comparable to the pH7 treatment.

Addition of 0.5 mM CaCl_2 in 3 mg/mL mucilage corresponds to the addition of 0.16 mmol Ca per g dry maize RM. When comparing this quantity with the amount of Ca in maize RM (mmol/g), it is \tilde{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 upon addition of 0.5 mM CaCl_2 suggests that some cross-linking sites in maize RM freed upon purification were occupied again upon addition of 0.5 mM CaCl_2 . In contrast, the drop of the viscosity upon the adjustment of CaCl_2 concentrations to ≥ 2.5 mM corresponds to the results of *Brax et al. (2020)* and expresses the suppression of intermolecular charge-charge repulsion between the polymers resulting from their contraction through complexation with the added Ca ions and thus the screening of their charges (*Koocheki et al., 2013*). These rearrangements of the polymers upon Ca addition confirm 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.

We expected in hypothesis 1 that the viscosity of maize RM would increase at pH 9 due to net electrostatic repulsion between the deprotonated carboxylic groups (pKa ~2) on the polymer chains and decreases at low pH due to a partial suppression of electrostatic repulsion as measured for chia and flax SM (Capitani et al., 2015; Chen et al., 2006). In contrast, the decrease of the viscosity measured at pH 9 suggests that another mechanism dominates the pH driven viscosity changes in maize RM, namely hydrogen bonding. Hydrogen bonding may play a role to stabilize the network as was measured in a neutral polysaccharide extracted from maca roots (Lee et al., 2020). This may also be an explanation for our results of maize RM and could again reveal that physical properties of maize RM are not driven by deprotonated functional groups, as their fraction in the whole network may be too low. Indeed, only 3-5% uronic acids were measured in maize RM (Brax et al., 2020; Nazari 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

Surface tension of untreated mucilage did not increase significantly upon EtOH precipitation as expected in hypothesis 2, which suggests that mucilage polymers play a dominant role in decreasing the surface tension in contrast with molecular components removed by EtOH precipitation. 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 (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 polygalacturonic acid, 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 micro-domains possibly present in polysaccharides can accumulate at the liquid-air interface and thus decrease the surface tension (Henni et al., 2005). The

orientation of hydrophobic moieties towards the liquid-air surface would also explain the hydrophobicity of dried mucilage.

Adjustment of the ionic strength at 15 mM at pH7 to compensate the ion depletion after EtOH precipitation did not vary significantly the surface properties of dry and wet mucilage. The slight increase of the surface tension could come from added NaCl, as electrolytes raise the surface tension of water due to the electrostatic image repulsive interaction between electrolyte ions and air (Hoorfar et al., 2006; Ohshima and Matsubara, 2004). By increasing strongly the surface tension with a narrow distribution of the replicate values with increasing Ca content, the addition of CaCl₂ and Ca-induced changes of the network dominate the effect of mucilage polymers, which were shown to decrease the surface tension. 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 CaCl₂ leads to an increase of the surface tension with increasing CaCl₂ 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).

Although surface tension did not increase significantly from pH7 upon pH modification to pH4 or pH9, its slight increase could come from the depletion of the charges at the surface. This negative adsorption is smaller for added HCl and NaOH than for added CaCl₂ as HCl and NaOH are not as strong electrolytes as CaCl₂ and were added in smaller amounts to control the pH. pH4 and 9 correspond to the addition of 0.1 mM HCl or NaOH, whereas adjustment of CaCl₂ concentrations started at 0.5 mM.

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 0.1 and 0.5 mg/L corresponds to 33 and 167 µg lecithin per g dry maize RM. Studies on the effect of surfactants on polymers generally focus on much higher concentrations in the range of mg/mL corresponding to concentrations below, around and above the critical aggregation concentration (CAC), which is generally slightly below the critical micelle concentration (CMC) and in the order of 1 mg/mL 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. This suggests that a similar mechanism affected both properties at the higher lecithin content. Reduction of surfactant efficiency by polymers has already been measured for polygalacturonic acid (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, although this does not fully explain the increase of the surface tension. At very low concentration, lecithin may increase the cohesion between polymers allowing ion-dipole association between hydrophilic groups of the polymer and the ionic headgroup of the surfactant, and hydrophobic interactions between the hydrophobic segments of the

polymer and the hydrophobic tail of the phospholipid (Goddard, 1993). Thus, the surface tension increases. The extent of this mechanism should be limited to the charges on the polymers: 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

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. 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 (SOM) surface in soil depending on various cation-water molecule 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. The narrow distribution of the replicate contact angle values of $\geq 2.5 \text{ mM CaCl}_2$ containing samples in contrast to the Ca free pH7 and 0.5 mM CaCl_2 containing maize RM suggests a homogenizing effect of CaCl_2 on the network organisation in the swollen state and upon drying. 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 CaCl_2 concentration of 0.5 mM , the orientation of the polymers in the dried network follows this trend but not as homogeneously as for the higher concentrations, explaining the wide distribution of the values. Although strong interactions with Ca and rearrangement of the chains with coiling reduce viscosity, the contracted chains seem after drying all the same less flexible to reorientate towards water as those without the contraction effect. One explanation is that the removal of hydration water upon drying probably increases the strength of Ca-polymer bridges.

Reason for the increased wettability and tight distribution of the replicate values upon addition of acid or base, which contradicts hypothesis 3, may not be the protonation or deprotonation of the functional groups such as in uronic acids, because they present only a very small percentage of all sugars in maize

RM. Therefore, it is not clear if their (de)protonation is sufficient to explain the drastic change of the wettability of dried maize RM upon changes in pH. One reason could be that, upon drying at room temperature, NaOH, and in an increased manner HCl, builds hygroscopic nano or micro domains, which the water quicker hydrate.

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). Low pH in comparison to bulk soil solution, accumulation of Ca and Mg ions and depletion of K ions as well as an increase of the osmotic potential in rhizosphere soil solution were measured upon time (Lorenz et al., 1994; Vetterlein and Jahn, 2004). We found that depending on the nature of the solutes, maize RM is subjected to various structural modifications leading to tremendous changes of its properties. Surface tension varied almost by a factor of 2 from 45 mN m^{-1} – 70 mN m^{-1} depending on the chemical treatment and mucilage's viscosity even ranged across two orders of magnitude from 10 mPa s – 1000 mPa s . On the one side, the Ohnesorge number – an important parameter for the break-up point/force of liquid filaments in the drying rhizosphere (Carminati et al., 2017) – is inversely proportional to the square root of surface tension and proportional to the viscosity, and on the other side, the hydraulic conductivity is roughly proportional to viscosity. Thus, we can expect that chemical environmental rhizosphere conditions may strongly alter hydraulic rhizosphere processes by affecting mucilage's physico-chemical properties.

Change of mucilage's properties in a calcium-rich soil solution could modify the way mucilage affects rhizosphere hydraulic processes in multiple ways: at first, 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 rhizosphere. 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). Secondly, in the unsaturated case, the viscosity of mucilage strongly controls the spatial configuration and formation of liquid bridges across the pore space. The theory of Ohnesorge (1936) was used to show how mucilage's high viscosity and low surface tension allows long liquid filaments to persist even at very negative water potential (Carminati et al., 2017). Mucilage viscosity and surface tension are therefore central parameters in controlling the break-up point of these filaments in the rhizosphere and thus the connectivity of the liquid phase. 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. Thus, a drop of mucilage's viscosity would increase the water flow under saturated conditions while an increase of its surface tension would reduce the formation of liquid bridges under unsaturated conditions.

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 degradation, for example) could decrease the hydrophobicity of the dry rhizosphere and accelerate water absorption.

A relationship between the various effects of mucilage properties on rhizosphere processes according to the chemical environment might be brought in relationship with plant growth and soil water content: viscosity of mucilage decreases and its surface tension increases upon high 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 (Carminati et al., 2017). 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.

One 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. 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. Although this study finally brings light upon interactions between mucilage and its chemical environment, it also raises the question of the ecological role of mucilage in a chemically changing rhizosphere.

Declarations

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Figures

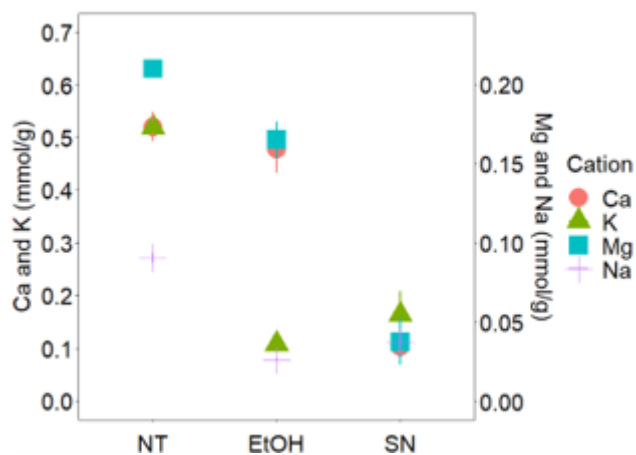


Figure 1

Cation content in untreated (NT) maize RM, after EtOH precipitation (EtOH) and in its supernatant (SN) in mmol cation per g dry mucilage. Error bars show standard deviation.

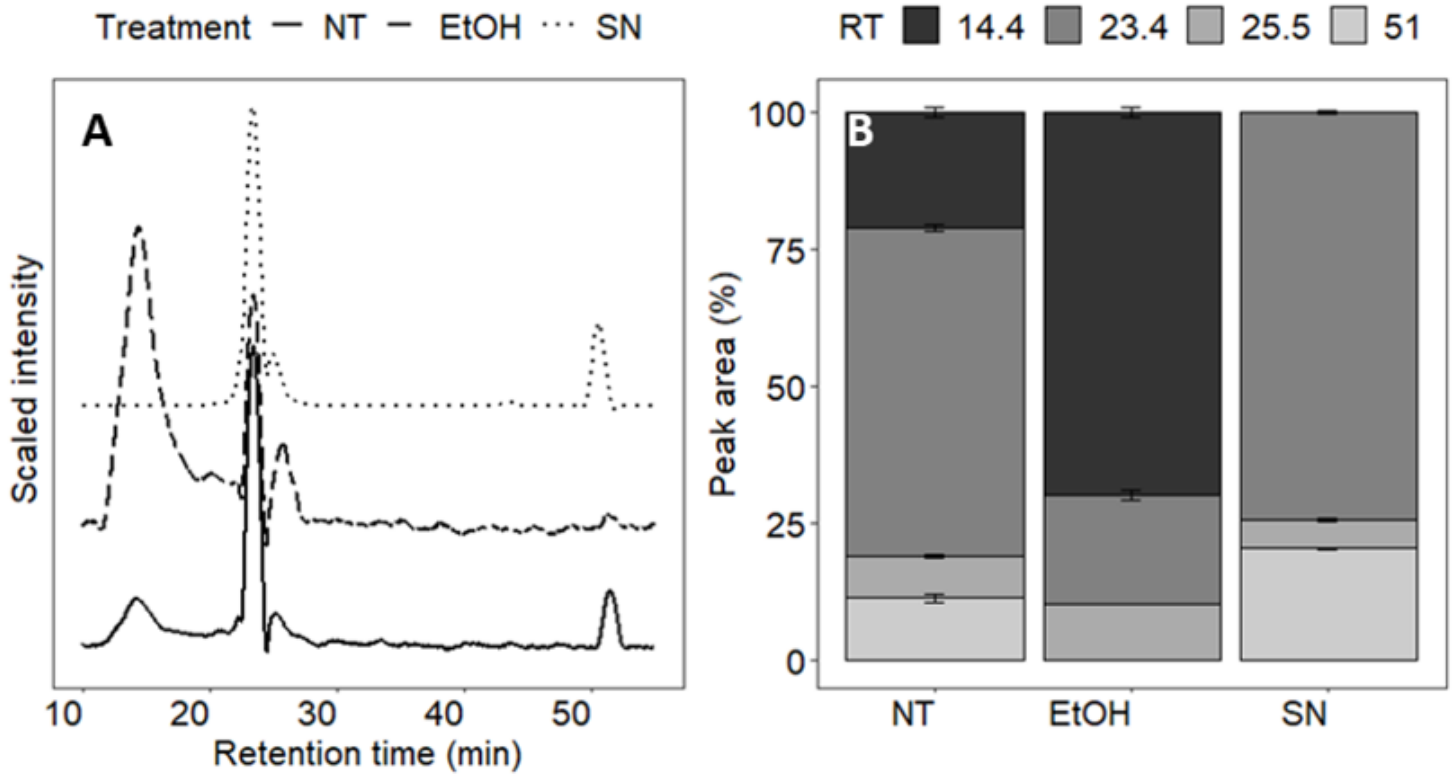


Figure 2

Size exclusion chromatograms (A) and corresponding peak areas according to the peak retention times (RT) (B) of maize root mucilage untreated (NT), ethanol precipitated (EtOH) and its ethanol supernatant (SN). Error bars show standard deviation.

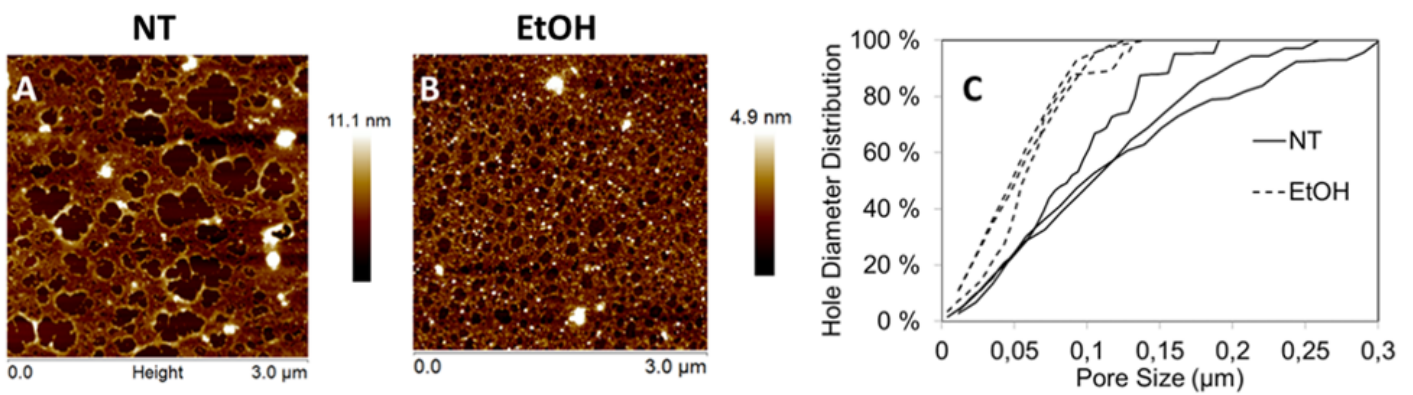


Figure 3

Examples of AFM height images for diluted untreated (NT) (A) and EtOH precipitated (B) maize RM ($3 \mu\text{g}/\text{cm}^2$) and corresponding cumulative geometric hole pore distribution of the holes in the dried mucilage film (C) represented for three replicates for each treatment.

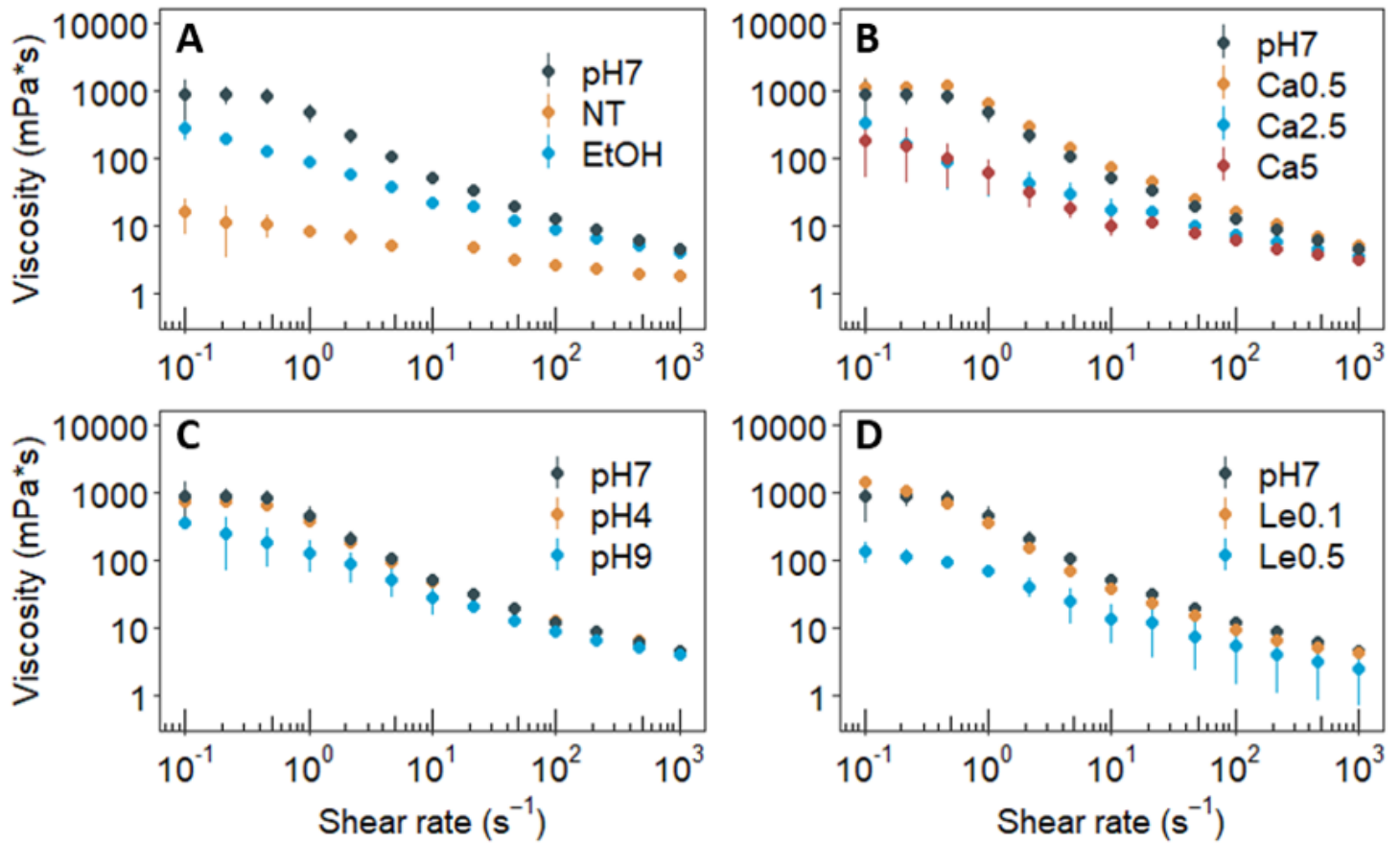


Figure 4

Viscosity curves of maize RM ($3 \text{ mg}/\text{mL}$) nontreated and after EtOH purification in MQ water and at ionic strength 15 mM NaCl ($\text{pH}7$) (A), after calcium addition of $0.5 - 5 \text{ mM CaCl}_2$ (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L^{-1} (D). Error bars show standard deviation.

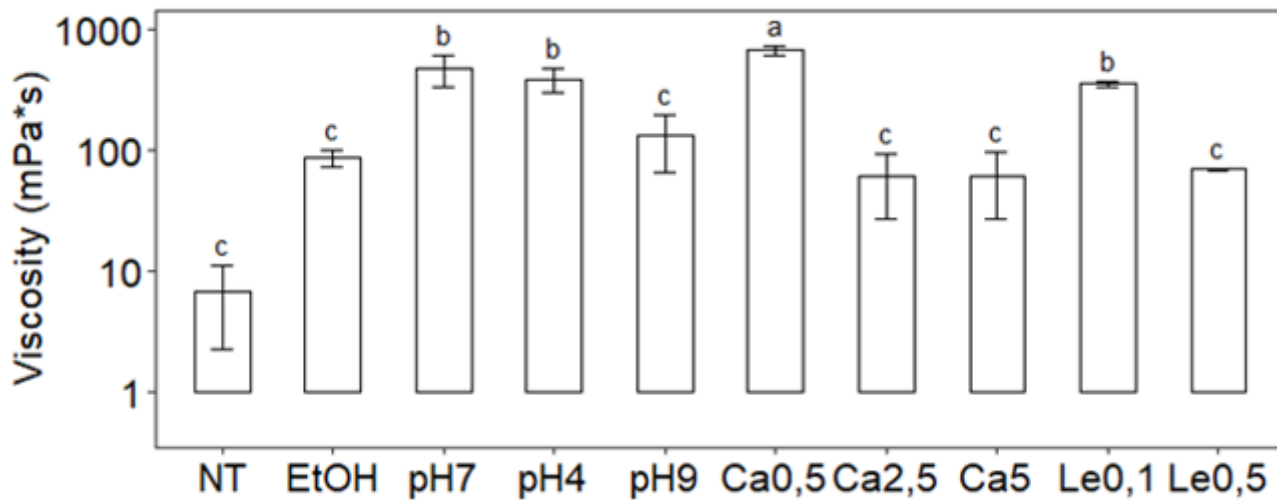


Figure 5

Viscosity of maize RM (3 mg/mL) after various treatments at shear rate 1 s^{-1} . Error bars show standard deviation and significant differences are indicated by different letters.

Figure 6

Surface tension in mN/m measured at a concentration of 3 mg/mL for maize RM nontreated and after EtOH purification in MQ water and at ionic strength 15 mM (pH7) (A), after calcium addition of 0.5 – 5 mM CaCl_2 (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L^{-1} (D). Multiple pairwise comparison was done with post-hoc Tukey for A and with Games-Howell test for B-D.

Figure 7

Contact angle 5 s after drop application for 0.138 mg/cm^2 dry mucilage per area for maize RM (3 mg/mL) nontreated and after EtOH purification in MQ water and at ionic strength 15 mM (pH7) (A), after calcium addition of 0.5 – 5 mM CaCl_2 (B), upon pH change (C) and after lecithin addition of 0.1 and 0.5 mg L^{-1} (D). Multiple pairwise comparison with post-hoc Tukey tests were done for A, B and D and with Games-Howell test for C.