Functions of root mucilage for plant and soil: Quantifying its exudation, characterizing its composition, and assessing its influence on plant water and nitrogen uptake and rhizosphere microorganisms

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Meisam Nazari

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1st Referee: Prof. Dr. Michaela Dippold

2nd Referee: Prof. Dr. Timothy Beissinger

3rd Referee: Prof. Dr. Sandra Spielvogel

4th Referee: Dr. Kyle Mason-Jones

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Summary

Plants modify the chemical, biological, and physical properties of the soil around their roots by releasing a set of substances called rhizodeposits. Mucilage, a viscoelastic high-molecular-weight substance exuded by root tips, constitutes about half of rhizodeposits. Mucilage has many important functions for plants and soil such as easing root penetration, aggregating soil particles, ameliorating soil aluminum toxicity, improving the rhizosphere water content and water-holding capacity, and being a substrate for microbial utilization. Polysaccharides, proteins, minerals, and lipids build up the chemical structure of mucilage, although there is no general overview of the proportion and functions of each mucilage component. Despite many services provided by mucilage, our understanding of the influence of genetics and environment (i.e., climate and soil) on mucilage exudation amount and composition is limited, restricting the incorporation of mucilage traits into breeding programs. Moreover, mucilage is mostly known for its hydraulic and physical functions in the rhizosphere. Studies relating mucilage to microbial processes in the rhizosphere are rare. In addition, several studies performed on mucilage highlight its functions for facilitating plant and nutrient uptake from dry soils, but there is no experimental evidence that mucilage actually does this because these experiments used artificial conditions in which mucilage was mixed with soil in the absence of plant.

Based on the above-mentioned research gaps, the present thesis aimed to analyze plant mucilage components and their functions in the rhizosphere (Study 1), quantify and characterize mucilage polysaccharide composition and exudation amount in maize from contrasting climatic regions (Study 2), investigate the effect of soil, climate, and variety on quantity and quality of maize root mucilage exudation (Study 3), analyze the function of mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat (Study 4), and investigate the function of mucilage for plant water and nitrogen uptake from a dry soil (Study 5).

Study 1 indicated that mucilage is composed of polysaccharides (78.4%), proteins (7.3%), minerals (5.6%), and lipids (3.1%), each playing important roles in the rhizosphere. Study 2 revealed that maize nodal root mucilage polysaccharide is composed of galactose (~39–42%), fucose (~22–30%), mannose (~11–14%), arabinose (~8–11%), xylose (~1–4%), glucose (~1–4%), and glucuronic acid (~3–5%). The Indian (900 M Gold) and Kenyan (DH 02) maize genotypes exuded 135 and 125% higher amounts of mucilage than the central European maize genotypes (Kentos and KXB 8383), respectively. Moreover, there was a significant positive relationship between the mucilage exudation amount and the vapor pressure deficit of the genotypes' agroecological zone of origin. Study 3 demonstrated that the Kenyan semi-arid tropical climatic conditions and loam Luvisol soil from Germany induced 35.8% and 73.7% higher mucilage exudation rate than the German humid temperate climatic conditions and sandy-clay loam Acrisol soil from Kenya, respectively. Furthermore, higher proportions of the uronic acids were observed in the mucilage of the varieties grown in the sandy-clay loam soil and under the semi-arid tropical climatic conditions. Study 4 showed that plant mucilage and microbial

extracellular polymeric substances (EPS) have no consistent differences in viscosity, surface tension, and polysaccharide, protein, neutral monosaccharide, and uronic acid composition. The high mucilage concentrations at the root tip led to maximally 10^9 bacterial cells grown per day and the low mucilage concentrations at the 28.35 mm zone above the root tip led to 3×10^{10} bacterial cells grown per day. Study 5 indicated that the lowest mucilage water-holding capacities belonged to the drought-susceptible maize varieties Keops (291 times its dry weight) and Kentos (599 times its dry weight). The drought-resistant maize varieties DH02 and DH04 had the highest leaf nitrogen contents of 35.1 SPAD and DH04 with 38.3 SPAD, respectively. Further, the mucilage water-holding capacity was significantly positively correlated with the leaf nitrogen content ($r = 0.56$).

In conclusion, plant breeders can exploit the functions of each mucilage component (polysaccharides, proteins, minerals, lipids) to promote agricultural and environmental sustainability (Study 1). Maize mucilage exudation amount is a function of the climatic conditions of the genotypes' agroecological zone of origin where the breeding is performed, possibly because of the role of mucilage in delaying the onset of hydraulic failure during periods of high vapor pressure deficit. We propose that maize mucilage exudation amount has a genetic basis and genotypes from semi-arid agroecological zones are important sources of genetic material for advantageous mucilage traits (Study 2). However, maize can increase its mucilage exudation rate in response to warm climatic conditions and in microbially fertile soils to adapt to water stress and support the rhizosphere microorganisms, respectively. We suggest that uronic acids of mucilage play a substantial role in maize resistance to water stress, because of their interconnections with Ca^{2+} modifying the mucilage and rhizosphere hydraulics (Study 3). Furthermore, mucilage and EPS have similar physical and chemical properties, suggesting comparable functions for these biogels and supporting the potential of mucilage to function as a biofilm matrix like EPS. We recommend that the function of mucilage as a biofilm matrix has been underestimated and should be considered in conceptual plant and soil models (Study 4). The maize varieties capable of exuding a mucilage that is able to hold huge amounts of water could take up more nitrogen from the dry soil, conveying the importance of mucilage water-holding capacity for improving the uptake of nutrients from drying soils (Study 5). Finally, in the context of the second green revolution, we suggest that plant breeders consider such an important belowground trait as mucilage in their breeding programs toward agricultural and environmental sustainability.

Zusammenfassung

Pflanzen verändern die chemischen, biologischen und physikalischen Eigenschaften des Bodens um ihre Wurzeln herum, indem sie eine Reihe von Substanzen freisetzen, die als Rhizodepots bezeichnet werden. Mucilage, eine viskoelastische Substanz mit hohem Molekulargewicht, die von Wurzelspitzen abgesondert wird, macht etwa die Hälfte der Rhizoablagerungen aus. Mucilage hat viele wichtige Funktionen für Pflanzen und Boden, wie z. B. die Erleichterung der Durchwurzelung, die Aggregierung von Bodenpartikeln, die Verbesserung der Aluminiumtoxizität des Bodens, die Verbesserung des Wassergehalts und der Wasserhaltekapazität der Rhizosphäre und die Tatsache, dass sie ein Substrat für die mikrobielle Nutzung sind. Polysaccharide, Proteine, Mineralien und Lipide bilden die chemische Struktur der Mucilage, obwohl es keinen allgemeinen Überblick über die Anteile und Funktionen der einzelnen Mucilagebestandteile gibt. Trotz vieler Dienste, die Mucilage leistet, ist unser Verständnis des Einflusses von Genetik und Umwelt (d. h. Klima und Boden) auf die Menge und Zusammensetzung der Mucilageexsudation begrenzt, was die Einbeziehung der Mucilagemerkmale in Zuchtprogramme einschränkt. Darüber hinaus ist Mucilage vor allem für seine hydraulischen und physikalischen Funktionen in der Rhizosphäre bekannt. Studien, die Mucilagestoffe mit mikrobiellen Prozessen in der Rhizosphäre in Verbindung bringen, sind selten. Darüber hinaus betonen mehrere Studien, die an Mucilage durchgeführt wurden, seine Funktionen zur Erleichterung der Pflanzen- und Nährstoffaufnahme aus trockenen Böden, aber es gibt tatsächlich keinen experimentellen Beweis dafür, dass Mucilage dies wirklich tut, da diese Experimente künstliche Bedingungen verwendeten, bei denen Mucilage ohne Abwesenheit mit Erde vermischt wurde der Pflanze.

Ausgehend von den oben genannten Forschungslücken zielte die vorliegende Arbeit darauf ab, Pflanzenmucilagebestandteile und ihre Funktionen in der Rhizosphäre zu analysieren (Studie 1), die Mucilagepolysaccharidzusammensetzung und die Exsudationsmenge in Mais aus unterschiedlichen Klimaregionen zu quantifizieren und zu charakterisieren (Studie 2), die Auswirkung zu untersuchen von Boden, Klima und Sorte auf Quantität und Qualität der Ausscheidung von Maiswurzelmucilage (Studie 3), Analyse der Funktion von Mucilage als Biofilmmatrix, die den mikrobiellen Lebensraum der Rhizosphäre formt (Studie 4), und Untersuchung der Funktion von Mucilage für Pflanzenwasser und Stickstoffaufnahme aus trockenem Boden (Studie 5).

Studie 1 zeigte, dass Mucilage aus Polysacchariden (78,4 %), Proteinen (7,3 %), Mineralien (5,6 %) und Lipiden (3,1 %) besteht, die jeweils eine wichtige Rolle in der Rhizosphäre spielen. Studie 2 ergab, dass das Polysaccharid des Knotenwurzelmucilages von Mais aus Galactose (~39–42 %), Fucose (~22–30 %), Mannose (~11–14 %), Arabinose (~8–11 %), Xylose (~1–4 %) Glucose (~1–4 %) und Glucuronsäure (~3–5 %). Die indischen (900 M Gold) und kenianischen (DH 02) Mais-Genotypen sonderten 135 bzw. 125 % höhere Mengen an Mucilage ab als die mitteleuropäischen Mais-Genotypen (Kentos und KXB 8383). Darüber hinaus bestand ein signifikant positiver Zusammenhang zwischen der Mucilageausscheidungsmenge und dem

Dampfdruckdefizit der agrarökologischen Herkunftszone der Genotypen. Studie 3 zeigte, dass die kenianischen halbtrockenen tropischen Klimabedingungen und Lehm Luvisol aus Deutschland eine um 35,8 % bzw. 73,7 % höhere Mucilageexsudationsrate induzierten als die deutschen feucht-gemäßigten Klimabedingungen bzw. sandig-lehmiger Acrisol-Boden aus Kenia. Außerdem wurden höhere Anteile der Uronsäuren in den Mucilagestoffen der Sorten beobachtet, die auf sandig-lehmigen Böden und unter semiariden tropischen Klimabedingungen angebaut wurden. Studie 4 zeigte, dass Pflanzenmucilage und mikrobielle extrazelluläre polymere Substanzen (EPS) keine konsistenten Unterschiede in der Viskosität, Oberflächenspannung, und Polysaccharid-, Protein-, neutralen Monosaccharid- und Uronsäurezusammensetzung aufweisen. Die hohen Mucilagekonzentrationen an der Wurzelspitze führten zu einem Wachstum von maximal 10^9 Bakterienzellen pro Tag und die niedrigen Mucilagekonzentrationen in der Zone von 28,35 mm über der Wurzelspitze führten zu einem Wachstum von 3×10^{10} Bakterienzellen pro Tag. Studie 5 zeigte, dass die trockenheitsanfälligen Maissorten Keops (das 291-fache seines Trockengewichts) und Kentos (das 599-fache seines Trockengewichts) die geringste Wasserhaltekapazität der Mucilagestoffe aufwiesen. Die dürreresistenten Maissorten DH02 und DH04 hatten die höchsten Blattstickstoffgehalte von 35,1 SPAD bzw. DH04 mit 38,3 SPAD. Darüber hinaus korrelierte die Wasserhaltekapazität des Mucilages signifikant positiv mit dem Stickstoffgehalt der Blätter ($r = 0,56$).

Zusammenfassend lässt sich sagen, dass Pflanzenzüchter die Funktionen jeder Mucilagekomponente (Polysaccharide, Proteine, Mineralien, Lipide) nutzen können, um die landwirtschaftliche und ökologische Nachhaltigkeit zu fördern (Studie 1). Die Ausscheidungsmenge von Maismucilage ist eine Funktion der klimatischen Bedingungen der agrarökologischen Ursprungszone der Genotypen, in der die Züchtung durchgeführt wird, möglicherweise aufgrund der Rolle des Mucilages bei der Verzögerung des Beginns des hydraulischen Versagens in Zeiten mit hohem Dampfdruckdefizit. Wir schlagen vor, dass die Ausscheidungsmenge von Maismucilage eine genetische Grundlage hat und Genotypen aus halbtrockenen agrarökologischen Zonen wichtige Quellen für genetisches Material für vorteilhafte Mucilageeigenschaften sind (Studie 2). Mais kann jedoch seine Mucilageexsudationsrate als Reaktion auf warme klimatische Bedingungen und in mikrobiell fruchtbaren Böden erhöhen, um sich an Wasserstress anzupassen bzw. die Mikroorganismen der Rhizosphäre zu unterstützen. Wir schlagen vor, dass Uronsäuren des Mucilages eine wesentliche Rolle bei der Resistenz von Mais gegenüber Wasserstress spielen, da ihre Verbindungen mit Ca^{2+} den Mucilage und die Hydraulik der Rhizosphäre modifizieren (Studie 3). Darüber hinaus haben Mucilage und EPS ähnliche physikalische und chemische Eigenschaften, was auf vergleichbare Funktionen für diese Biogele hindeutet und das Potenzial von Mucilage unterstützt, als Biofilmmatrix wie EPS zu fungieren. Wir empfehlen, dass die Funktion von Mucilage als Biofilmmatrix unterschätzt wurde und in konzeptionellen Pflanzen- und Bodenmodellen berücksichtigt werden sollte (Studie 4). Die Maissorten, die einen Mucilage absondern können, der große Mengen Wasser halten kann, könnten mehr Stickstoff aus dem trockenen Boden aufnehmen, was die Bedeutung der Wasserspeicherkapazität des Mucilages für die Verbesserung der Nährstoffaufnahme aus

trocknenden Böden verdeutlicht (Studie 5). Zusammenfassend schlagen wir im Zusammenhang mit der zweiten grünen Revolution vor, dass Pflanzenzüchter einem so wichtigen unterirdischen Merkmal wie Mucilage im Hinblick auf landwirtschaftliche und ökologische Nachhaltigkeit mehr Aufmerksamkeit schenken.

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Abbreviations

ANOVA	Analysis of variance
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
C	Carbon
C_{in}	Mucilage available to bacterial degradation
CUE	Carbon use efficiency
DW	Dry weight
eDNA	Extracellular deoxyribonucleic acid
EPS	Extracellular polymeric substances
f_c	Carbohydrate fraction of mucilage
GC-MS	Gas chromatography-mass spectrometry
HSD	Honestly Significant Difference
IS	Internal standard
LED	Light-emitting diode
M_B	Bacterial cell biomass
M_{cell}	Bacterial cell carbon mass
N_{cell}	Bacterial cell number
NMP	1-methyl-2-pyrrolidone
NS	Non-significant
PGA	Polygalacturonic acid
RCBD	Randomized complete block design
SPAD	Single-photon avalanche diode
TFA	Trifluoroacetic acid
VPD	Vapor pressure deficit

I Extended summary

Introduction

Plants fix carbon dioxide (CO_2) from the atmosphere and convert it into carbohydrates through photosynthesis. About 30% of plants photosynthetic output is deposited into the soil around their roots, the rhizosphere, as rhizodeposits (Walker et al., 2003; Jones et al., 2009). Rhizodeposits refer to a category of substances including root exudates, sloughed-off cells and tissues, soluble lysates, and mucilage (Jones et al., 2009; Oburger and Jones, 2018; Tian et al., 2020; Figure 1). Approximately half of plant rhizodeposits is mucilage (Chaboud, 1983; Walker et al., 2003), which is a viscoelastic high-molecular-weight substance exuded by root tips (McCully, 1999; Sasse et al., 2018).

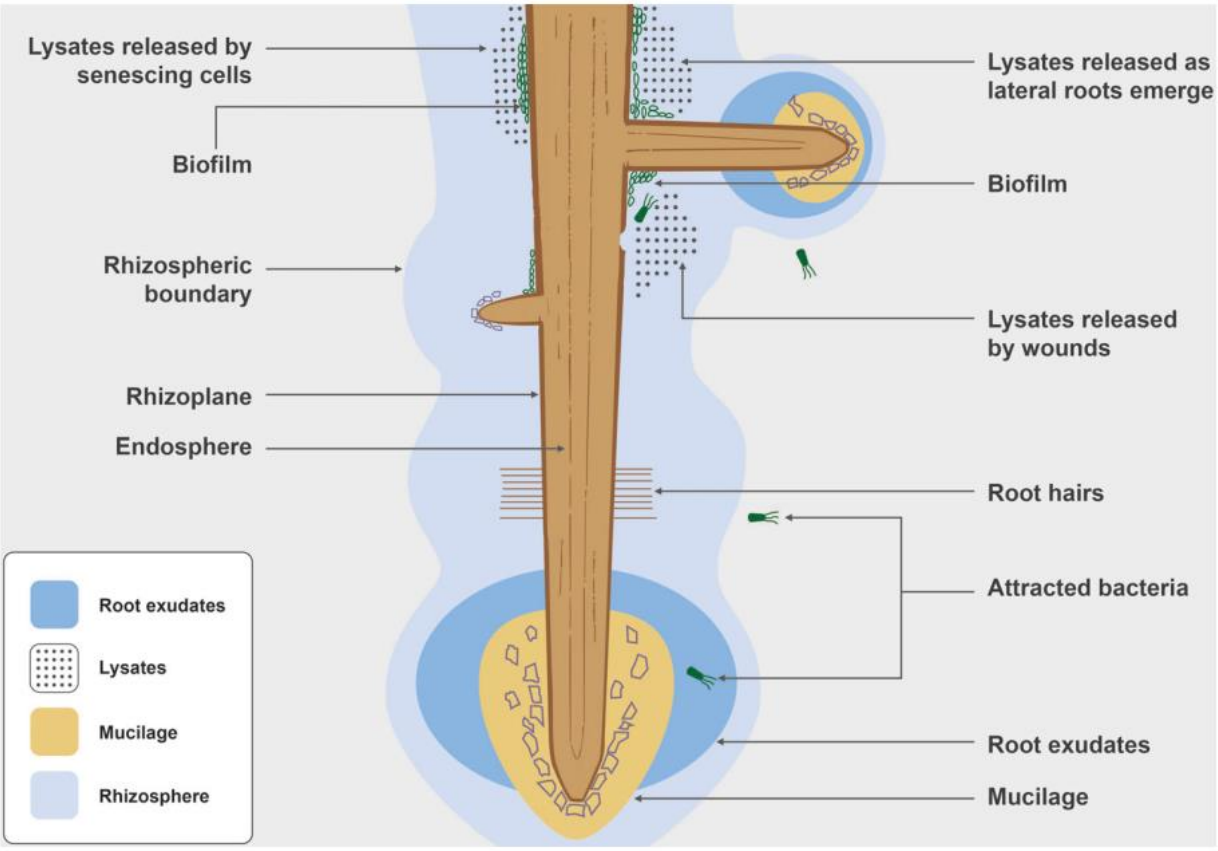

Figure 1. Different types of rhizodeposits and their origin in the rhizosphere (after Tian et al., 2020).

Mucilage is mainly composed of polysaccharides, but also contains proteins, lipids, and minerals (Carminati and Vetterlein, 2013; Alizedeh Behbahani et al., 2017; Amicucci et al., 2019). Mucilage plays important roles in the formation of rhizosheath, improving the root-soil connection, easing the penetration of the growing root, ameliorating aluminum toxicity, and aggregating soil particles (McCully, 1999; Czarnes et al., 2000; Dakora and Phillips, 2002; Iijima et al., 2003; Sasse et al., 2018). The high viscosity and low surface tension of mucilage enhance the soil liquid-phase connectivity, increase the rhizosphere water content, and facilitate root water and nutrient uptake (Carminati et al., 2010; Ahmed et al., 2015; Naveed et al., 2017; Benard et al.,

2018; Benard et al., 2019; Zarebanadkouki et al., 2019). Mucilage is capable of holding between 25 and 600 times its dry weight water, leading to improved water-holding capacity and hydraulic conductivity in the rhizosphere of drying soils (McCully and Boyer, 1997; Capitani et al., 2013; Kroener et al., 2014; Carminati et al., 2017). Mucilage is also a substrate for microbial utilization (Mary et al., 1993; Ahmed et al., 2018; Bennet et al., 2020). Moreover, mucilage exuded by the crown roots of a landrace of maize namely Sierra Mixe (*Zea mays* Y.) harbors nitrogen-fixing bacteria that provide 29-82% the plant's nitrogen need (Van Deynze et al., 2018; Amicucci et al., 2019).

Although a lot of studies have so far been performed on mucilage functions in the rhizosphere, review studies on the functions of each mucilage component (polysaccharides, proteins, minerals, lipids) are lacking. Moreover, there is no general overview of the proportion of each mucilage component, demanding for analytical studies. Study 1 – *Plant mucilage components and their functions in the rhizosphere* – aimed to fill both knowledge gaps by analyzing the proportion of each mucilage component and reviewing the functions of the distinct components in the rhizosphere.

Despite the importance of root mucilage for plant and soil, breeding programs have largely focused on aboveground plant traits (Lammerts Van Bueren et al., 2011). Our understanding of the genetic basis of mucilage exudation amount and composition is limited, restricting the incorporation of this important trait into breeding programs (Monchgesang et al., 2016; Kuijken et al., 2015; Fernández-Aparicio et al., 2016). In fact, identifying the genetic basis of root mucilage exudation amount and its polysaccharide composition is a crucial step toward agricultural sustainability (Bennett et al., 2020). To do so, different plant genotypes grown under the same conditions need to be investigated (Micallef et al., 2009). Study 2 – *Mucilage polysaccharide composition and exudation in maize from contrasting climatic regions* – aimed to quantify and characterize the nodal root mucilage of eight maize (*Zea mays* L.) genotypes originating from the different regions of the world grown under the same abiotic and biotic conditions in a field experiment. Furthermore, it is necessary to study the influence of different environmental conditions (i.e., climate and soil) on mucilage exudation rate and polysaccharide composition (Blizard and Sparks 2020; Oburger and Jones 2018). Study 3 – *Soil, climate, and variety impact on quantity and quality of maize root mucilage exudation* – aimed to quantify and characterize the nodal root mucilage of two maize varieties from Kenya and Germany grown in the soils and simulated climatic conditions of their region of origin.

Mucilage is mostly recognized for its hydraulic and physical functions in the rhizosphere. Studies associating mucilage to microbial processes in the rhizosphere are scarce. For instance, mucilage was indicated to be an energy source for rhizosphere microorganisms (Mary et al., 1993; Ahmed et al., 2018). Extracellular polymeric substances (EPS) are similarly gelatinous high-molecular-weight substances produced by microorganisms and function as a biofilm matrix (Flemming and Wingender, 2010). EPS support the establishment of microbial assemblages in soils by providing a moist environment, a protective barrier, and serving as carbon and nutrient sources (Kumar et

al., 2007; Flemming and Wingender, 2010; Vardharajula and Sk, 2014). EPS can hold 15–20 times more water than their dry weight and considerably increase the water-holding capacity of soils (Chenu, 1993; Adessi et al., 2018). The appearance and also some functional similarities of plant mucilage and microbial EPS suggest that mucilage is also a potential biofilm matrix that shapes the rhizosphere microbial habitat. However, there is no analytical evidence that mucilage resembles EPS, functions like EPS, and shapes the rhizosphere microbial habitat. Study 4 – *Biogels in soils: Plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat* – aimed to analyze and review existing evidence to determine physical, chemical, and functional similarities between mucilage and EPS and to model the spatial and temporal extent of the mucilage biofilm along the root axis as well as microbial abundance and EPS production in the mucilage biofilm.

Several studies implemented on mucilage highlight its functions for facilitating plant water and nutrient uptake from dry soils. However, there is no experimental evidence that mucilage really does this, because these experiments used artificial conditions in which mucilage was mixed with soil in the absence of plant. Recent studies showed that the drought-resistant maize and barley (*Hordeum vulgare* L.) plants exude higher amounts of mucilage than the drought-susceptible ones (Carter et al., 2019; Nazari et al., 2020). This gives us a great opportunity to realistically investigate the effect of mucilage on plant water and nutrient uptake in dry soils. Study 5 – *Root mucilage improves plant nitrogen uptake from dry soil* – aimed to investigate the role of mucilage in plant water and nitrogen uptake from a dry soil using four drought-resistant and drought-susceptible maize varieties differing in mucilage quantity and quality.

Summary of objectives

In summary, the main objectives of the present thesis were to

(1) analyze plant mucilage components and their functions in the rhizosphere (Study 1)**,**

(2) quantify and characterize mucilage polysaccharide composition and exudation in maize from contrasting climatic regions (Study 2),

(3) investigate the effect of soil, climate, and variety on quantity and quality of maize root mucilage exudation (Study 3),

(4) analyze the function of mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat (Study 4),

(5) investigate the function of mucilage for plant water and nitrogen uptake from a dry soil (Study 5).

Materials and methods

Study 1

In this study, 67 published papers were reviewed to get a detailed overview of the functions of the distinct mucilage components (polysaccharides, proteins, minerals, lipids) in the rhizosphere. Moreover, 56 data points (n = 56) were extracted from 16 published papers to calculate the average proportion of each mucilage component. All extracted data points were related to crude mucilage.

Study 2

A field experiment was implemented as a randomized complete block design near Bayreuth, Bavaria, Germany. Eight maize genotypes from contrasting climatic regions of the world were used. The genotypes were planted manually in May 2019 in strips of 0.2 m × 9 m with 75 cm row distance between the strips and grown until the end of tassel emergence (BBCH 59) for harvesting nodal root mucilage. Each maize genotype consisted of three replicates and the row of each genotype was replicated three times (n = 3). Detailed information on the genotypes, their regions of origin, and their breeding methods is presented in Table 1.

Table 1. Detailed information on the maize genotypes, their agroecological zones of origin, and breeding methods.

Region	Country	Climate	Temperature (°C)[a]	Relative humidity (%)[a]	P (mm)[b]	Genotype	Breeding method
Central Europe	Germany	Temperate	15.8	77.7	132-560	Kentos	Hybrid
	France	Temperate	15.7	60	105-660	KXB 8383	Hybrid
Southern Europe	Turkey	Mediterranean	16.4	59	86-332	Keravnos	Hybrid
	Italy	Mediterranean	18.4	72.5	240-1100	Kerubino	Hybrid
Africa	Kenya	Semi-arid	24.8	75	62-720	DH 02	Hybrid
	Kenya	Tropical	24.6	83	62-867	DH 04	Hybrid
Asia	India	Semi-arid	25.7	72.3	324-2044	900 M Gold	Hybrid
	India	Semi-arid	25.7	72.3	324-2044	30 V 92	Hybrid

a: Temperature and relative humidity are the mean for the maize growing season in the related agroecological zone within the past 10 years

b: P (precipitation) is the sum over the entire growing season as range from minimum to maximum of observed precipitation within the past 10 years.

Mucilage was sampled at the end of tassel emergence (BBCH 59) from each of the three rows according to Ahmed et al (2015). Briefly, the nodal roots covered with mucilage were cut and placed in aluminum trays. Soil and plant debris were removed from the nodal roots by distilled water. Then, the mucilage-covered roots were submerged in distilled water to reach full saturation. The hydrated mucilage was aspirated from the roots using syringes. The mucilage was collected in 20 ml vials, frozen at - 18 °C, and subsequently freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany). The freeze-dried mucilage samples were analyzed to determine the neutral monosaccharide and uronic acid proportion by gas chromatography (Agilent 7820A GC, Agilent

Technologies, Waldbronn, Germany) and mass spectrometry (Agilent 5977B, Agilent Waldbronn, Germany) according to Banfield et al (2018).

The vapor pressure deficit (VPD) of the genotypes' agroecological zones during the growing period was calculated according to Seager et al (2015):

$$VPD\ (kPa) = 0.611 \times exp\left(\frac{17.5 \times T}{240.987 + T}\right) - 0.611 \times exp\left(\frac{17.5 \times T_d}{240.987 + T_d}\right) \qquad Eq\ (1)$$

Where, T is the mean temperature (°C) and T_d is the dew point (°C).

T and relative humidity (RH%) were used to calculate the T_d:

$$T_d = \frac{243.04 \times \left(ln\left(\frac{RH}{100}\right) + \frac{17.625 \times T}{243.04 + T}\right)}{17.625 - \left(ln\left(\frac{RH}{100}\right) + \frac{17.625 \times T}{243.04 + T}\right)} \qquad Eq\ (2)$$

Study 3

A three-factorial (variety × soil × climate) pot experiment was performed as a randomized complete block design consisting of four replicates (n = 4) in the climate chambers of the Department of Plant Ecology and Ecosystems Research of the Georg-August University of Göttingen, Göttingen, Germany. A drought-susceptible maize variety from Germany (Kentos) and a drought-resistant maize variety from Kenya (DH02) were used as representatives of maize varieties grown in Germany (humid temperate climate) and Kenya (semi-arid tropical climate). Nazari et al (2020) indicated that the variety DH02 exudes significantly higher amounts of mucilage than the variety Kentos. The maize varieties were grown in the soils collected from Hohenpölz in Germany and Kitui in Kenya. Detailed information on the used soils is presented in Table 2.

Table 2. Properties of the soils collected from the German (Hohenpölz) and Kenyan (Kitui) farms.

Site	Collection depth (cm)	Soil type	Sand (%)	Silt (%)	Clay (%)	TOC (%)	TN (%)	MBC (µg g^{-1})	WHC (%)	pH (H_2O)
Hohenpölz	25	Luvisol	36	42	22	1.77	0.19	493	63.3	6.4
Kitui	25	Acrisol	59	9	32	0.62	0.07	103	32.6	5.4

The plants were grown in two climate chambers (York® 2300, Johnson Controls, USA) to simulate the climatic conditions in which the maize varieties are grown in Germany and Kenya. In each climate chamber, totally 16 pots were kept containing the two different maize varieties in each of the two different soils, which were replicated four times. The light sources in the chambers were 400W Eye Clean Ace metal halide bulbs with a near-daylight spectral composition (Eye Lighting International, Ohio, USA). A photoperiod of 12 hours day and 12 hours night was applied.

Mucilage was sampled at the growth stage with nine or more nodes visible (BBCH 39) from five nodal roots of each maize plant according to Ahmed et al (2015). The newly emerged nodal roots

(not yet in the soil) were immersed in distilled water for 24 hours until the mucilage was fully saturated. Thereafter, the saturated mucilage was aspirated from the nodal root tips using a 5 ml pipettor. The mucilage samples were collected in 50 ml vials and frozen at -18 °C. All collected mucilage samples were freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany) and stored in a desiccator. Mucilage exudation rate was expressed as the dry weight (DW) of freeze-dried mucilage per root tip per day. The freeze-dried mucilage samples were analyzed to determine the neutral monosaccharide and uronic acid proportion by gas chromatography (Agilent 7820A GC, Agilent Technologies, Waldbronn, Germany) and mass spectrometry (Agilent 5977B, Agilent Waldbronn, Germany) according to Banfield et al (2018).

Study 4

To analyze the similarities of plant mucilage and microbial EPS (the biogels), 376 data points were collected from 83 published papers. The viscosity and surface tension were considered physical properties. The total polysaccharide, total protein, neutral monosaccharide, and uronic acid proportions were considered chemical properties. The studied neutral monosaccharides included galactose, fucose, glucose, mannose, arabinose, rhamnose, and xylose, and uronic acids included glucuronic and galacturonic acid.

To model the mucilage spatial and temporal distribution around the growing root, experimental and literature data including the below parameters were used:

Soil porosity = 50% (assumed); root diameter = 3.3 mm (measured); mucilage exudation rate = 1.41 mg dry weight per day and root tip (measured); maximum hydration ratio of mucilage = 425:1 (wet mass: dry mass, measured), assuming a 39% water saturation upon exudation at the root tip and a rapid saturation to 100% within 6 h (Sealey et al., 1995); root elongation rate = 30 mm per day (Schmidt et al., 2013); and a maximum decomposition rate of the mucilage = 50% in 7 days (Ahmed et al., 2018).

The bacterial abundance and EPS production around the growing root were modeled as follows:

The mucilage available to bacterial degradation (C_{in}) was assumed to produce bacterial biomass under carbon limitation. The growth and biomass yield of several rhizosphere bacteria using glucose are comparable to the growth achieved by mucilage as a sole carbon source (Knee et al., 2001). Here, we considered the carbohydrate fraction of mucilage obtained in this study (f_c = 0.77) to be available for bacterial consumption (considering the mass-fraction of carbon for simple sugars; e.g., glucose w_c = 0.4). The upper bound on mucilage-derived carbon (C_{in}) that can be allocated to produce bacterial cell biomass (M_B) was obtained by considering an average carbon use efficiency (CUE) for a range of carbon sources ($M_B = f_c \times w_c \times \text{CUE} \times C_{in}$). We assumed that maintenance costs were negligible and all cell biomass could be produced within a day. For the calculation presented here, we used an average CUE of 0.6 based on genome scale metabolic predictions (Saifuddin et al., 2019). To estimate the number of cells (N_{cell}) that could feed on degraded mucilage, we divided the cell biomass carbon by an average bacterial cell carbon mass

(M_{cell}) of 10 fg C per cell ($N_{cell} = M_B/M_{cell}$) (Khachikyan et al., 2019). We estimated the EPS produced (M_{EPS}) by a given bacterial abundance using an EPS yield per unit of cellular biomass ($M_{EPS} = M_B \times Y_{EPS}$ with $Y_{EPS} = 10$ mg g^{-1}) (Shene et al., 2008). Our model calculation assumed that all cells produce EPS.

Study 5

A pot experiment was done as a randomized complete block design consisting of six replicates (n = 6) in a plant growth chamber. Two drought-resistant maize varieties from Kenya (DH02 and DH04) and two drought-susceptible maize varieties from Germany (Kentos and Keops) were used. The soil used was a loam Luvisol collected from an agricultural farm located in Hohenpölz, Bavaria, Germany. The plants were grown in the growth chamber with a photoperiod of 12 hours day and 12 hours night. The average temperature and relative humidity in the growth chamber were 26 °C ± 1 and 60% ± 5 and the light sources were 243W light-emitting diode (LED) with a near-daylight spectral composition (Kind LED Growth Lights, California, USA). Drought was simulated by setting the soil water content to 30% water-holding capacity for a week from the growth stage beginning of stem elongation (BBCH 30) to the growth stage nine or more nodes visible (BBCH 39). The soil water content was kept at 70% water-holding capacity for about two weeks from sowing to BBCH 30.

Mucilage was sampled from nodal roots of the maize varieties at the growth stage nine or more nodes visible (BBCH 39) according to Ahmed et al (2015). Briefly, emerged nodal roots were immersed in distilled water for 24 hours to get maximum mucilage hydration. Then, the hydrated mucilage was harvested from the nodal root tips using a 5 ml pipettor. The mucilage samples were collected in 50 ml vials, weighed, and freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany). Mucilage exudation rate was expressed as the dry weight of freeze-dried mucilage per nodal root tip per day. Mucilage water-holding capacity was calculated as the weight of fully hydrated mucilage – the weight of freeze-dried mucilage / the weight of freeze-dried mucilage and expressed as times its dry weight.

Leaf stomatal conductance was measured by a leaf porometer (Decagon Devices, USA) at the abaxial surface of the center of the fully developed sixth leaf. Plant transpiration rate was measured by weighing the pots expressed as the weight of lost water per hour. A portable hand-held SPAD 502 Plus device (Konica Minolta, Japan) was used at the center of the fully developed sixth leaf to measure leaf nitrogen content (Blackmer and Schepers, 1995; Rorie et al., 2011; Makarian et al., 2016). For measuring leaf stomatal density, pieces of plant leaves were bleached in a 10% sodium hypochlorite solution for 24 hours to remove the mesophyll. The abaxial surface of the center of the fully developed sixth leaf of each plant was observed using a light microscope (Olympus BX40, Olympus Optical Co., Ltd., Tokyo, Japan) in order to count stomata. All of these measurements were performed at the growth stage nine or more nodes visible (BBCH 39). The ^{15}N natural abundance of the freeze-dried mucilage was measured as an index of plant nitrogen fixation using the Delta XP isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany)

at the Centre for Stable Isotope Research and Analysis of the Georg-August University of Göttingen.

Key findings

Study 1

In total, mucilage is composed of 78.4% polysaccharides, 7.3% proteins, 5.6% minerals, and 3.1% lipids. Each of these components have important functions for the rhizosphere (Table 3).

Table 3. Constituents of plant mucilage and the functions of each in the rhizosphere.

Mucilage constituent	Functions
Polysaccharides	• Energy source for microorganisms • Water absorption • Exchange of cations • Adherence to solid surfaces (i.e., soil particles and nematodes)
Proteins	• Disassembly of the mucilage polysaccharide for microbial use • Response to abiotic and biotic stresses (i.e., heat stress and plant pathogens) • Assistance in nutrient uptake (e.g., phosphorus and iron)
Minerals	• Exchange of cations • Improvement of soil liquid-phase connectivity and water content
Lipids	• Alternation of soil hydraulic properties • Desorbing adsorbed phosphorus on soil particles • Easing the penetration of roots into the soil

Study 2

Maize nodal root mucilage polysaccharide is composed of galactose (~39–42%), fucose (~22–30%), mannose (~11–14%), arabinose (~8–11%), xylose (~1–4%), glucose (~1–4%), and glucuronic acid (~3–5%). The proportion of all sugars (except mannose) significantly differed between some of the genotypes. The Indian (900 M Gold) and Kenyan (DH 02) genotypes exuded 135 and 125% higher amounts of mucilage than the central European genotypes (Kentos and KXB 8383), respectively (Figure 2). There was a significant positive association between the mucilage exudation amount and the vapor pressure deficit of the genotypes' agroecological zone (Figure 3).

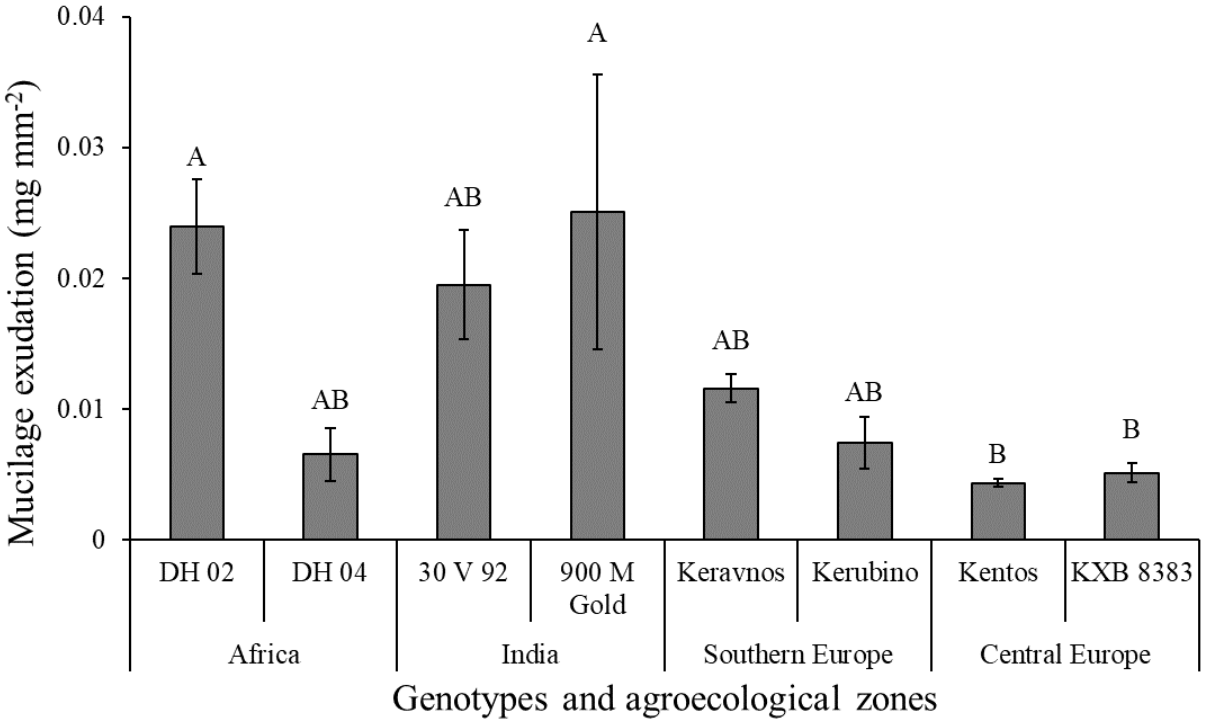

Figure 2. Mucilage exudation amount (dry weight) of the investigated maize genotypes normalized by root surface area. Different letters above the bars indicate significant differences between the exudation amounts (Tukey's HSD, at $P \leq 0.05$). Error bars indicate the standard error of the mean (n = 3).

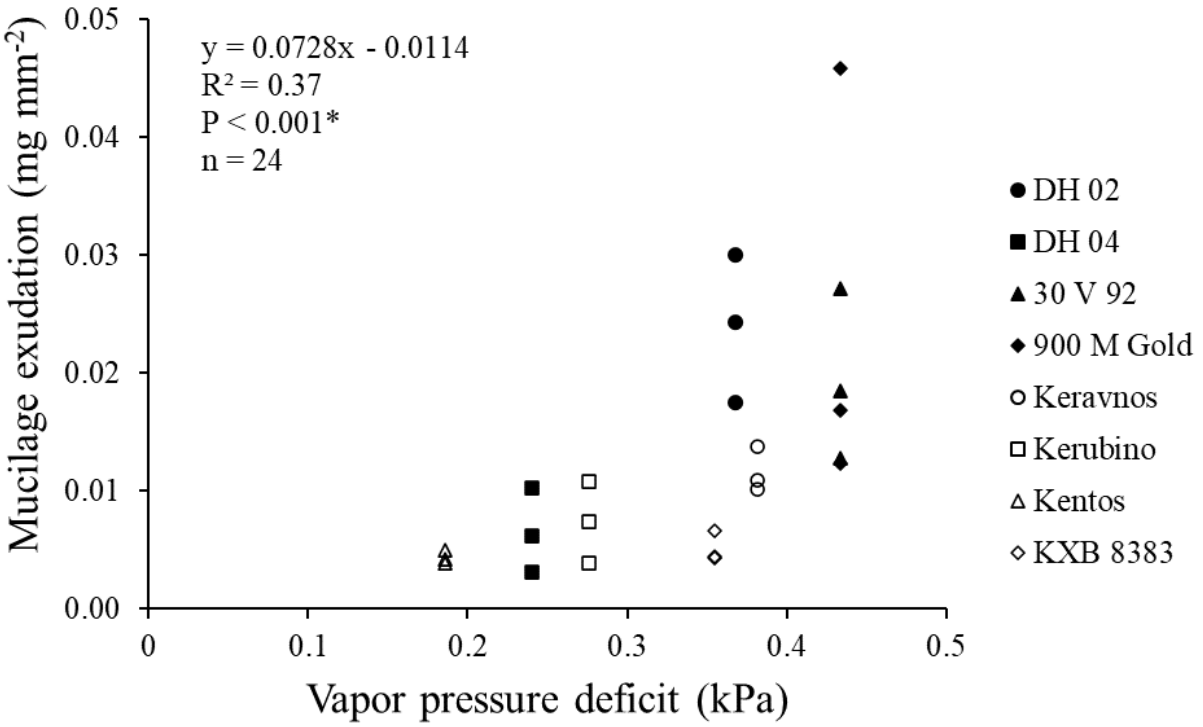

Figure 3. Association between the genotypes' mucilage exudation amount and the vapor pressure deficit (VPD) of their agroecological zones (∗ = significant at $P \leq 0.05$). The VPD for the region where the field study was performed (Bavaria, Germany) was 0.19 kPa.

Study 3

The mucilage exudation rate of maize nodal roots was significantly affected by climate, soil, and variety. The Kenyan semi-arid tropical climatic conditions led to 35.8% higher mucilage exudation rate compared to the German humid temperate climatic conditions. Growing the varieties in the loam Luvisol from Germany induced 73.7% higher mucilage exudation rate than growing in the sandy-clay loam Acrisol soil from Kenya. The drought-resistant Kenyan maize variety exuded 58.2% more mucilage than the drought-susceptible German variety. On average, mucilage polysaccharides were composed of 40.6% galactose, 26.2% fucose, 13.1% mannose, 11% arabinose, 3.5% glucose, 3.2% xylose, 1.3% glucuronic acid, and 1% an unknown uronic acid. Among these sugars, only significantly higher proportions of the uronic acids were observed in the mucilage of the varieties grown in the sandy-clay loam soil and under the semi-arid tropical climatic conditions. A graphical summary of the main results of this study is illustrated in Figure 4.

Graphical abstract

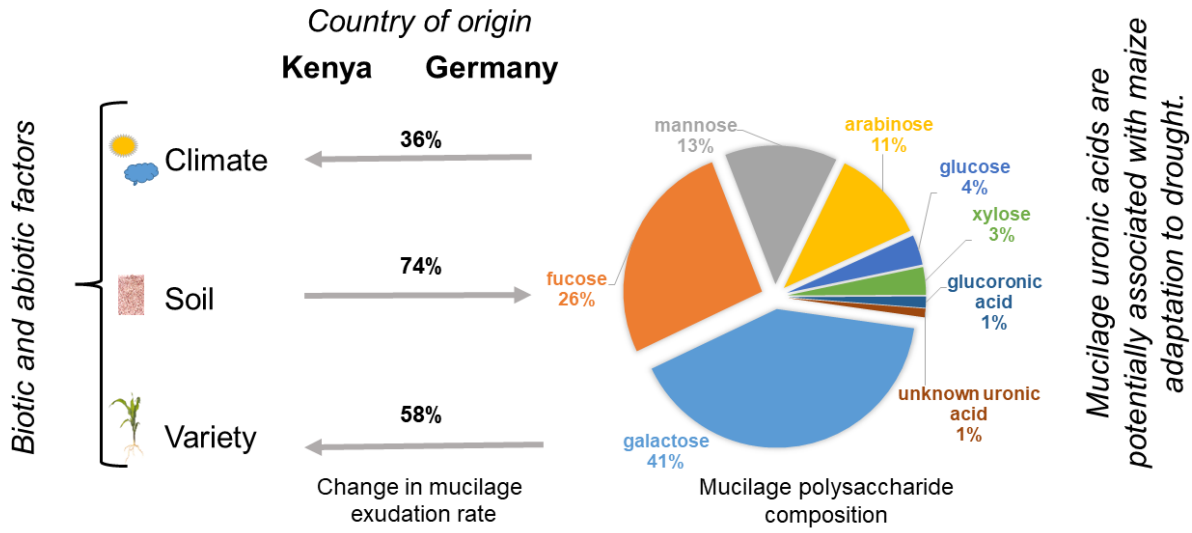

Figure 4. Graphical summary of the main results of Study 3.

Study 4

Plant mucilage and microbial EPS had no consistent differences in viscosity, surface tension, and polysaccharide, protein, neutral monosaccharide, and uronic acid composition. All major functions of EPS for biofilm formation are also provided by mucilage. A model for the spatial and temporal extent of a potential mucilage biofilm around a maize root segment is illustrated in Figure 5. The high mucilage concentrations at the root tip led to maximally 10^9 bacterial cells grown per day and the low mucilage concentrations at the 28.35 mm zone above the root tip led 3×10^{10} bacterial cells grown per day. The bacterial cells could potentially produce about 0.01 mg EPS per day at the root tip and 0.29 mg EPS per day within the 28.35 mm zone of daily growth. A model for the spatial and temporal bacterial abundance and EPS production along a maize root segment is shown in Figure 6.

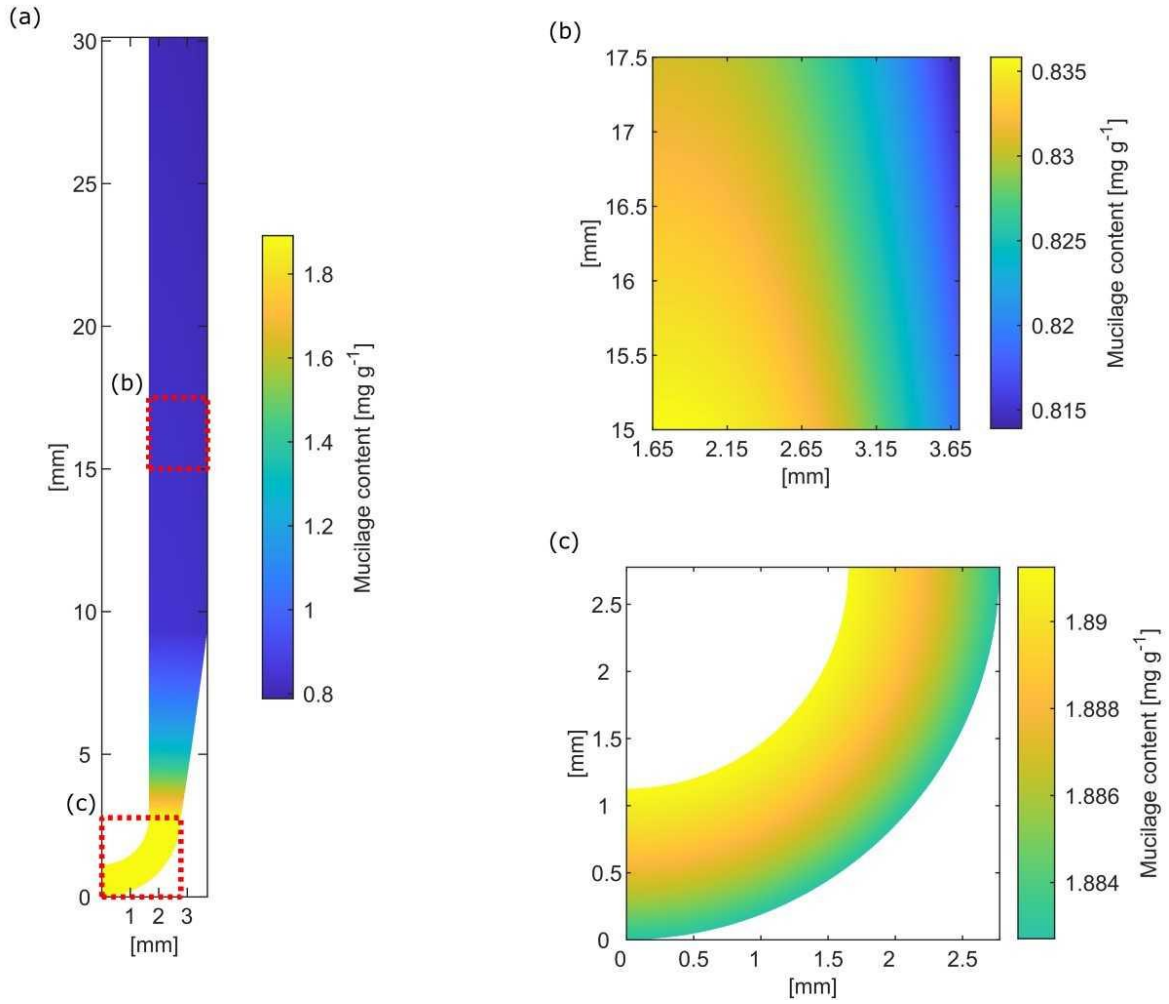

Figure 5. Spatial and temporal model of mucilage around a maize root segment grown within 1 day (= 30 mm) [(A) along the root; (B) lateral; (C) at the root tip]. Mucilage-affected soil is indicated by yellow to blue color along the root and by yellow to green at the root tip, reflecting the increasing radial extent by swelling as well as the decreasing content of mucilage by decomposition.

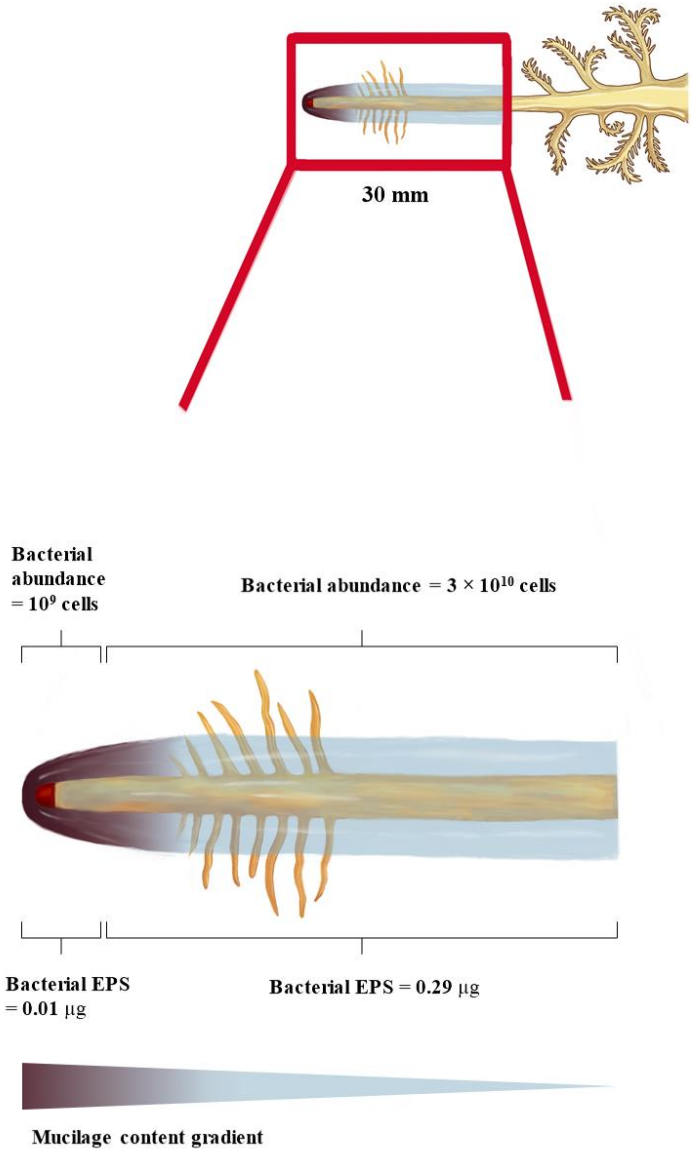

Figure 6. Spatial and temporal model of bacterial abundance and EPS production along a maize root segment grown within 1 day (= 30 mm). Mucilage content is reduced away from the root tip by bacterial decomposition. Mucilage extent enhances away from the root tip due to swelling.

Study 5

Mucilage exudation rate was 186% higher in the variety DH02 than in the variety Kentos (Figure 7A). Moreover, the variety Keops had 264% and 160% higher mucilage exudation rate compared to the varieties Kentos and DH04, respectively. The lowest mucilage water-holding capacities belonged to Keops (291 times its dry weight) and Kentos (599 times its dry weight) (Figure 7B). DH02 with 35.1 SPAD and DH04 with 38.3 SPAD had the highest leaf nitrogen contents (Figure 8A). The lowest ^{15}N natural abundance (- 1.25 ‰) belonged to the variety Kentos (Figure 8B).

The leaf nitrogen content was positively correlated with the mucilage water-holding capacity (r = 0.56). The leaf stomatal conductance and plant transpiration rate did not significantly correlate with the mucilage properties.

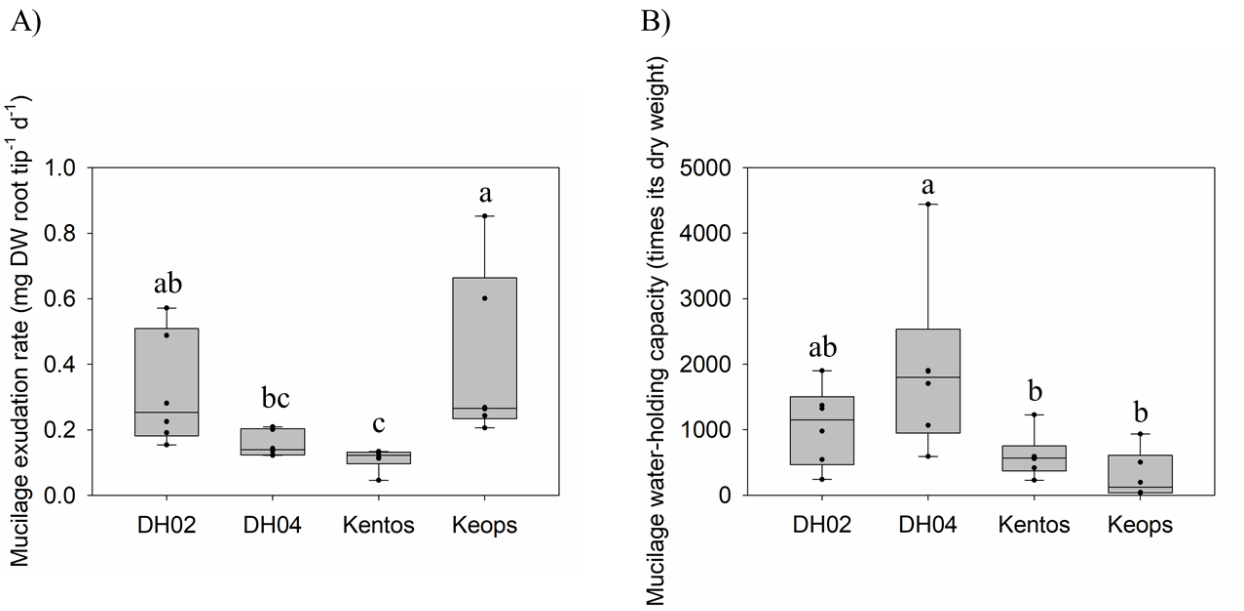

Figure 7. Mucilage exudation rate (A) and mucilage water-holding capacity (B) of nodal roots of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box defines the 25th and 75th percentiles.

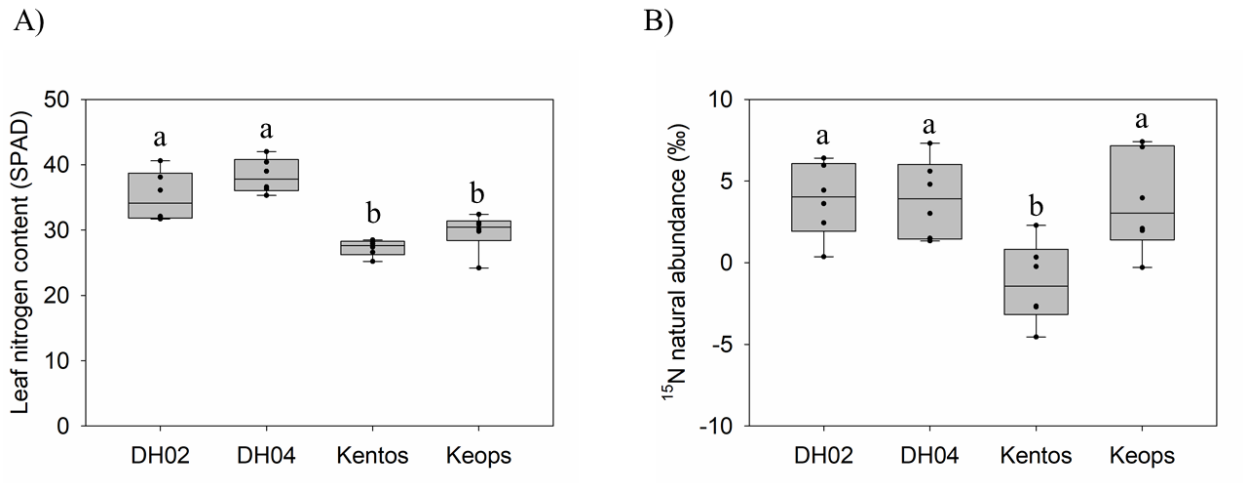

Figure 8. Leaf nitrogen content (A) and mucilage ^{15}N natural abundance (B) of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box defines the 25th and 75th percentiles.

Conclusions

Study 1

Polysaccharides, proteins, minerals, and lipids build up the composition of mucilage, each playing necessary roles in the rhizosphere. Based on the breeding goal, plant breeders can exploit the functions of each mucilage component to support agricultural and environmental sustainability.

Study 2

Maize mucilage exudation amount is associated with the climatic conditions of the genotypes' agroecological zone of origin where the breeding is done. Therefore, selection for environments with high vapor pressure deficit can favor higher mucilage exudation amount, plausibly due to the function of mucilage to delay the onset of hydraulic failure during periods of high vapor pressure deficit. We suggest that maize mucilage exudation amount has a genetic basis and genotypes from semi-arid agroecological zones are valuable sources of genetic material for beneficial mucilage traits.

Study 3

Maize can increase its mucilage exudation rate in response to warm climatic conditions and in microbially fertile soils to adapt to water stress and support the rhizosphere microorganisms,

respectively. It seems that uronic acids play a vital role in maize resistance to water stress, because of their interconnections with Ca^{2+} altering the mucilage and rhizosphere hydraulic properties.

Study 4

Mucilage and EPS have similar physical and chemical properties, suggesting comparable functions for these biogels and supporting the potential of mucilage to function as a biofilm matrix like EPS. In contrast to an EPS-based biofilm (heterotrophic), the C needed to form the biofilm is provided directly by the autotrophic plant, forming large volumes of stable microbial habitats in the rhizosphere. We suggest that the function of mucilage as a biofilm matrix has to be considered in conceptual plant and soil models.

Study 5

Mucilage from the drought-resistant varieties could hold large amounts of water, implying the specific function of this mucilage trait in drought resistance, i.e., in maintaining the rhizosphere wet for the survival of the rhizosphere microorganisms and plant in dry conditions. Moreover, our results indicated that the varieties capable of exuding a mucilage that holds huge amounts of water could take up more nitrogen from the dry soil, conveying the importance of mucilage water-holding capacity for improving the uptake of nutrients from drying soils. Plant breeders may consider root mucilage water-holding capacity in their breeding programs for sustaining plant nutrient uptake and productivity in arid and semi-arid agroecosystems.

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II Publications and manuscripts

Study 1: Plant mucilage components and their functions in the rhizosphere

Meisam Nazari

Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, 37073, Göttingen, Germany

Correspondence: meisam.nazari@forst.uni-goettingen.de, meisam.nazari1991@gmail.com.

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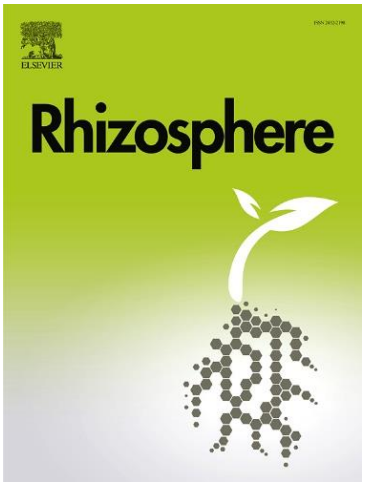

Abstract

This study aimed at analyzing the proportion of root and seed mucilage components and their functions in the rhizosphere. In total, polysaccharides (78.4%), proteins (7.3%), minerals (5.6%), and lipids (3.1%) constitute 94.3% of the mucilage composition. Mucilage polysaccharides are an energy source for microorganisms, absorb water, exchange cations, and adhere to solid surfaces in the rhizosphere. Mucilage proteins disassemble the mucilage polysaccharide for microbial utilization, respond to abiotic and biotic stresses, and mobilize nutrients in the rhizosphere. Mucilage minerals include monovalent and divalent cations capable of being exchanged for other cations in the rhizosphere. Mucilage lipids improve plant water uptake and desorb adsorbed phosphorus on soil particles in the rhizosphere. These important functions can support plant health and agricultural and environmental sustainability. Plant breeders can benefit from the advantages of a specific mucilage component and enhance its proportion in the mucilage of given plant species.

Keywords: mucilage composition, rhizosphere functions, soil properties, polysaccharide

Introduction

Plants produce a viscoelastic high-molecular-weight substance called mucilage (Chaboud, 1983; Sasse et al., 2018, Figure 1). Mucilage is a polysaccharide-rich substance, but also contains proteins, minerals, and lipids (Koocheki et al., 2013; Alizedeh Behbahani et al., 2017; Amicucci et al., 2019). Depending on the plant species, mucilage is secreted by roots, seeds, leaves, and stems (Galloway et al., 2020). Mucilage secreted by seeds and roots has a variety of beneficial functions in the rhizosphere. For instance, the seed-coat mucilage increases the seed's water availability and resistance against drought, plays an important part in soil seed bank maintenance, and is utilized as a carbon source by beneficial rhizosphere microorganisms (Huang et al., 2004; Yang et al., 2012; Galloway et al., 2020). The root mucilage improves soil aggregation, reduces the friction against the growing root, improves the rhizosphere water content and root water uptake under drought, and is a carbon source for soil microorganisms (Czarnes et al., 2000; Benizri et al., 2007; Iijima et al., 2003; Carminati et al., 2010; Ahmed et al., 2015). Moreover, mucilage ameliorates the rhizosphere toxicity caused by aluminum (Al^{3+}), and heavy metals such as cadmium (Cd^{3+}), and copper (Cu^{3+}) (Morel et al., 1986; Archamabault et al., 1996; Deiana et al., 2003). Mucilage also contributes to the formation of rhizosheath in some plant species, which has ecologically important functions like water and nutrient uptake in the rhizosphere (McCully, 1999; Sasse et al., 2018).

So far, research has mainly focused on the physical and hydraulic functions of mucilage in the rhizosphere (Carminati et al., 2010; Vetterlein et al., 2020). Recently, mucilage secreted by crown roots of a Mexican landrace maize (*Zea mays* Y.) was indicated to harbor nitrogen-fixing bacteria capable of fixing 29%–82% of the plant's nitrogen requirement (Van Deynze et al., 2018; Bennet et al., 2020). The nitrogen-fixing characteristic of mucilage is becoming a hot topic and receiving special attention, because it can increase maize (*Zea mays* L.) yield in regions with low soil fertility and can be an alternative for chemical nitrogen fertilizer. Moreover, plant breeding is endeavoring to develop the nitrogen-fixing system of the landrace maize in other cereals (Sheoran et al., 2021).

Despite the existence of many studies on the functions of mucilage in the rhizosphere, review studies on the functions of distinct mucilage components (i.e., polysaccharides, proteins, minerals, lipids) in the rhizosphere are lacking. Furthermore, the proportion of each mucilage component may differ depending on the plant species, which demands for analytical studies in order to get a holistic view on the proportion of each mucilage component. This study aimed at filling both knowledge gaps through analyzing the proportion of each mucilage component and by reviewing the functions of each component in the rhizosphere. The goal of this study was to expand and deepen the existing knowledge of the rhizospheric mucilage composition and the distinct functions of each mucilage component in the rhizosphere.

Figure 1. Mucilage secreted by the crown root of maize at the end of stem elongation (BBCH 39).

Methodology

Altogether, 56 datasets were extracted from 16 articles published between 1974 and 2018 for analyzing the proportion of each mucilage component (Weber et al., 1974; Gould and Northcote, 1986; Fedeniuk and Biliaderis, 1994; Chaboud and Rougier, 1991; Barbary et al., 2009; Razavi et al., 2009; Karazhiyan et al., 2011; Naqvi et al., 2011; Koocheki et al., 2012; Farahnaky et al., 2013; Paynel et al., 2013; Jouki et al., 2014; Uschapovsky et al., 2015; Behbahani and Imani Fooladi, 2018). Search engines used for finding the articles were the Web of Knowledge and Google Scholar. Key words used for searching included plant mucilage, composition, function, polysaccharide, protein, mineral, and lipid. Of these datasets, 12 belonged to the polysaccharide, 16 to the protein, 13 to the lipid, and 15 to the mineral component of the mucilage of 15 different plant species. Only articles that had investigated the composition of seed and root mucilage were investigated. All data were attributed to crude mucilage. Articles not fulfilling these criteria were not considered. The values presented in the pie chart are arithmetic mean.

Polysaccharides

Polysaccharides constitute 78.4% of the rhizosphere mucilage (Figure 2). Mucilage polysaccharides have essential functions in the rhizosphere, which are discussed below.

Energy source for rhizosphere microorganisms

Mucilage polysaccharides secreted by roots and seeds can differ depending on the plant species, but are in general composed of neutral monosaccharides such as galactose, fucose, mannose, glucose, arabinose, and xylose and uronic acids such as galacturonic acid and glucuronic acid (Rautengarten et al., 2008; Phan et al., 2016; Amicucci et al., 2019; Nazari et al., 2020). All of these sugars are a potential energy source for fueling soil microorganisms and microbial processes in the rhizosphere. Several pea (*Pisum sativum* L.) rhizosphere bacteria such as *Rhizobium*

leguminosarum and *Burkholderia cepacian* are capable of hydrolyzing the pea root mucilage polysaccharides and utilizing the derived sugars (Knee et al., 2001). By utilizing the sole pea mucilage polysaccharide, these bacteria reached cell densities of 3–25 times higher than the control (Knee et al., 2001). Moreover, polysaccharides of maize root mucilage are utilized by soil microorganisms, especially by bacteria (Mary et al., 1993; Ahmed et al., 2018). Interestingly, diazotrophic bacteria inhabiting maize mucilage can utilize the abundant sugars found in the mucilage polysaccharide such as fucose, arabinose, and xylose to fuel the energy-expensive nitrogen-fixing process (Bennet et al., 2020). As a future research prospect, it would be interesting to disentangle the effects of mucilage on rhizosphere microbial community structure and functions through metagenomics and metatranscriptomics.

Absorption of water

Mucilage can absorb up to 25 to 600 times its dry weight water (Huang and Gutterman, 1999; Capitani et al., 2013; Nazari et al., 2020). Rapid and strong hydration is one of the most striking features of mucilage, which is attributed to its hydrophilic polysaccharides, especially to negatively charged uronic acids (Sola et al., 2019). It is demonstrated that eliminating uronic acids like galacturonic acid from the seed mucilage of Arabidopsis (*Arabidopsis thaliana* L.) removes the expansion ability of the mucilage when exposed to water (Western et al., 2001; Dean et al., 2007; North et al., 2014). The self-assembly of uronic acids in the seed mucilage of Arabidopsis is responsible for the hydration and dehydration (wetting-drying) of the mucilage in the rhizosphere (Williams et al., 2020). However, more research on the mucilage of other plant species (i.e., maize) is required to generalize the finding.

Exchange of cations

One of the important characteristics of mucilage is its ability to exchange cations. This feature can be ecologically significant, i.e., in ameliorating Al^{3+} toxicity and sodium (Na^{+}) salinity in the rhizosphere (Horst, 1995; Edmond Ghanem et al., 2010). It is demonstrated that negatively charged uronic acids of mucilage are the major part responsible for ion exchange and toxic ion sequestration in the rhizosphere (Sola et al., 2019; Zhou et al., 2020). Calcium (Ca^{2+}) is the most abundant cation found in the root and seed mucilage of some plant species such as maize, cress (*Lepidium sativum* L.), saltmarsh mallow (*Kosteletzkya virginica* L.), and Arabidopsis, being strongly bound to the uronic acids (Edmond Ghanem et al., 2010; Brax et al., 2019; Sola et al., 2019). Ca^{2+} can be exchanged for other divalent cations such as Cu^{2+}, Cd^{2+}, lead (Pb^{2+}), and zinc (Zn^{2+}) and also for monovalent cations such as potassium (K^{+}) and Na^{+} (Pellerin and O'Neill, 1998; Edmond Ghanem et al., 2010; Sola et al., 2019; Zhou et al., 2020).

Adherence to surfaces

Mucilage is a glue-like viscous substance with adhesive properties (Sasse et al., 2018; Galloway et al., 2020). Upon release to the rhizosphere, mucilage can stick to solid surfaces and soil particles (Tsai et al., 2017; Holz et al., 2018). This has important ecological functions in the rhizosphere

such as soil aggregation, trapping root-parasitic nematodes, and attracting beneficial microorganisms (Czarnes et al., 2000; Tsai et al., 2019). Mucilage adherence to solid surfaces is attributed to major polysaccharide components such as xylose, arabinose, and uronic acids (Naran et al., 2008; Sola et al., 2019; Tsai et al., 2019). Intermolecular connections between uronic acids and Ca^{2+} seem to increase the adhesion strength of mucilage secreted by Arabidopsis seed (Sola et al., 2019). It is suggested that the relevance of mucilage adhesive properties to its uronic acid and Ca^{2+} interconnections is also investigated in the mucilage of other plant species (i.e., maize).

Proteins

Proteins constitute 7.3% of the rhizosphere mucilage (Figure 2). Mucilage proteins also play important roles in the rhizosphere, which are discussed in the following.

Disassembly of the mucilage polysaccharide

Mucilage polysaccharide is mainly composed of neutral monosaccharides and uronic acids (Rautengarten et al., 2008; Phan et al., 2016; Amicucci et al., 2019; Nazari et al., 2020). These sugars need to be released in order to feed the mucilage-inhabiting and rhizosphere microorganisms. Mucilage secreted by maize roots and Arabidopsis seed contains endogenous glycosyl hydrolase protein enzymes such as α-N-arabinofuranosidase, xylanase, α-L-fucosidase, β-1,4 xylosidase, β-mannosidase, glucosidase, and β-galactosidase, capable of disassembling the mucilage polysaccharide and releasing the sugars for microbial utilization (Tsai et al., 2017; Pozzo et al., 2018; Voiniciuc et al., 2018). These mucilage enzymes can potentially act in the decomposition of rhizosphere organic matter, which remains to be studied.

Response to abiotic and biotic stresses

A proteomic study of maize root mucilage revealed that the mucilage encompasses a remarkable number of proteins responsive to abiotic and biotic stresses (Ma et al., 2010). Proteins responsible for the alleviation of abiotic stresses include heat shock proteins and chaperonins, temperature-sensitive histone proteins, water stress proteins, and salt stress proteins. Major protein groups against biotic stresses include chitinases and peroxidases. Chitinases and peroxidases play significant parts in suppressing soil-borne plant pathogenic fungi and their biocontrol (Ordentlich et al., 1988; Mittler, 2002; Gohel et al., 2005). Chitinase enzymes were also found in the root mucilage of pea, rapeseed (*Brassica napus* L.), and Arabidopsis (Basu et al., 2006; Wen et al., 2007). Roots of maize genotypes originating from semi-arid agroecosystems were indicated to produce higher amounts of mucilage than those originating from temperate agroecosystems (Nazari et al., 2020). It would be interesting to investigate the correlation between mucilage exudation amount and its drought-alleviating proteins. Moreover, mucilage efficiency against soil-borne plant pathogenic fungi has to be checked in a large-scale (i.e., through field experiments) for use in organic agriculture.

Assistance in nutrient uptake

Maize root mucilage contains several protein enzymes that can improve the plant's nutrient availability and uptake (Ma et al., 2010). These proteins include phosphorus-mobilizing (i.e., acid phosphatase) and iron-mobilizing (i.e., aldehyde oxidase and aconitate hydratase). Phosphorus and iron are two important limiting factors for plant growth in many soils. Nearly 30–80% of soils total phosphorus is composed of organic phosphorus esters (Neumann and Romheld, ¨ 2007). Mucilage acid phosphatase may mobilize phosphorus in the rhizosphere and improve the plant's phosphorus uptake. However, the contribution quality and quantity of these protein enzymes in mobilizing phosphorus and iron in the rhizosphere remain to be investigated.

Minerals

Minerals constitute 5.6% of the rhizosphere mucilage (Figure 2). In general, mucilage contains Ca^{2+}, magnesium (Mg^{2+}), Cu^{2+}, Zn^{2+}, and K^{+} (Redgwell, 1983; Edmond Ghanem et al., 2010; Kaur et al., 2018). These cations can be exchanged for harmful divalent and monovalent cations in the rhizosphere such as Pb^{2+}, Cd^{2+}, and Na^{+}. Moreover, Ca^{2+} connects mucilage uronic acids, giving the mucilage a viscose gel-like form (Brax et al., 2019; Sola et al., 2019). The high viscosity of mucilage can improve rhizosphere water content, soil particle connectivity, soil liquid-phase connectivity, and root water uptake under drought (Young 1995; Carminati et al., 2010; Ahmed et al., 2015; Benard et al., 2019; Zarebanadkouki et al., 2019). It is recommended that researchers pay more attention to the remarkable cation exchange capacity of mucilage for the remediation of soils contaminated with heavy metals.

Lipids

Lipids constitute 3.1% of the rhizosphere mucilage (Figure 2). Major lipids found in the root mucilage of maize, lupin (*Lupinus angustifolius* L.), and wheat (*Triticum aestivum* L.) are phosphatidylcholines, which are mainly composed of saturated fatty acids (Read et al., 2003). Root mucilage lipids help the plant in taking up water from soil pores in the rhizosphere through altering the soil hydraulic properties (Read et al., 2003; Zeppenfeld et al., 2017). Furthermore, mucilage lipids can desorb already-adsorbed phosphorus on soil particles in the rhizosphere (Read et al., 2003). Mucilage lipids also act as lubricant and ease the penetration of roots into the soil (Zeppenfeld et al., 2017). So far, there have been a few studies on mucilage lipids than its other components. Therefore, more research is required to shed more light on further functions of this mucilage component.

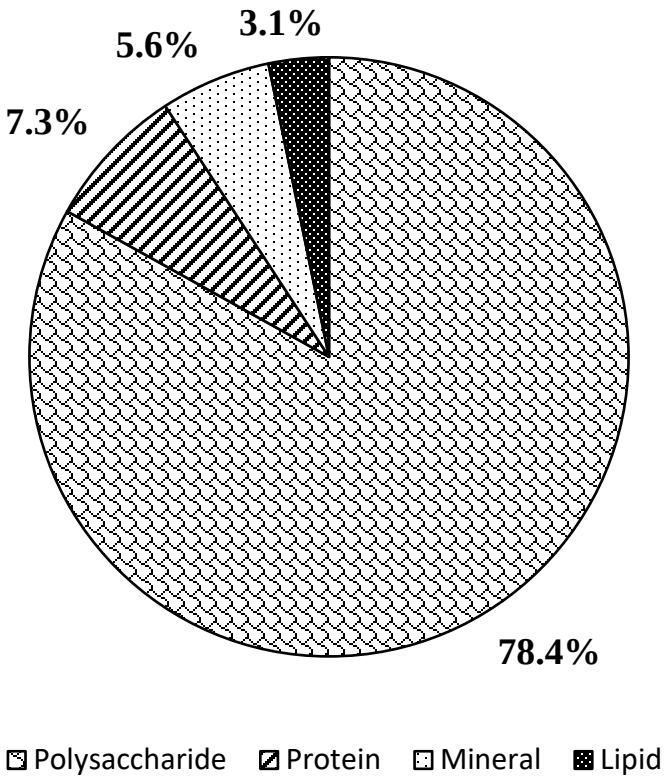

Figure 2. Proportion of each component of mucilage secreted by roots and seed of a set of plant species (n = 56).

Table 1. Plant mucilage components and the functions of each component in the rhizosphere.

Mucilage components	Functions
Polysaccharides	• Energy source for microorganisms • Water absorption • Exchange of cations • Adherence to solid surfaces (i.e., soil particles and nematodes)
Proteins	• Disassembly of the mucilage polysaccharide for microbial use • Response to abiotic and biotic stresses (i.e., heat stress and plant pathogens) • Assistance in nutrient uptake (e.g., phosphorus and iron)
Minerals	• Exchange of cations • Improvement of soil liquid-phase connectivity and water content
Lipids	• Alternation of soil hydraulic properties • Desorbing adsorbed phosphorus on soil particles • Easing the penetration of roots into the soil

Conclusions

This study analyzed the components of mucilage secreted by rhizospheric plant organs (roots and seed) and the functions of each component in the rhizosphere (Table 1). In total, polysaccharides (78.4%), proteins (7.3%), minerals (5.6%), and lipids (3.1%) constitute 94.3% of the mucilage composition. Altogether, the mucilage components have necessary functions in the rhizosphere

that can support plant health and, in a larger scale, agricultural and environmental sustainability. Plant breeders can exploit the advantages of a specific mucilage component by incorporating that component in their breeding programs.

Declaration of competing interest

The author declares no conflict of interest.

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Study 2: Mucilage polysaccharide composition and exudation in maize from contrasting climatic regions

Meisam Nazari[1], Sophie Riebeling[1], Callum C. Banfield[1], Asegidew Akale[2], Margherita Crosta[2], Kyle Mason-Jones[3], Michaela A. Dippold[1] and Mutez Ali Ahmed[1,2]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany,

[2]Chair of Soil Physics, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany,

[3]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands

Correspondence: meisam.nazari@forst.uni-goettingen.de

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Abstract

Mucilage, a gelatinous substance comprising mostly polysaccharides, is exuded by maize nodal and underground root tips. Although mucilage provides several benefits for rhizosphere functions, studies on the variation in mucilage amounts and its polysaccharide composition between genotypes are still lacking. In this study, eight maize (*Zea mays* L.) genotypes from different globally distributed agroecological zones were grown under identical abiotic conditions in a randomized field experiment. Mucilage exudation amount, neutral sugars and uronic acids were quantified. Galactose (~39–42%), fucose (~22–30%), mannose (~11–14%), and arabinose (~8–11%) were the major neutral sugars in nodal root mucilage. Xylose (~1–4%), and glucose (~1–4%) occurred only in minor proportions. Glucuronic acid (~3–5%) was the only uronic acid detected. The polysaccharide composition differed significantly between maize genotypes. Mucilage exudation was 135 and 125% higher in the Indian (900 M Gold) and Kenyan (DH 02) genotypes than in the central European genotypes, respectively. Mucilage exudation was positively associated with the vapor pressure deficit of the genotypes' agroecological zone. The results indicate that selection for environments with high vapor pressure deficit may favor higher mucilage exudation, possibly because mucilage can delay the onset of hydraulic failure during periods of high vapor pressure deficit. Genotypes from semi-arid climates might offer sources of genetic material for beneficial mucilage traits.

Keywords: agroecological zones, genotype, maize, mucilage, root exudation, vapor pressure deficit

Introduction

Plant roots have intensive physical, chemical and biological interactions with the surrounding soil (Haichar et al., 2008; Micallef et al., 2009; Bender et al., 2016; Leff et al., 2018). Roots exude a diverse range of metabolites that modify the physical and chemical environment of the rhizosphere (Hinsinger et al., 2009; Carminati and Vetterlein, 2013; Oleghe et al., 2017) and mediate plant-plant and plant-microbe interactions (Badri and Vivanco, 2009; Dutta et al., 2013; Williams and de Vries, 2020).

Plants exude about 25% of their total photosynthetic output into the rhizosphere, of which approximately half is in the form of mucilage (Chaboud, 1983; Walker et al., 2003). Mucilage is a gelatinous high-molecular-weight substance produced by almost all plants (Ahmed et al., 2014). Maize (*Zea mays* L.) exudes mucilage both from its nodal and underground roots. Maize nodal root mucilage has very similar properties to its underground root mucilage (Chaboud, 1983; Osborn et al., 1999). Mucilage produced by the nodal roots of some maize landraces harbors nitrogen-fixing bacteria, contributing to the fixation of 29% to 82% of the plant's nitrogen nutrition (Van Deynze et al., 2018; Amicucci et al., 2019; Bennett et al., 2020). This nitrogen fixation can increase maize yield and nitrogen use efficiency, especially in regions where agriculture suffers from poor soil fertility. Moreover, mucilage has been shown to increase the rhizosphere water content (Young, 1995; Carminati et al., 2010; Naveed et al., 2019), attenuate the gradients in soil water potential, and thereby facilitate root water uptake during soil drying (Ahmed et al., 2014). However, mucilage becomes water repellent upon drying (Ahmed et al., 2015; Zickenrott et al., 2016) and delays rewetting of the rhizosphere (Zarebanadkouki et al., 2018). Mucilage has also been implicated in reducing friction against the growing root (Iijima et al., 2003), ameliorating aluminum toxicity (Horst, 1995), and stabilizing soil aggregates (Czarnes et al., 2000). Mucilage is also a substrate for microbial decomposition (Mary et al., 1993; Ahmed et al., 2018a) and can provide a unique microbial habitat (Ahmed et al., 2018c). Mucilage likely contributes to plant resistance to water stress, and may be particularly important in water-limited environments (Ahmed et al., 2018b), and those with high vapor pressure deficit (VPD), where mucilage may delay the onset of hydraulic failure during drought (Grossiord et al., 2020).

Mucilage is mainly composed of polysaccharides but also contains proteins, minerals, and lipids (Carminati and Vetterlein, 2013; Koocheki et al., 2013; Alizadeh Behbahani et al., 2017). Though the monosaccharide composition and polysaccharide structure of mucilage has been previously studied, only recently have the first links been made between mucilage chemistry and its functional properties (Amicucci et al., 2019). This work has shown that mucilage chemical composition has important implications for the structure of colonizing microbial communities (Amicucci et al., 2019). Elucidating the polysaccharide composition of mucilage will therefore be a crucial step for understanding mucilage functions and the interaction of roots with their environment. Previous efforts to elucidate the mucilage composition of maize nodal roots have revealed that this mucilage is rich in fucose, galactose, and arabinose (Van Deynze et al., 2018; Amicucci et al., 2019). However, the observed similarity in the mucilage composition of these previous studies could be

attributed to the use the same maize landrace, Sierra Mixe, Mexico (*Zea mays* Y.). Thus, similarities or differences in mucilage composition between maize genotypes remain unknown. Current knowledge indicates that the root exudates of different genotypes of the same plant species can differ in composition (Bulgarelli et al., 2015; Iannucci et al., 2017). However, these results largely consider low molecular weight organic compounds, which are apidly consumed by soil microbial communities (Gunina and Kuzyakov, 2015). In contrast, the longer mean resistance time of mucilage in the rhizosphere (Mary et al., 1993) suggests that it can have greater spatial and temporal impact on root-soil interactions (Ahmed et al., 2018a).

Plant breeding has mainly focused on aboveground traits while only a few attempts have targeted root traits that support plant-soil interactions (Lammerts Van Bueren et al., 2011). Our knowledge of the genetic basis for the amount and composition of mucilage exudation is limited (Monchgesang et al., 2016), hindering the exploitation of this trait for breeding purposes (Kuijken et al., 2015; Fernández-Aparicio et al., 2016). Thus, an important research task is to identify the genetic basis of mucilage exudation and polysaccharide composition in support of future maize breeding and agricultural sustainability (Bennett et al., 2020). Addressing this issue requires disentangling a complex set of factors including different plant genotypes and growth conditions (Micallef et al., 2009). The first step of such an approach—testing different plant genotypes under constant soil and climatic conditions—is the scope of this study.

The aims of this study were to quantity and characterize the nodal root mucilage of eight maize genotypes from different agroecological zones grown under the same abiotic and biotic conditions. To characterize the mucilage, neutral sugar and uronic acid compositions were analyzed by gas chromatography-mass spectrometry (GC-MS). The mucilage exudation amount was measured for quantitative assessment. We hypothesized that mucilage polysaccharide composition and exudation amount are adapted to the agroecological zone in which a variety was bred, with VPD being a key variable controlling mucilage composition and amount. Thus, these genetically inherited traits will be evident even if these genotypes are grown under identical abiotic environmental conditions in a field experiment.

Materials and methods

Field experiment

The field experiment was performed as a randomized complete block design consisting of three replicates of each genotype at the teaching facility "Landwirtschaftliche Lehranstalten" near Bayreuth, Bavaria, Germany (49° 55' 47" North, 11° 33' 8" East and 344 m above sea level). The soil was classified as loamy silt with 67% sand, 11% silt, and 22% clay. Bayreuth has a temperate climate with mean annual temperature and annual precipitation of 8.3 °C and 638 mm, respectively. The maximum temperature during the 2019 maize season was 36.76 °C on 25th of July (daily average: 26.24 °C) and the minimum temperature was -4.98 °C on 12th of April (daily average: 1.29 °C). Minimum precipitation was zero mm per day with nearly rain-free periods

beginning of April and end of May until first half of June. The maximum rainfall was on 7th of August with 22.91 mm. The VPD for Bayreuth during the maize growing period was 0.19 kPa.

The maize genotypes were sown manually in the first week of May 2019 in strips of 0.2 m × 9 m with 75 cm row distance between the strips. On each side of the strip, there were three rows of plants of a commercial maize genotype to avoid border effects. The row of each genotype was replicated three times (n = 3). Weeds were manually removed but no fertilizers or pesticides were applied. Due to the hot weather and absence of rain in June, we applied irrigation for the whole experiment. Mucilage replicates were sampled from each of the three rows (n = 3) at the end of tassel emergence (BBCH 59). To gain a sufficient amount of mucilage for follow up analysis, several plants per row were sampled randomly.

Genotypes of maize

Eight maize genotypes from contrasting agroecological zones of the world were used (Table 1). The genotype Kentos is a silage maize developed by the breeding company KWS SAAT SE & Co KGaA (Einbeck, Germany) for the German market. Germany is classified as a cool temperate climate. The genotype KXB 8383 is also produced and marketed by the French branch of the KWS company as grain maize for distribution in France. While suited to a temperate climate, this genotype is also tolerant to the Mediterranean climate in the south of the country. The Italian branch of KWS produces the genotype Kerubino at a breeding station near Venice. The climate there is warm temperate. Keravnos is a Turkish maize genotype produced by KWS Türk. Turkey has a Mediterranean climate with hot, dry summers. The genotypes DH 02 and DH 04 are produced by the Kenya Seed company (Kitale, Kenya). The genotype DH 02 comes from dryland regions in the southeast of the country (annual precipitation < 700 mm). The genotype DH 04 is bred for the lowland regions where climate is tropical (annual precipitation < 900 mm). The Indian 900 M Gold and 30 V 92 genotypes are produced by Dekalb and Pioneer Hi-Bred companies, respectively. Both companies have their breeding station in Hyderabad, India, with a semi-arid and very hot steppe climate (annual precipitation < 800 mm).

Table 1. Information on the maize genotypes, their agroecological zones, and breeding methods.

Region	Country	Climate	Temperature (°C)[a]	Relative humidity (%)[a]	P (mm)[b]	Genotype	Breeding method
Central Europe	Germany	Temperate	15.8	77.7	132-560	Kentos	Hybrid
	France	Temperate	15.7	60	105-660	KXB 8383	Hybrid
Southern Europe	Turkey	Mediterranean	16.4	59	86-332	Keravnos	Hybrid
	Italy	Mediterranean	18.4	72.5	240-1100	Kerubino	Hybrid
Africa	Kenya	Semi-arid	24.8	75	62-720	DH 02	Hybrid
	Kenya	Tropical	24.6	83	62-867	DH 04	Hybrid
Asia	India	Semi-arid	25.7	72.3	324-2044	900 M Gold	Hybrid
	India	Semi-arid	25.7	72.3	324-2044	30 V 92	Hybrid

a: Temperature and relative humidity are the mean for the maize growing season in the related agroecological zone within the past 10 years

b: P (precipitation) is the sum over the entire growing season as range from minimum to maximum of observed precipitation within the past 10 years.

Sampling of mucilage

Mucilage samples were taken at the end of tassel emergence (BBCH 59) from each of the three rows (n = 3) using the method of Ahmed et al (2015). Maize second and third nodal roots covered in mucilage were selected (Figure 1). The nodal roots were cut from the stem and placed in aluminum trays. In the laboratory, soil and plant residues on the nodal roots were removed by distilled water in a coarse sieve. Thereafter, the mucilage-covered roots were submerged in distilled water until the mucilage was fully water-saturated. After one day, the excess water was discarded through a sieve with a mesh size of 500 µm. The hydrated mucilage was aspirated from the nodal roots with syringes. The remaining mucilage on the root tip was removed with fine forceps. The mucilage was collected in 20 ml vials, frozen at - 18 °C, and subsequently freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany).

Figure 1. Mucilage secreted by nodal roots of the maize genotype Kerubino at the end of tassel emergence after a rainfall event.

Preparation of samples

Freeze-dried mucilage (20 mg) was weighed into centrifuge tubes. Lipids were sequentially pre-extracted by 20 ml methanol, followed by 20 ml dichloromethane:methanol (1:1, v:v) and by 20 ml dichloromethane. The pre-extractions were performed in an ultrasonic bath (35 kHz, 25 °C, 80 W nominal power, Bandelin Sonorex RK100, Berlin, Germany) for 10 min, followed by centrifugation at 1500 × g for 10 min. The pellet was dried under nitrogen gas and weighed. Only chemicals of p.a. grade or better were used for the subsequent analyses. Analysis follows in its principles the method of Zhang and Amelung (1996).

For neutral sugar and uronic acid analyses, 10 ml 4 M trifluoroacetic acid (TFA) was added to each sample, capped and hydrolyzed at 105 °C for 8 h. The hydrolysate was filtered through glass fiber filters (GF6, Whatman GmbH, GE Healthcare, Freiburg, Germany) with 5 ml of ultrapure water, and the first internal standard (0.05 mg allose) was added. Thereafter, the sample was dried in a rotary evaporator at 40 °C and 30 mbar. To completely remove TFA residues, 0.5 ml of water was added to the conical flask and evaporated again. This step was repeated once more. Samples were transferred to reaction vessels with three times 0.5 ml of water and dried under pure nitrogen

gas. To split the samples into two parts for measurement of neutral sugars and uronic acids, they were re-dissolved in 500 µl of water, sonicated for 10 min in the ultrasonic bath, and then an aliquot of 250 µl was transferred to new reaction vessels. The samples were evaporated again and stored at -20 °C prior to derivatization.

Derivatization and measurement of neutral sugars

Seven volumes (ranging from 10–800 µl) of an external standard stock solution of 0.5 mg ml^{-1} of allose (D+), arabinose (D-), fucose (L-), galactose (D+), glucose (D+), mannose (D+), rhamnose (L+), ribose (D-), and xylose (D+), were pipetted into reaction vessels and dried under a nitrogen gas stream. Samples and external standards were derivatized in a two-step procedure to aldononitrile acetates. First, 300 µl of hydroxylamine hydrochloride and 4-(dimethylamine) pyridine (32 mg ml^{-1}) dissolved in pyridine-methanol (4:1, v:v) was added to the samples and standards, capped and heated to 80 °C for 30 min. After cooling down, 1 ml acetic anhydride was added to all vessels, which were then heated again to 80 °C for 30 min, following the procedure of Zhang and Amelung (1996). After the derivatization, 15 µg of the second internal standard (IS2) methyl tridecanoate was added to derivatized and purified samples (filtered through anhydrous Na_2SO_4) and standards and they were transferred with a mixture of ethyl acetate/n-hexane (1:1, v:v) to 300 µl inserts in GC vials.

The analytes were separated by gas chromatography (Agilent 7820A GC, Agilent Technologies, Waldbronn, Germany) and detected by mass spectrometry (Agilent 5977B, Agilent Waldbronn, Germany). Helium with a constant flow rate of 1.1 ml min^{-1} was used as the carrier gas through an OV 17-MS capillary column (Macherey Nagel, Düren, Germany, 30 m length, 250 µm inner diameter, 0.25 µm film thickness). An aliquot of 1 µl per sample was injected with a split ratio of 30:1 at an injector temperature of 250 °C. The oven program started at 100 °C, held for 1 min, then heated by 20 °C min^{-1} to 175 °C and held at this temperature for 3 min. The temperature was increased further by 4 °C min^{-1} to 235 °C, held for 3 min and finally raised to 300 °C at 50 °C min^{-1} and held for 7 min. Further detailed GC parameters can be found in the supplementary materials to Banfield et al. (2018). The mass-sensitive detector was run in scan mode (50-550 amu) with electron ionization energy of 70 eV.

Derivatization and measurement of uronic acids

Eight volumes (ranging from 10–800 µl) of an external standard stock solution containing 1 mg ml^{-1} galacturonic acid, glucuronic acid and 3-O-methylglucose were pipetted into reaction vessels and treated as samples. 0.05 mg of 3-O-methylglucose were added to the samples as an internal standard. Derivatization was performed in two steps: First, 200 µl NMP was added as a solvent and 200 µl methoxyamine hydrochloride solution (20 mg ml^{-1} pyridine) was used for methyloxime formation. Vessels were heated to 75 °C for 30 min. Thereafter, 400 µl N,O-bis(trimethylsilyl)trifluoroacetamide was applied for silylation of the hydroxyl groups to trimethylsilyl groups. Vessels were heated for 5 min at 75 °C. Details were as described by Banfield et al. (2018). After derivatization, 50 µg of hexadecane were added as the IS2 and the

samples were transferred, including their derivatization reagents, into GC vials for measurement within 8 h of derivatization.

The analytes were separated by gas chromatography (Agilent 7890 GC, Agilent Waldbronn, Germany) and detected in a mass-sensitive detector (Agilent 7000B Triple Quadrupole MS, Agilent Waldbronn, Germany). The GC was equipped with a DB-5MS column (30 m length, 250 µm inner diameter, 0.25 µm film thickness). Helium with a flow rate of 1.5 ml min^{-1} was used as the carrier gas. An aliquot of 1 µl was injected at 250 °C and 0.68 bar at a split ratio 50:1. The oven program started at 145 °C, held for 0.5 min and then heated to 160 °C at 10 °C min^{-1}, held again for 0.5 min and heated at 6 °C min^{-1} to 185 °C. The temperature was raised to 185 °C at a rate of 6 °C min^{-1}, held for 0.5 min and increased to 300 °C at 100 °C min^{-1}. The detector was set to scan mode (all fragments from 50–550 amu) and electron ionization energy of 70 eV.

Integration and quantification of sugars and uronic acids

Total ion current chromatogram peaks were integrated with the Agilent Mass Hunter Quantitative Data Analysis software (Agilent Technologies, Waldbronn, Germany), always ensuring peak identity by comparison of characteristic fragments with the external standards. Analyte peak areas were normalized to the peak areas of the sample's respective IS2 peak (methyl tridecanoate, hexadecane). Quantification was performed based on a linear regression of the external standards' peak areas to the external standard amounts. Furthermore, a recovery correction using IS1 provided absolute quantification of polysaccharide-derived monosaccharide content.

Measurement of mucilage exudation amount and saturation water content

Mucilage exudation amount was expressed as dry mass of collected mucilage per unit area of the nodal root surface (g mm^{-2}). The root surface was estimated using the formula for the surface of a cylinder $A = 2\pi \times r \times h$. The diameter and length of the roots were measured with calipers. In addition, the saturation water content of mucilage was calculated by the subtraction of the mucilage wet weight from dry weight divided by its dry weight. The calculation of mucilage exudation and saturation water content was carried out with the average of 10 nodal roots per genotype.

Calculation of vapor pressure deficit

The following equation was used to calculate VPD during the growing season of maize for each agroecological zone (Marengo et al., 2008; Seager et al., 2015):

$$VPD\ (kPa) = 0.611 \times exp\left(\frac{17.5 \times T}{240.987 + T}\right) - 0.611 \times exp\left(\frac{17.5 \times T_d}{240.987 + T_d}\right) \qquad Eq\ (1)$$

Where, T is the mean temperature (°C) and T_d is the dew point (°C).

T and relative humidity (RH%) were used to calculate the T_d:

$$T_d = \frac{243.04 \times \left(ln\left(\frac{RH}{100}\right) + \frac{17.625 \times T}{243.04 + T}\right)}{17.625 - \left(ln\left(\frac{RH}{100}\right) + \frac{17.625 \times T}{243.04 + T}\right)} \qquad Eq\ (2)$$

It is noted that T and RH were the mean for the maize growing season in each agroecological zone.

Statistical analysis

The statistical design follows a randomized block design with blocks expressed as rows in our experiment and each block serving as one of the field replicates. Each block contained one row with each genotype representing the levels of our treatments. All data were analyzed by SPSS 25 (SPSS Inc., Chicago, IL, USA). The data were tested for homogeneity of variance by Levene's test and normality by visual inspection of Q-Q plots, respectively, and transformed appropriately if they did not meet these prerequisites. One-way analysis of variance (ANOVA) was used to test for significant differences of the means between the genotypes at a significance level (α) of 0.05. Tukey's HSD (Honestly Significant Difference) test was used for pair-wise comparison of the arithmetic means. Linear regression ($\alpha = 0.05$) was used to detect associations between the genotypes' mucilage exudation amount and also saturation water content and the VPD of their agroecological zones. Linear regression was also used to find associations between polysaccharide composition and VPD. Multiple regression ($\alpha = 0.05$) was used to identify correlations between the mucilage saturation water content and its mucilage polysaccharide composition.

Results

Mucilage Polysaccharide Composition

The hexoses galactose (~39–42%), fucose (~22–30%), mannose (~11–14%), and glucose (~1–4%) were found in the nodal root mucilage of all maize genotypes (Figure 2). The hexose rhamnose was below the limit of detection. With exception of mannose and rhamnose, there were significant differences in hexose composition between the maize genotypes (at $P \leq 0.05$, Table 2). The Kenyan genotype DH 02 had the lowest overall proportion of hexoses, whereas 3.5% higher hexose proportions were found in the genotypes 30 V 92, 900 M Gold, Keravnos, Kerubino, and Kentos (at $P \leq 0.05$, Figure 3). The Indian genotypes had the highest sum of galactose and fucose, the two most abundant monomers, but showed significantly contrasting partitioning of these two monomers (at $P \leq 0.05$, Figure 4). The genotype 30 V 92 had the highest proportion of galactose (42.6%) and the lowest proportion of fucose (24.1%), whereas vice versa 900 M Gold displayed highest fucose (30.2%) and lowest galactose (38.1%) proportions within the set of studied genotypes. Besides this, there is generally a remarkable similarity in the proportion of galactose and fucose and only minor differences in distribution of other hexose monomers were observed. Kentos and DH 02 had the highest (4.2 and 3.9%, respectively) and 900 M Gold the lowest proportion of glucose (1.8%) (Figure 4).

The pentoses comprised arabinose (~8–11%) and xylose (~1–4%), whereas ribose was below the limit of detection (Figure 2). The maize genotypes significantly differed in pentose composition (at $P \leq 0.05$, Table 2). Kentos and 30 V 92 had the lowest proportion of pentoses (4.5% less than DH 02 and KXB 8383, at $P \leq 0.05$, Figure 3). In the case of 30 V 92 this was due to a 2.9% lower arabinose proportion and in the case of Kentos by 2.7% lower xylose proportion relative to DH 02

or KXB 8383 (at $P \leq 0.05$, Figure 5). The Kenyan genotype DH 02 and the central European genotype KXB 8383 had the highest arabinose proportions. Glucuronic acid (~3–5%) was the only uronic acid detected. The proportion of glucuronic acid of the total sugar monomers was significantly different among the maize genotypes (at $P \leq 0.05$, Figure 5), with the lowest proportion in the Southern European genotype Keravnos (3% of monomers) and highest in the Indian genotype 30 V 92 and the central European genotype Kentos (5.9% of monomers). No consistent relationship was detected between the mucilage polysaccharide composition of the genotypes and the vapor pressure deficit (VPD) of their agroecological zones of origin (Supplementary Table 1).

Table 2. Analysis of variance for the mucilage sugar composition, exudation, and saturation water content of the maize genotypes (one-way ANOVA, at $P \leq 0.05$).

Sources of variation	df	Sum of squares	Mean square	F	P-value
Hexoses	7	42.66	6.09	6.09	< 0.001 *
Pentoses	7	0.06	0.01	16.64	< 0.0001 *
Glucuronic acid	7	27.54	3.93	4.06	< 0.01 *
Galactose	7	60.19	8.59	10.37	< 0.0001 *
Fucose	7	142.63	20.33	11.59	< 0.0001 *
Mannose	7	0.39	0.04	2.59	NS
Glucose	7	0.35	0.05	4.68	< 0.01 *
Arabinose	7	0.04	0.006	11.70	< 0.0001 *
Xylose	7	0.33	0.04	10.19	< 0.0001 *
Mucilage exudation	7	1.87	0.26	8.06	< 0.0001*
Mucilage saturation water content	7	123058.57	17579.79	4.01	< 0.01 *

* = significant; NS = not significant

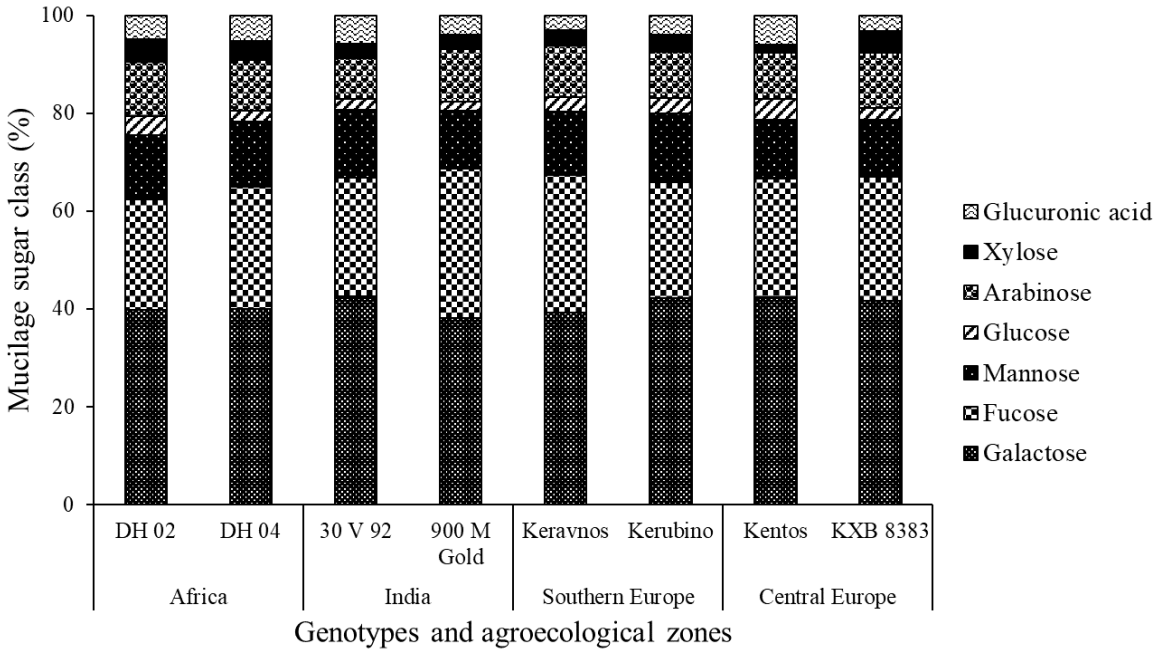

Figure 2. Proportion of the neutral monosaccharides and uronic acids in the nodal root mucilage of the maize genotypes. Displayed is the proportion of the individual monosaccharides or uronic acids as proportion of the total monosaccharide and uronic acid content of the respective mucilage sample. Values represents averages of 3 field replicates (n = 3).

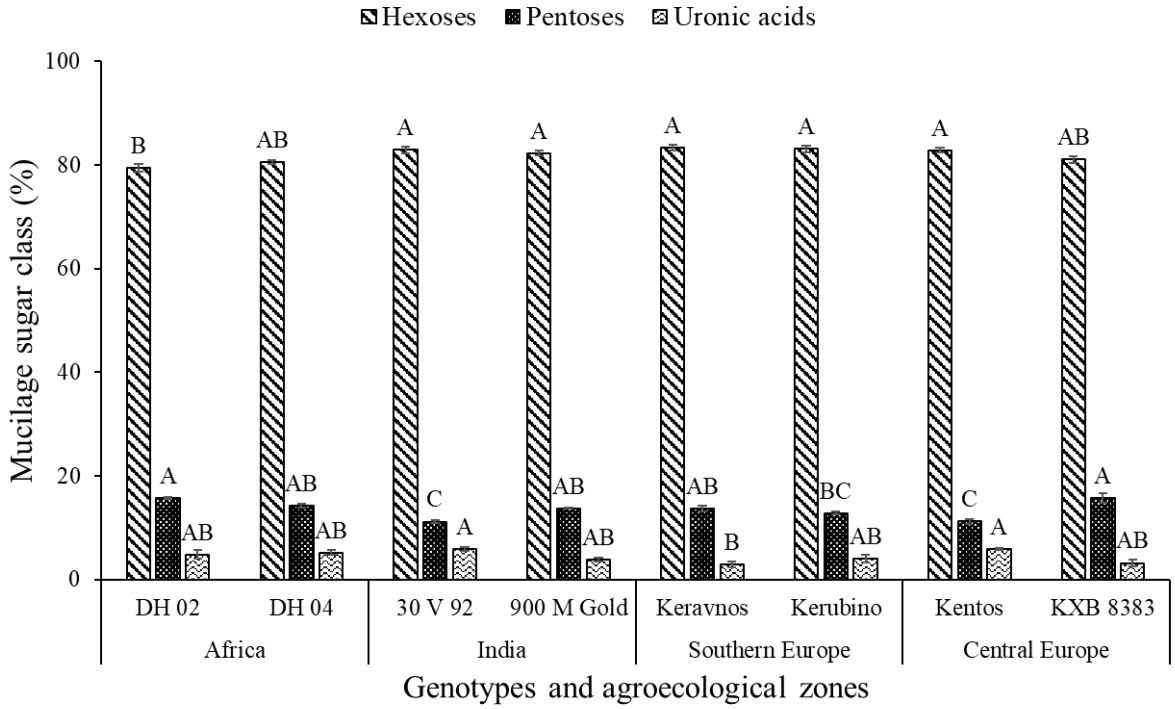

Figure 3. Proportion of the sugar compound classes in the nodal root mucilage of the maize genotypes. Different letters on each bar show a statistically significant difference (Tukey's HSD, at $P \leq 0.05$). Error bars indicate the standard error of the mean (n = 3).

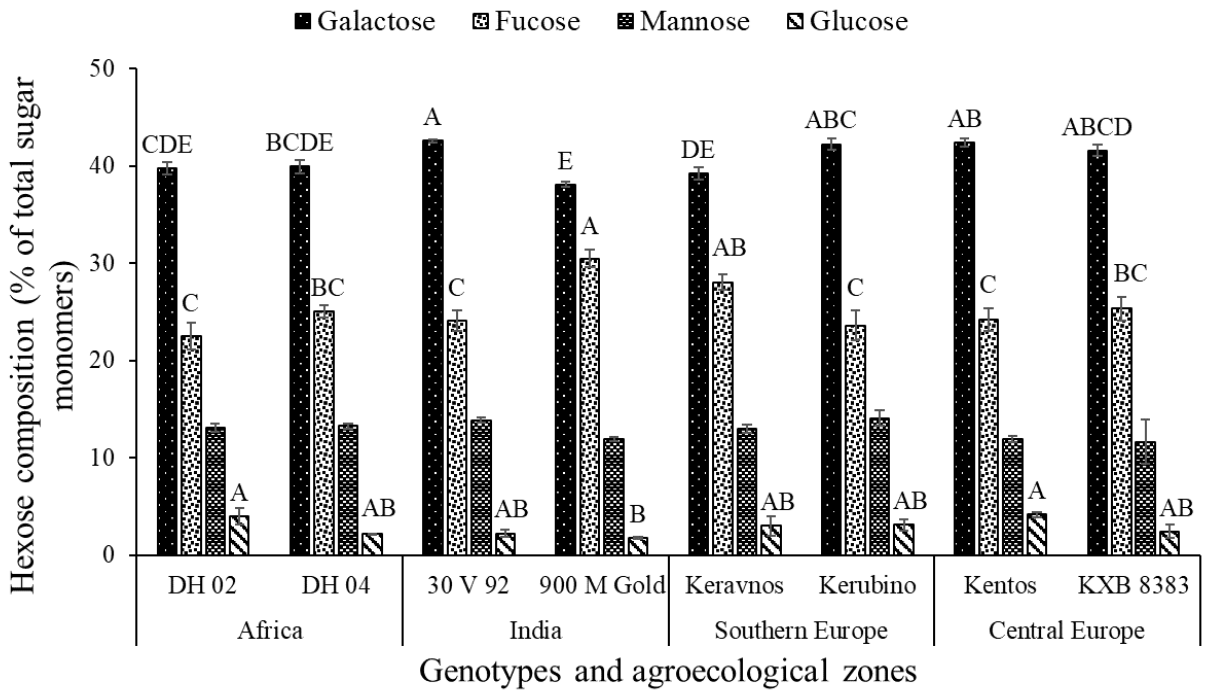

Figure 4. Proportion of hexose monosaccharides in the nodal root mucilage of the maize genotypes. Different letters on each bar show a statistically significant difference (Tukey's HSD, at $P \leq 0.05$). Error bars indicate the standard error of the mean (n = 3).

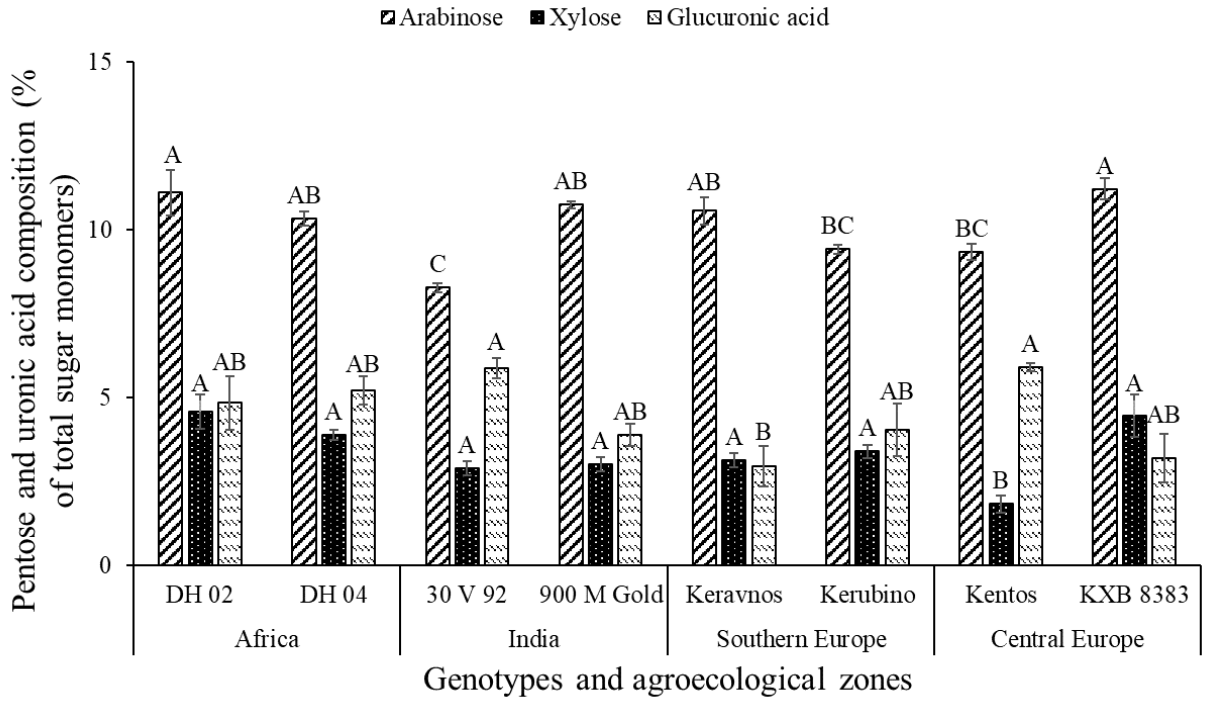

Figure 5. Proportion of pentose monosaccharides and glucuronic acid in the nodal root mucilage of the maize genotypes. Different letters on each bar show a statistically significant difference (Tukey's HSD, at $P \leq 0.05$). Error bars indicate the standard error of the mean (n = 3).

Mucilage Exudation Amount and Saturation Water Content

Mucilage exudation amount deviated significantly between the maize genotypes of contrasting origin investigated in this study (Table 2 and Figure 6A). The Indian genotype 900 M Gold and the Kenyan genotype DH 02 had a 135 and 125% higher mucilage production than the central European genotypes Kentos and KXB 8383. Mucilage exudation was positively correlated with the VPD of the agroecological zone for which the respective genotype was bred (Figure 7).

The maize mucilage absorbed on average 200 times its own dry weight in water. The mucilage saturation water content differed significantly between the maize genotypes. The mucilage of the southern European genotype Keravnos had the highest saturation water content, whereas the mucilage of the Kenyan genotype DH 02 had the lowest (Figure 6B). No significant relationship between the mucilage saturation water content of the genotypes and the VPD of their agroecological zones was found (Supplementary Figure 1). Moreover, no significant relationship between the mucilage saturation water content and its polysaccharide composition was detected (Supplementary Table 2).

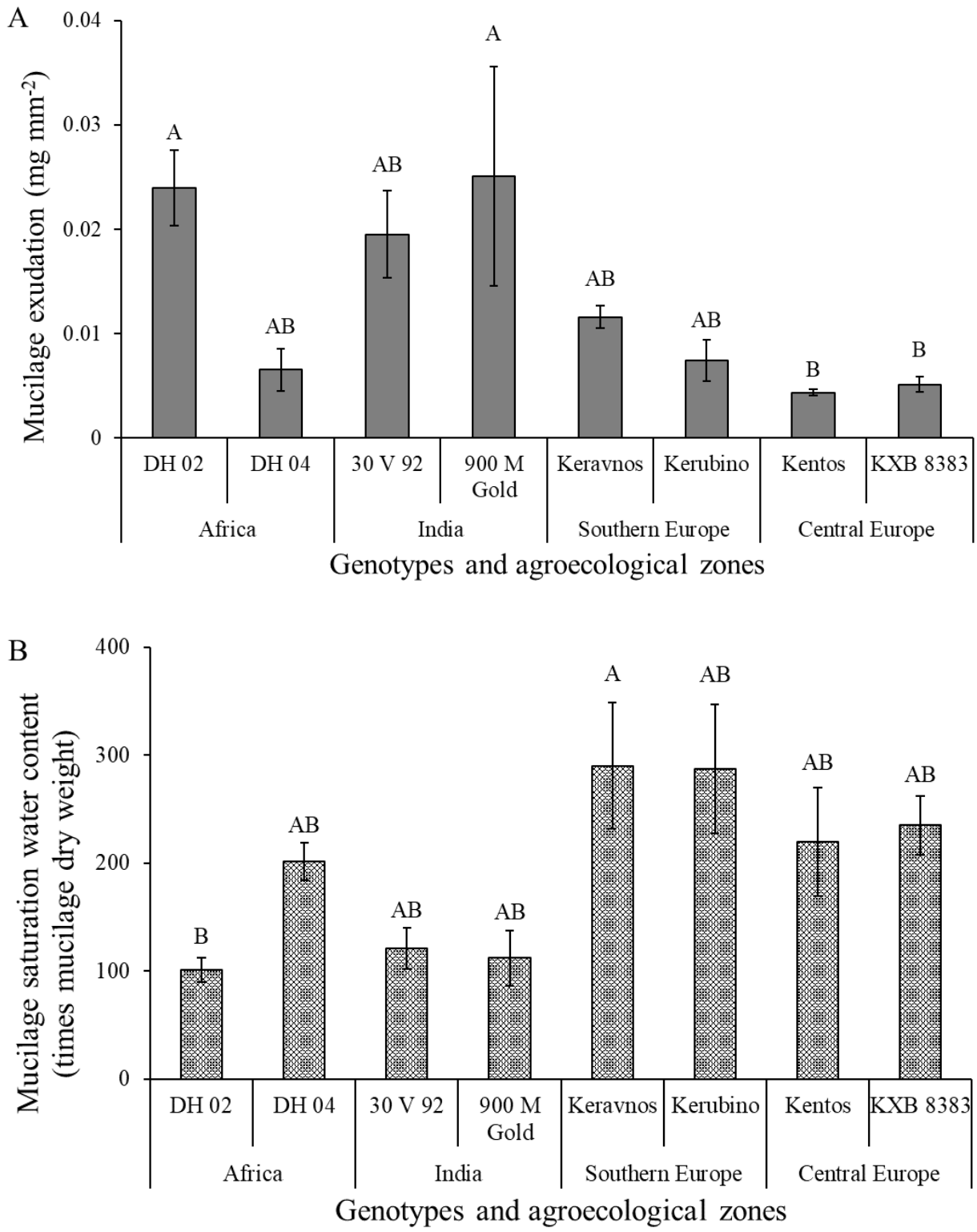

Figure 6. (A) Mucilage dry weight of the different maize genotypes normalized by root surface area. **(B)** Mucilage saturation water content of the maize genotypes. Different letters above the bars show significant differences between the respective water contents and exudation amounts (Tukey's HSD, at $P \leq 0.05$). Error bars indicate the standard error of the mean (n = 3).

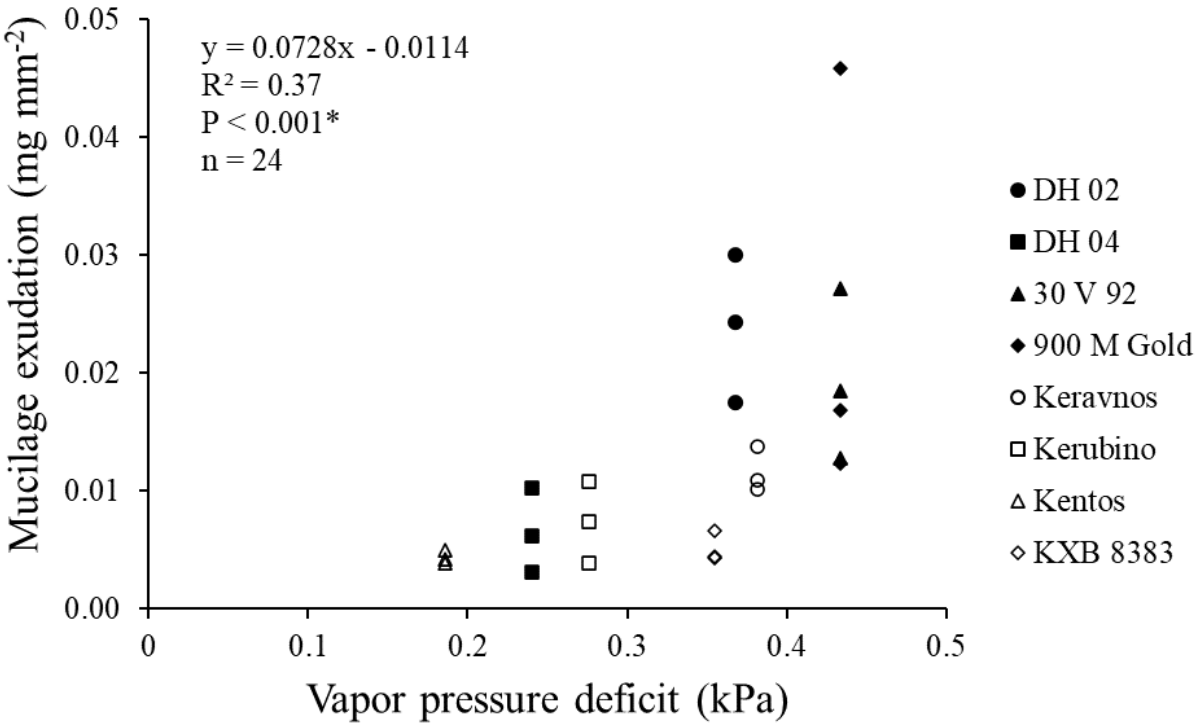

Figure 7. Relationship between the genotypes' mucilage exudation and the vapor pressure deficit (VPD) of their agroecological zones (∗ = significant at $P \leq 0.05$). The VPD for the region where the maize genotypes were grown for this study (Bavaria, Germany) was 0.19 kPa.

Discussion

Mucilage polysaccharide composition

Galactose, fucose, mannose, and arabinose were the major neutral sugars in the nodal root mucilage of the maize genotypes, whereas xylose and glucose were shown to be of minor proportion. Sugar monomers liberated by acid hydrolysis are frequently used in soil science as biomarkers, assuming plants mainly produce pentose-rich polymers whereas microbes preferentially synthetize hexoses (Kögel-Knabner et al. 2002). This has led to proxies like the GM/AX ratio (galactose + mannose / arabinose + xylose) as a proxy for microbial versus plant origin of soil organic matter (Spielvogel et al. 2016). Our study (Figure 3) demonstrates that mucilage sugar monomer composition would clearly fall into the microbial category and hence the utilization of such proxies, especially in the rhizosphere, needs to be used with caution (Gunina and Kuzyakov, 2015).

In previous studies, the polysaccharide composition of maize nodal root mucilage was similarly found to comprise fucose, galactose, arabinose, and mannose as the main neutral sugars (Van Deynze et al., 2018; Amicucci et al., 2019). The similarity in this pattern, especially the dominance of galactose, suggests that the polymeric structure of mucilage does not deviate greatly between genotypes from the polymeric structure proposed by Amicucci et al. (2019) i.e. a galactose backbone (most abundant monomer), which is highly fucosylated (2nd most abundant monomer)

but also partially xylosylated, with further arabinan and mannoglucoronan branches. Amicucci et al. (2019) and Van Deynze et al. (2018) linked the polymeric structure of the nodal root mucilage to a functional role in attracting a specific plant-growth promoting microbial community. The general similarities in monomer composition suggest that this potentially linked function of nodal root mucilage to rapidly attract N-fixing microbial communities is present over a large set of genotypes and retained during progressive breeding from the landrace to the hybrids.

The proportion of xylose and glucose in the present study deviated from previous studies (Van Deynze et al., 2018; Amicucci et al., 2019). Whether glucosylation of the galactose backbone is partially replacing the xylosylation remains speculative and awaits confirmation by further LC-MS analysis in follow-up studies.

In terms of the uronic acids, glucuronic acid was the only uronic acid found in the mucilage of the maize genotypes, which is in agreement with the study of Van Deynze et al (2018). Amicucci et al (2019) found in addition low amounts of galacturonic acid (1%) and a much higher proportion of glucuronic acid (11.3%) in the nodal root mucilage. As the proportion of carboxylic groups (i.e. uronic acid monomers) accounts for the negative charge of the mucilage polymer, the landrace maize Sierra Mixe can be assumed to have much higher interactions via bivalent Ca^{2+} ion bonds (Brax et al. 2019). The ratio of specific Ca^{2+} ion bonds to unspecific electrostatic chain interactions was identified as a crucial parameter for hydration-dehydration kinetics as well as water flow through the mucilage, but only with biogels having considerably higher uronic acid content than the maize mucilage characterized in this study (Brax et al. 2019). Therefore, the relevance of the uronic acid-based Ca^{2+} interactions seems to be of rather minor importance for the nodal root mucilage of maize. Brax et al. (2019) found also a clear effect of the Ca^{2+} concentration on the gel properties, which might suggest an adaptation of the uronic acid content to the Ca^{2+} saturation of the soil solution. Although we do not have specific soil data available for the breeding stations of the genotypes presented here, it is most likely that the spectrum of soils covers calcareous as well as strongly decalcified acidic soils. Aboveground biogels, such as low-methoxy pectins, would show strongly contrasting rheological and hydraulic properties depending on the Ca^{2+} concentration of the soil solution. Nodal root mucilage of maize, with low uronic acid content but instead forming its 3D-structure with covalent as well as unspecific electrostatic interactions (Brax et al. 2019), will instead maintain similar structural and physicochemical features irrespective of the soil properties. However, this observation suggests that, although polygalacturonic acid (PGA) has been used in previous experiments to represent mucilage, PGA is in fact a rather weak analogue of nodal root mucilage (Zickenrott et al. 2016, Ellerbrock et al., 2019, Brax et al. 2019).

Generally, differences in sugar monomer composition between the literature and our results need to be interpreted carefully, as they could either be related to genotype-specific differences or result from methodological differences. These differences could include plant growth stage at the time of mucilage collection, root type targeted for mucilage collection, mucilage collection method, hydrolysis time, hydrolysis temperature, purification methods, and derivatization methods (Chaboud and Rougier, 1984; Amicucci et al., 2019). Furthermore, a regulatory response of the

plant to abiotic environmental conditions may have had significant influence on the mucilage composition. Conditions simulating the Sierre Mixe region of origin in Oaxaca (Mexico) (Amicucci et al, 2019), those in a greenhouse in Wisonsin (Van Deynze et al. 2019), and our field experiment in Bayreuth (Germany) would differ in temperature and humidity (VPD) as well as soil properties, with potential implications for the monomer composition (Amicucci et al, 2019; Van Deynze et al, 2018).

Our set of eight genotypes comprises a substantial breadth of breeders (KWS SAAT, Kenya Seed, Dekalb, and Pioneer Hi-Bred) and agroecological zones. We therefore expect that the variability of quantified sugar monomer composition, even between landraces and hybrids, would be much lower if a single standardized method were applied (Annette et al., 2020). Without detailed experimental comparisons of these methods, no final conclusion on the extent of possible methodological bias can be made. This calls for an inter-laboratory comparison of the relatively novel methodological approaches for sugar monomer analysis of mucilage and other biogels.

This study used one standardized methodological approach, and thus the identified differences in monomer composition between the eight genotypes, even if rather small, is robust. Nonetheless, the genotype-specific monosaccharide fingerprint of the mucilage could not be linked by a consistent relationship to the VPD in their agroecological zones of origin. Thus, further factors besides local climatic conditions might be responsible for the adaptation of the polysaccharide composition of the mucilage.

Compared to most previous studies, this experiment was not done in sterile hydroponic or greenhouse conditions on artificial substrates, but was a field experiment on natural soil and thus additional factors besides the plant genotype need to be considered. Ahmed et al. (2018a,b) have already shown that mucilage in soil may provide beneficial habitats for microbes. This was even more specifically demonstrated for nitrogen-fixing microorganisms in the aboveground mucilage on nodal roots (Triplett, 1996; Estrada et al., 2002; Montanez et al., 2009; Van Deynze et al., 2018; Amicucci et al., 2019). Evidence of mucilage C use for bacterial and fungal growth in soil (Ahmed et al. 2018 a,b) as well as the specific mutualistic adaptation of defined glycosylhydrolases of microbial community members to the mucilage (Pozzo et al. 2018) suggest that the polysaccharide composition of the maize genotypes may have coevolved with beneficial soil microbial communities. Such microbiome-related carbohydrate adaptations would explain the absence of any correlation of monosaccharide composition with the VPD. However, more research with multiple factorial designs (e.g., full factorial designs growing genotypes on their native as well as contrasting soils) would be required to confirm mucilage-microbiome co-evolution. However, our data at least indicate that the nodal root mucilage polysaccharide composition may have a genetic basis, as the hybrids grown here under identical environmental conditions displayed genotype-specific polysaccharide patterns. Amicucci et al (2019) observed significant differences neither between the polysaccharide linkages nor within the monosaccharide composition of the mucilage of Sierra Mixe maize grown under two different environmental conditions, which provides an

indication for a potential genetic basis for the polysaccharide composition of nodal root mucilage in maize.

Mucilage exudation amount and saturation water content

The amount of mucilage exuded by maize genotypes from agroecological zones with higher VPD (Kenya and India) was considerably higher than the mucilage exudation of genotypes from zones with lower VPD (central Europe). There was a highly significant positive relationship between the mucilage exudation amount and the VPD of the genotypes' agroecological zones of origin. Vapor pressure deficit (VPD) is an indirect measure of water stress for plants (Dai, 2013). Water depletion and thereby plant water stress increase with increasing VPD (Grossiord et al., 2020). The observed higher mucilage exudation in the Kenyan and Indian genotypes could be attributed to various factors. A very plausible explanation is the more frequent water deficiency in these regions, due to high temperatures and evapotranspiration (Mallick et al., 2007; Mutiga et al., 2010). Maize nodal roots are aboveground only for a short time and later enter the soil and become belowground roots, where they play a dominant role in water uptake (Ahmed et al., 2018d). Mucilage increases the rhizosphere water content (Young 1995; Carminati et al., 2010). Higher mucilage exudation by the genotypes of the agroecological zones with higher VPD supports our hypothesis that mucilage exudation is inadvertently selected by plant breeding in water-stressed regions and displays an adaptive trait to drought – most likely to dry soils. This is consistent with the role of mucilage in sustaining water uptake and preventing root dehydration under dry conditions (Ahmed et al. 2018c). Furthermore, mucilage also reduces friction against the growing root in soil (Iijimma et al., 2003). The maize genotypes from the semi-arid agroecological zones may exude high quantities of mucilage to ease seeking water in compacted soil layers. However, further research is required to experimentally verify this.

Another possible explanation can be a poor nutrient status in the soil of Kenya and India (Sharma et al., 2009; Mugo et al., 2020, Stewart et al. 2020), which represent our high-VPD agroecosystems. It has been shown that the landrace maize Sierra Mixe, originally from a nitrogen-depleted region in Mexico, gets a considerable amount of its nitrogen demand through biological nitrogen fixation by diazotrophs abundant in mucilage produced by its huge nodal roots (Van Deynze et al., 2018; Amicucci et al., 2019). It was also shown that maize nodal roots take up large amounts of nitrogen from the soil due to their length, high surface area, and a great number and density of lateral roots (Dechorgnat et al., 2018). Therefore, high mucilage exudation – especially of nodal roots – can also be an adaptive trait to nitrogen deficient soils, provided that breeding was not performed under optimal nitrogen supply.

Indications that mucilage exudation has probably a genetic component are also found in studies in Mexico, which showed that the ancient maize genotype teosinte (*Zea mays spp. mexicana*) exudes lower amounts of mucilage from its nodal roots compared to its domesticated landrace Sierra Mixe (Van Deynze et al., 2018). Our study indicates that genotype also governs mucilage exudation in commercial varieties.

The saturation water content of mucilage reported here (on average 200 times mucilage dry weight) is in agreement with previous studies, which fall in the range of 27-589 times dry weight (McCully and Boyer, 1997; Huang and Gutterman, 1999; Capitani et al., 2013). This very high water retention capability of mucilage is the basis for one of its key rhizosphere functions: providing a moist root environment, especially in dry and water-deficient conditions (Ahmed et al. 2018 a). A multiple regression model was, however, not able to identify any significant relationships between the mucilage polysaccharide composition and its saturation water content. It seems possible that the mucilage water absorption capacity depends on other mucilage components such as protein and lipid content. This suggests further investigation to consider the potentially important functional roles of the minor chemical components of mucilage, the proteins and the lipids. Undoubtedly, we need to identify desirable biochemical and biophysical properties of mucilage for targeted breeding of beneficial mucilage composition and exudation amounts suited to defined agroecological zones.

Conclusion

This study investigated the polysaccharide composition, exudation amount, and saturation water content of nodal root mucilage of maize genotypes from three continents. Galactose, fucose, mannose, and arabinose were the major neutral sugars in the mucilage of these maize genotypes. Xylose and glucose were minor components. Glucuronic acid was the sole uronic acid found in the mucilage of the maize genotypes. Significant differences were detected among the maize genotypes in the mucilage polysaccharide composition, exudation amount, and saturation water content. Mucilage exudation amount of maize might be linked to an adaptation to the climatic conditions of the agroecological zones for which the genotypes were bred. However, as previous studies suggested a strong coevolution between mucilage chemical composition and beneficial microbial communities, the nutrient status of the respective soils may have affected the polysaccharide's monomer composition. This remains to be elucidated in further studies. This study suggests further experiments to include wild genotypes, landraces, and modern maize varieties and use these genotypes in multi-factorial field designs across agroecological zones globally. This may allow disentangling of the genotype × environment interactions that underlie mucilage composition and exudation. This would fundamentally extend our understanding of mucilage-related traits and may shed more light on their origin and selection.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Author contributions

MN and SR performed the lab experiments and parts of the field trial. CB supervised the lab experiments. AA and MC collected maize mucilage and arranged field-related works. KM-J assisted in manuscript preparation and scientific corrections. MD and MA designed and supervised

the experiments. MN analyzed the data and prepared the manuscript draft. All authors read the final draft of the manuscript and shared their comments.

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Supplementary material

Supplementary Table 1. Relationship between the genotypes' mucilage polysaccharide composition and the vapor pressure deficit (VPD) of their agroecological zones (linear regression, at $P \leq 0.05$, n = 24).

Dependent variable	Independent variable	df	Mean square	F	P-value	R^2
Galactose	VPD	1	9.40	3.23	0.086 NS	0.12
Fucose	VPD	1	26.83	4.10	0.055 NS	0.15
Mannose	VPD	1	0.015	0.010	0.92 NS	<0.0001
Glucose	VPD	1	4.19	4.55	0.055 NS	0.17
Arabinose	VPD	1	0.41	0.34	0.56 NS	0.015
Xylose	VPD	1	1.11	1.13	0.29 NS	0.049
Glucuronic acid	VPD	1	3.54	1.97	0.17 NS	0.082

NS = not significant

Supplementary Table 2. Relationship between the genotypes' mucilage saturation water content and its polysaccharide composition (multiple regression, at $P \leq 0.05$, n = 24).

Model/Variable	df	Mean square	F	P-value	R^2
Regression	6	13034.97	1.92	0.13 NS	0.40
Galactose	1	6814.69	0.80	0.37 NS	0.03
Fucose	1	31.514	0.00	0.87 NS	< 0.01
Mannose	1	825.55	0.09	0.53 NS	< 0.01
Glucose	1	5841.56	0.68	0.12 NS	0.41
Arabinose	1	886.38	0.10	0.94 NS	< 0.01
Xylose	1	4572.66	0.53	0.35 NS	< 0.01
Glucuronic acid	1	30882.46	4.18	0.058 NS	0.16

NS = not significant

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Study 3: Soil, climate, and variety impact on quantity and quality of maize root mucilage exudation

Meisam Nazari[1], Nataliya Bilyera[2], Callum C. Banfield[1, 2], Kyle Mason-Jones[3], Mohsen Zarebanadkouki[4], Rosepiah Munene[1], Michaela A. Dippold[1, 2]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[2]Geo-Biosphere Interactions, Department of Geosciences, University of Tübingen, Tübingen, Germany

[3]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands

[4]Soil Biophysics and Environmental Systems, Technical University of Munich, Freising, Germany

Correspondence: Meisam.nazari@forst.uni-goettingen.de; Meisam.nazari1991@gmail.com

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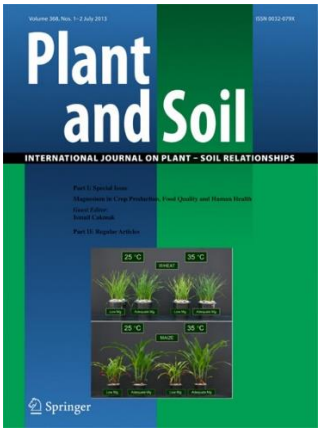


Abstract

Aims This study investigated the influence of climate and soil on the exudation rate and polysaccharide composition of aerial nodal root mucilage from drought-resistant and drought-susceptible maize varieties.

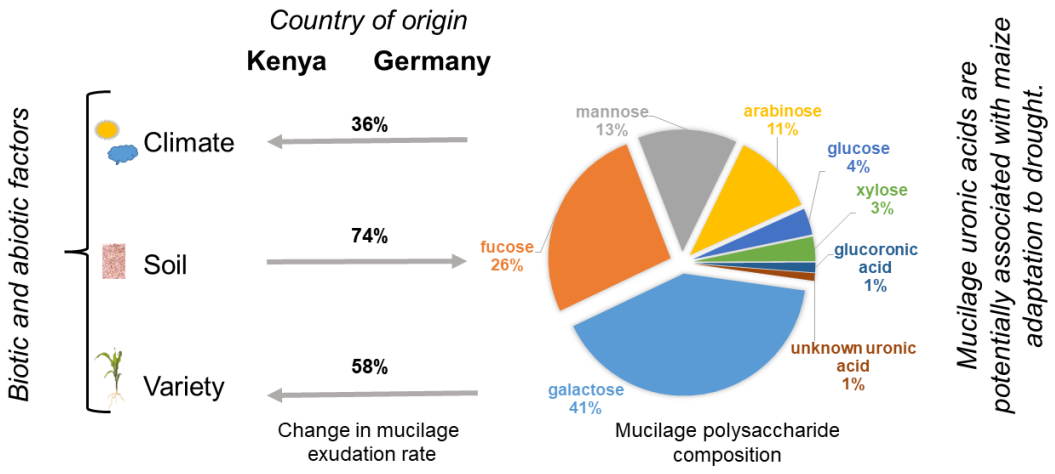
Methods Two maize varieties were grown in two different soils (sandy-clay loam Acrisol and loam Luvisol) under simulated climatic conditions of their agroecological zones of origin in Kenya and Germany. The exudation rate of mucilage from the aerial nodal roots was quantified as dry weight per root tip per day and the mucilage was characterized for its polysaccharide composition.

Results On average, the mucilage exudation rate was 35.8% higher under the Kenyan semi-arid tropical than under the German humid temperate climatic conditions. However, cultivation in the loam Luvisol soil from Germany led to 73.7% higher mucilage exudation rate than cultivation in the sandy-clay loam Acrisol soil from Kenya, plausibly due to its higher microbial biomass and nutrient availability. The drought-resistant Kenyan maize variety exuded 58.2% more mucilage than the drought-susceptible German variety. On average, mucilage polysaccharides were composed of 40.6% galactose, 26.2% fucose, 13.1% mannose, 11% arabinose, 3.5% glucose, 3.2% xylose, 1.3% glucuronic acid, and 1% an unknown uronic acid. Overall, significantly higher proportions of the uronic acids were found in the mucilage of the plants grown in the Kenyan sandy-clay loam soil and under the Kenyan semi-arid tropical climatic conditions.

Conclusions Maize is able to enhance its mucilage exudation rate under warm climatic conditions and in soils of high microbial activity to mitigate water stress and support the rhizosphere microbiome, respectively.

Keywords: drought resistance, monosaccharides, mucilage, rhizodeposition, plant adaptation, uronic acids

Graphical abstract



Introduction

Plant roots exude various metabolites to mediate the microbial, physical, and chemical processes in the rhizosphere (Carminati and Vetterlein 2013; Williams and de Vries 2020). Up to 20-40% of plants' photosynthetic carbon is allocated to root exudates, nearly half of which exuded as mucilage from root tips (Badri and Vivanco 2009; Chaboud 1983; Walker et al. 2003). Mucilage is a gelatinous high-molecular-weight substance containing mainly polysaccharides (78.4%) but also other substances such as proteins (7.3%), minerals (5.6%), and lipids (3.1%) (Nazari 2021).

Several ecologically remarkable functions have been linked to mucilage in the rhizosphere, such as aggregation of soil particles, lubrication of roots for better penetration and growth, enhancement of soil water holding capacity, facilitation of root water and nutrient uptake under dry conditions, amelioration of heavy metals, provision of a carbon source for microorganisms, and enhancing contact between the root surface and soil through rhizosheath formation (Ahmed et al. 2015; Benizri et al. 2007; Carminati et al. 2010; Czarnes et al. 2000; Iijima et al. 2003; Nazari 2021; Sasse et al. 2018; Zarebanadkouki et al. 2019). Moreover, the nodal root mucilage of the maize landrace Sierra Mixe (*Zea mays* Y.) from Mexico harbors diazotrophic bacteria that fix atmospheric nitrogen and provide 29%–82% of the plant's nitrogen demand (Bennett et al. 2020; Van Deynze et al. 2018). Mucilage is also a potential biofilm matrix, similar to microbial extracellular polymeric substances (EPS), that shapes the rhizosphere microbial habitat (Benard et al. 2019; Nazari et al. 2022). Most of these functions are associated with the polysaccharide component of mucilage (Nazari 2021).

In the context of the second green revolution, which focuses on agricultural and environmental sustainability, belowground traits (i.e., root architecture, root hairs, mucilage, and rhizosphere) have received significant attention as a new avenue to enable efficient and sustainable use of limited water and soil nutrient resources (Bennett et al. 2020; Lynch 2013). However, these objectives require a deep mechanistic understanding of belowground traits and their influence on the success of plants under deficit conditions. It is not yet understood how the exudation rate and composition of mucilage respond to varying external conditions of soil moisture and vapor pressure deficit. Additionally, few studies have compared mucilage exudation rates and composition between droughtsusceptible and drought-resistant varieties. It was recently shown that the amount of nodal root mucilage exudation in the drought-resistant maize (*Z. mays* L.) varieties from India and Kenya were significantly higher than in those from Germany and France, being positively associated with the vapor pressure deficit of the varieties' agroecological zones of origin (Nazari et al. 2020). Moreover, mucilage exudation was higher in a drought-resistant barley (*Hordeum vulgare* L.) variety than a conventional one (Carter et al. 2019). These studies postulate that higher amounts of mucilage exudation have been unintentionally selected in the course of breeding under drought conditions. Nazari et al. (2020) also found significant differences in the mucilage sugar composition among the maize varieties. Although these studies shed some light on the genetic basis of root mucilage traits, more research is required to expand the knowledge.

It also remains to be revealed whether or not mucilage exudation rate and polysaccharide composition are influenced by environmental conditions such as climate and soil (Blizard and Sparks 2020; Oburger and Jones 2018). This will assist plant breeders in selecting optimum crop traits for current and future agricultural conditions. Maize is a model plant for mucilage studies, exuding mucilage from its aerial nodal and underground roots.

This study used a fully crossed design to quantify and characterize the aerial nodal root mucilage of two maize varieties from Kenya and Germany grown in the soils and under the climatic conditions of their agroecological zones. The exudation rate of mucilage was quantified as dry weight (DW) per root tip per day and the mucilage was characterized for its polysaccharide composition.

Materials and methods

Soil and plant preparation

This study evaluated the effect of plant genotype, soil, and climatic conditions on the quantity and quality of root mucilage exudation in maize plants. To do so, a three-factorial (variety × soil × climate) pot experiment was implemented as a randomized complete block design consisting of four replicates in the climate chambers of the Department of Plant Ecology and Ecosystems Research of the Georg-August University of Göttingen, Göttingen, Germany. Two maize varieties, namely Kentos and DH02, were selected as representatives of maize varieties grown in Germany (humid temperate climate) and Kenya (semi-arid tropical climate), respectively. Kentos is a drought-susceptible but high-yielding maize variety developed by the KWS SAAT company (Einbeck, Germany) for cultivation in Germany and central Europe (See details at: https://www.kws.com/de/de/produkte/mais/sortenuebersicht/kws-kentos/) and DH02 is a drought-resistant maize variety developed by the Kenya Seed company (Kitale, Kenya) for cultivation in dryland regions of Kenya (See details at: https://kenyaseed.com/product/hybrid seed-maize-dh-02/) (Nazari et al. 2020).

The maize plants were grown in soils collected from depths of 0-25 cm of agricultural farms in Hohenpölz in Germany (49° 54′ N and 11° 08′ E) and Kitui in Kenya (1° 22′ S and 37° 59′ E). The soils collected from Germany and Kenya were a Luvisol with a loam texture and an Acrisol with a sandy-clay loam texture, respectively. Information on the properties of the soils is presented in Table 1 (Apostel et al. 2018). Regarding the soil properties in Table 1, the soil pH was determined at a 1/2.5 (w/v) soil-to-water ratio, the soil organic carbon and total nitrogen were measured by dry combustion using an elemental analyzer (Analytic, Jena), the soil microbial biomass carbon was determined by the fumigation-extraction method (Brookes et al. 1985; Wu et al. 1990), and the soil water-holding capacity was calculated as the weight of saturated soil – the weight of dry soil / the weight of dry soil × 100.

Table 1. Properties of the soils collected from the German (Hohenpölz) and Kenyan (Kitui) farms.

Site	Collection depth (cm)	Soil type	Sand (%)	Silt (%)	Clay (%)	TOC (%)	TN (%)	MBC (µg g^{-1})	WHC (%)	pH (H_2O)
Hohenpölz	25	Luvisol	36	42	22	1.77	0.19	493	63.3	6.4
Kitui	25	Acrisol	59	9	32	0.62	0.07	103	32.6	5.4

The plants were grown in pots of 15 cm height and 15 cm diameter filled uniformly with the dry soils. The weights of the Luvisol and Acrisol soils per pot were 2500 g and 3200 g, respectively. The maize seeds were pre-germinated on wet filter paper in the dark for 3 days and then one seedling was planted at a depth of 3 cm in each pot (on day 1 in the chamber). The maize plants were grown in two climate chambers (York® 2300, Johnson Controls, Milwaukee, Wisconsin), simulating the climatic conditions in which the plants are grown in Germany and Kenya. It is noted that the use of two climate chambers per treatment (four climate chambers) would be better for replication. However, adequate biological replicates in each chamber allows to obtain statistically valid results. The growth speed of the maize plants differed in the climate chambers, mainly due to the different temperatures, being 13.4 °C for the chamber simulating the humid temperate climate of Germany and 19.3 °C for the chamber simulating the semi-arid tropical climate of Kenya. In the chamber simulating the humid temperate climate, the maize plants were grown until day 21. On this day, the soil around the stems was moved aside to open up space for catching the upcoming aerial nodal roots. On day 28, the maize plants reached the growth stage of nine or more nodes visible on stem (BBCH 39). The aerial nodal roots of the 28-day old plants were immersed in water for mucilage collection. In the chamber simulating the semi-arid tropical climate, the maize plants were grown until day 14. On this day, the soil around the stems was moved aside to open up space for catching the upcoming aerial nodal roots. On day 21, the maize plants reached the growth stage of nine or more nodes visible on stem (BBCH 39). The aerial nodal roots of the 21-day old plants were immersed in water for mucilage collection.

The day/night temperature, relative humidity, and irrigation events were set in each climate chamber to mimic average temperature, relative humidity, and cumulative precipitation in Hohenpölz in Germany (May to June 2018-2020) and Kitui in Kenya (mid-April to mid-May 2018-2020) obtained from www.am.rlp.de and www.worldweatheronline.com, respectively. The average temperature, average relative humidity, cumulative precipitation, and vapor pressure deficit in the growing periods were 13.4 °C, 78.1%, 73.3 mm, and 0.15 kPa for Hohenpölz and 19.3 °C, 78%, 83.2 mm, and 0.27 kPa for Kitui, respectively. To assure uniform environmental conditions, the pots were rotated in the climate chambers. The climate chambers were equipped with fans by which the boundary layer on the leaves was kept minimum. Note that plant water stress and the probability of its hydraulic failure increase with increasing vapor pressure deficit (Grossiord et al. 2020). In each climate chamber, totally 16 pots were kept containing the two different maize varieties in each of the two different soils, which were replicated four times. The light sources in the chambers were 400 W Eye Clean Ace metal halide bulbs with a near-daylight

spectral composition (Eye Lighting International, Mentor, Ohio). A photoperiod of 12 hours day and 12 hours night was applied.

Sampling of mucilage

When plants were at the growth stage with nine or more nodes visible on stem (BBCH 39), mucilage was sampled from five nodal roots of each maize plant according to Ahmed et al. (2015). Mucilage was collected from five nodal roots to have adequate replicates and also enough mucilage for characterizing its polysaccharide composition. The collected mucilage was divided by 5 to get the exudation rate of mucilage per nodal root tip. It is also noted that the nodal roots were of similar lengths at the time of mucilage collection. The newly emerged nodal roots (not yet in the soil) were immersed in distilled water for 24 hours until the mucilage was fully hydrated. Thereafter, the hydrated mucilage was aspirated from the nodal root tips using a 5 ml pipettor. Any small amount of remaining mucilage on the nodal root was sampled with forceps. The mucilage samples were collected in 50 ml vials and frozen at -18 °C. All collected mucilage samples were freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany) and stored in a desiccator. Mucilage exudation rate was expressed as the dry weight of freeze-dried mucilage per nodal root tip per day.

Preparation of samples and standards

At least 2 mg freeze-dried mucilage was weighed into hydrolysis flasks, followed by adding 10 ml 4 M trifluoroacetic acid (TFA) to each sample and hydrolyzing at 105 °C for 4 h. After cooling the flasks down to room temperature, 0.5 mg ml^{-1} allose (250 mg) (D +) was added to each sample. Then, the hydrolysate was filtered through glass fiber filters (GF6, Whatman GmbH, GE Healthcare, Freiburg, Germany) into a conical flask. Each hydrolysis flask was rinsed three times with 5 ml Millipore water and filtered in order to assure complete transfer of the hydrolysate. After that, the samples were dried by rotary evaporators at 50 °C and 30 mbar. 0.5 ml Millipore water was added to the conical flask and evaporated, being repeated two more times for thorough removal of TFA residues. The samples were transferred into reaction vials by rinsing three times with Millipore water and then were dried under nitrogen gas.

A standard mixture of both neutral and acidic monosaccharides (analysis grade purity, each 1 mg ml^{-1} double-distilled and sterilized water), i.e., galactose (D +), glucose (D +), mannose (D +), rhamnose (L +), fucose (L -), arabinose (D -), xylose (D +), ribose (D -), allose (D +), glucuronic acid, and galacturonic acid was prepared in a volumetric flask and sonicated for 5 min. Seven volumes of 10, 25, 50, 100, 200, 400, and 800 µl of the standard solution were transferred into reaction vials and dried under nitrogen gas.

Derivatization and measurement of sugars

The analytes were derivatized to methoxime trimethylsilyl derivates based on the protocol developed by Roessner et al (2000). First, 200 µl *1-methyl-2*-pyrrolidone (NMP) was added to each sample and standard as a solvent. Then, 200 µl methoxyamine hydrochloride solution in pyridine (20 mg ml^{-1}) was added and samples were sonicated for 5 min, followed by heating to 75

°C for 30 min. Each sample and standard were vortexed once for 30 s during heating. After cooling down to room temperature, 400 µl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added to each sample and standard for silylation of the hydroxyl groups to trimethylsilyl groups. The samples and standards were heated to 75 °C for 5 min. Finally, 50 µl octadecane were added to each sample and standard (internal standard 2, IS 2) and then the samples were transferred into GC vials and tightly capped for measurement.

The analytes were separated by gas chromatography (Agilent 7820 GC, Agilent Waldbronn, Germany) and detected in a mass-sensitive detector (Agilent 5977B Single Quadrupole MS, Agilent Waldbronn, Germany). The GC was equipped with a DB-5MS column (45 m length, 250 mm inner diameter, 0.25 mm film thickness). Helium with a flow rate of 1.5 ml min^{-1} was used as the carrier gas (28 cm sec^{-1} average velocity). An aliquot of 1 µl was injected into the split inlet at 250 °C inlet temperature and a split ratio 50:1 for 2 min. The oven program started at 145 °C, held for 0.5 min and then heated to 160 at 10 °C min^{-1}, held again for 0.5 min and heated at 6 °C min-1 to 185 °C. The temperature was raised to 185 °C at a rate of 6 °C min^{-1}, held for 0.5 min and increased to 300 °C at 100 °C min^{-1}. The detector was set to scan mode (all fragments from 50 to 550 m/z) and an electron ionization energy of 70 eV was used for ionization and fragmentation.

Integration and quantification of sugars

Total ion current chromatogram peaks were integrated with the Agilent Mass Hunter Quantitative Data Analysis software (Agilent Technologies, Waldbronn, Germany), always ensuring peak identity by comparison of characteristic fragments with external and single substance standards. Analyte peak areas were normalized to the peak areas of each sample's IS2 peak (octadecane). Quantification was performed based on a linear regression of the external standards' peak areas to the external standard amounts. Furthermore, a recovery correction using allose (D +) provided absolute quantification of polysaccharide-derived monosaccharide content.

Statistical analysis

All data were analyzed by IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The data were checked for normality using the Shapiro-Wilk test and for the homogeneity of variances using Levene's test. The data not meeting these assumptions were logarithmically transformed. Three-way analysis of variance (ANOVA) was used to test for significant effects of the factors (soil, climate, variety) and their interactions at the significance level (α) of 0.05. Tukey's HSD (Honestly Significant Difference) test was used for pair-wise comparison of the means between the significant interactive factors at $\alpha = 0.05$. All charts were drawn using Microsoft Excel, version 2019. It is noted that a sample replicate was broken during sugar analysis, for which we took the average of the three remaining replicates due to insufficient mucilage to repeat the analysis.

Results

Mucilage exudation rate

Climate, soil, variety, and the interaction of soil and variety significantly affected the mucilage exudation rate at $\alpha = 0.05$ (Table 2). The mucilage exudation rate was 35.8% higher under the Kenyan semi-arid tropical climatic conditions than under the German humid temperate climatic conditions (Figure 1). On average, the German loam soil led to 73.7% higher mucilage exudation rate than the Kenyan sandy-clay loam soil (Figure 1). The Kenyan maize variety DH02 exuded 58.2% more mucilage compared to the German variety Kentos (Figure 1). The overall highest mucilage exudation rate (2.7 mg DW root tip^{-1} d^{-1}) belonged to the Kenyan variety DH02 grown in the German loam soil (Figure 1).

Table 2. Arithmetic means and probability values (p-values) derived from three-way ANOVA for mucilage exudation rates and sugar proportions of DH02 and Kentos maize varieties grown in two different soils and under two contrasting climatic conditions (n = 4).

Treatment	MER	Hex	Pen	UA	Gal	Fuc	Man	Glu	Ara	Xyl	GluA	UUA
Climate												
Semi-arid tropical	1.68	84.18	12.54	2.63	40.28	25.65	14.84	3.40	10.58	2.96	1.04	1.59
Humid temperate	1.17	83.83	14.35	2.12	41.58	27.70	11.89	3.66	11.35	3.68	1.66	0.45
Soil												
Sandy-clay loam	0.90	83.82	12.74	3.04	40.27	26.31	14.64	3.60	10.66	3.49	2.16	0.88
Loam Luvisol	1.95	84.19	14.15	1.71	41.59	27.05	12.09	3.46	11.28	3.15	0.54	1.17
Variety												
DH02	1.84	83.06	13.79	2.24	41.42	26.65	12.95	3.03	11.45	3.49	1.30	0.94
Kentos	1.01	84.95	13.10	2.51	40.43	26.71	13.78	4.03	10.49	3.14	1.40	1.11
p-values												
Climate	**0.02***	0.79	0.41	**0.04***	0.25	0.057	0.08	0.72	0.08	0.055	0.83	**<0.0001***
Soil	**<0.0001***	0.77	0.28	**<0.0001***	0.24	0.47	0.06	0.84	0.15	0.22	**<0.0001***	0.19
Variety	**0.003***	0.17	0.74	0.26	0.37	0.95	0.60	0.16	0.06	0.22	0.46	0.10
Climate × soil	0.23	0.06	0.16	**0.003***	0.053	0.85	0.25	0.76	0.06	0.16	0.84	**0.002***
Climate × variety	0.44	0.06	0.09	0.15	0.11	0.73	0.09	0.51	0.99	0.057	0.53	0.15
Soil × variety	**0.01***	0.18	0.54	0.052	0.62	0.42	0.06	0.56	0.054	0.19	0.50	0.06
Climate × soil × variety	0.37	0.10	0.18	0.19	0.18	0.057	0.059	0.19	0.07	0.24	0.49	0.22

Bold p-values with star indicate a statistically significant effect at $\alpha = 0.05$. MER mucilage exudation rate (mg DW root tip^{-1} d^{-1}), Hex hexoses (%), Pen pentoses (%), UA uronic acids (%), Gal galactose (%), Fuc fucose (%), Man mannose (%), Ara arabinose (%), Xyl xylose (%), GluA glucuronic acid (%), UUA an unknown uronic acid (%).

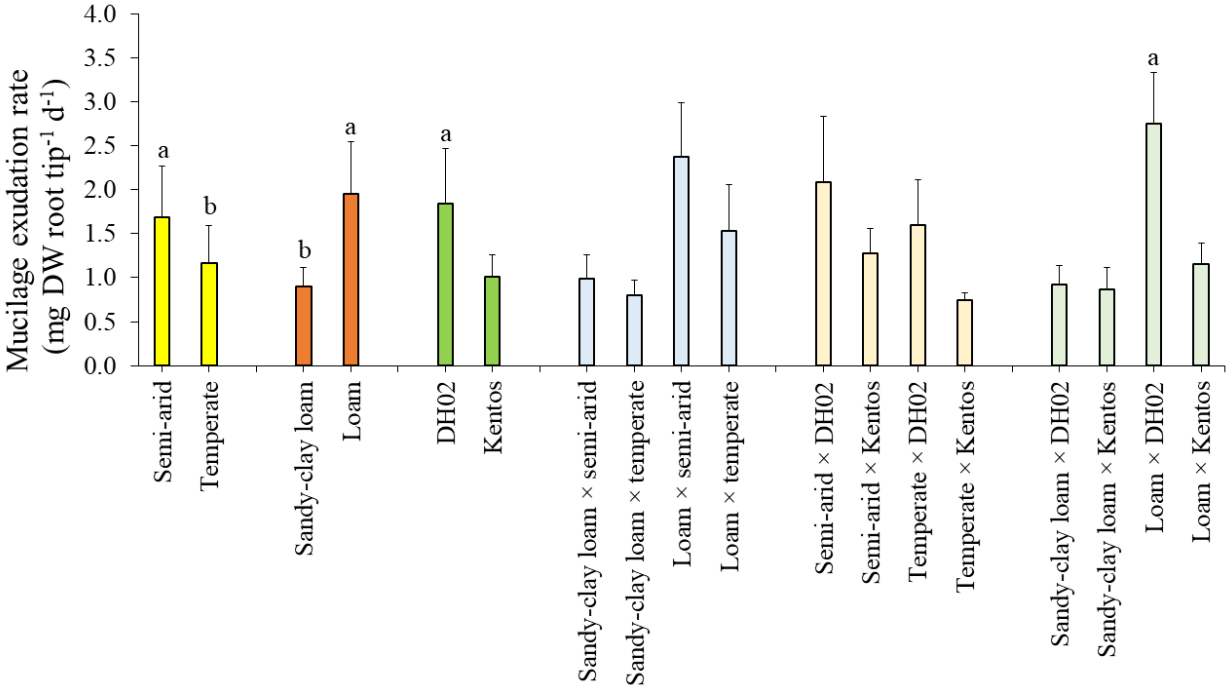


Figure 1. Effect of the factors climate, soil, variety, and their interactions on the mucilage exudation rate of maize aerial nodal roots. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. Semi-arid tropical: climate of Kitui, Kenya; humid temperate: climate of Hohenpölz, Germany; sandy-clay loam: infertile soil from Kitui, Kenya; loam: fertile soil from Hohenpölz, Germany; DH02: drought-resistant variety from Kenya; Kentos: drought-susceptible variety from Germany.

Mucilage polysaccharide composition

On average (all plants and treatments), hexoses (galactose, fucose, mannose, glucose), pentoses (arabinose, xylose), and uronic acids (glucuronic acid, an unknown uronic acid) constituted 83.4%, 14.3%, and 2.3% of the mucilage polysaccharide composition, respectively (Figure 2A). The mucilage polysaccharide was composed of 40.6% galactose, 26.2% fucose, 13.1% mannose, 11% arabinose, 3.5% glucose, 3.2% xylose, 1.3% glucuronic acid, and 1% an unknown uronic acid, not present in the external standard mixture but with a clear mass spectrum of a uronic acid (Figure 2B). The proportions of hexoses, pentoses, galactose, fucose, mannose, glucose, arabinose, and xylose were neither significantly affected by climate, soil, and variety nor by their interactions (Table 2).

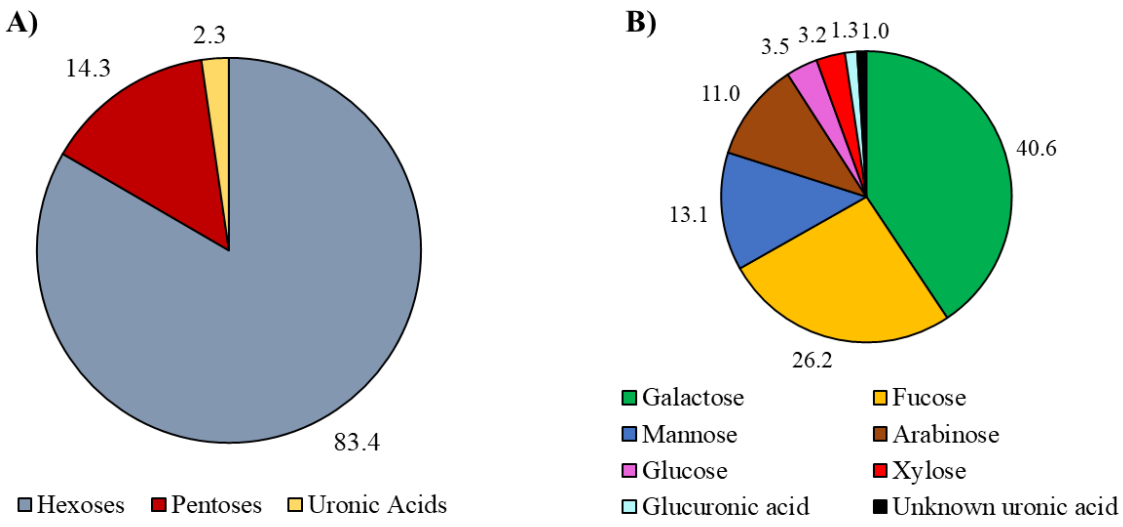


Figure 2. Average proportion (in %) of the hexoses, pentoses, uronic acids (A) and each monomer (B) in the nodal root mucilage of the maize varieties DH02 and Kentos (n = 32).

The proportion of uronic acids was significantly affected by climate, soil, and the interaction of climate and soil at $\alpha = 0.05$ (Table 2). The Kenyan semiarid tropical climatic conditions resulted in 0.51% greater uronic acid proportion in the exuded mucilage than the German humid temperate climatic conditions (Figure 3). The Kenyan sandy-clay loam soil induced 1.3% more uronic acids in the mucilage in comparison with the German loam soil (Figure 3). The highest uronic acid proportion (3.7%) was observed under the Kenyan semi-arid tropical climatic conditions and in the Kenyan sandy-clay loam soil (Figure 3).

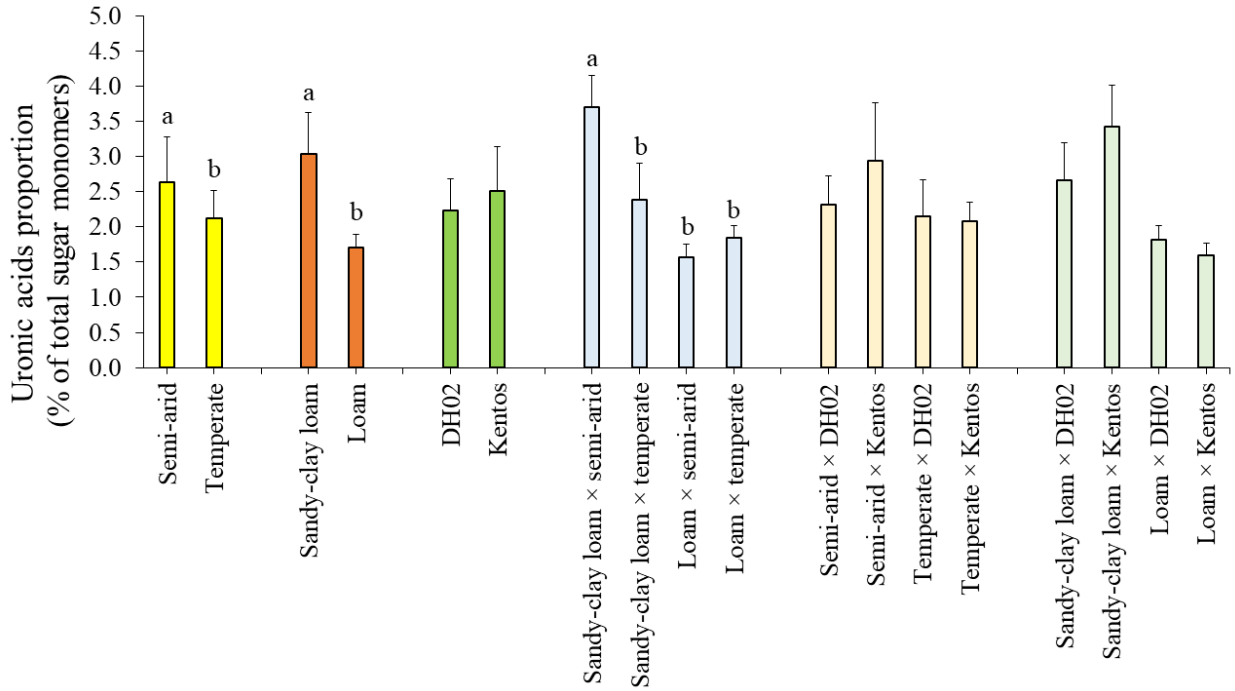


Figure 3. Effect of the factors climate, soil, variety, and their interactions on the proportion of uronic acids in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. Semi-arid tropical: climate of Kitui, Kenya; humid temperate: climate of Hohenpölz, Germany; sandy-clay loam: infertile soil from Kitui, Kenya; loam: fertile soil from Hohenpölz, Germany; DH02: drought-resistant variety from Kenya; Kentos: drought-susceptible variety from Germany.

Soil significantly affected the glucuronic acid proportion of the mucilage at $\alpha = 0.05$ (Table 2). The Kenyan sandy-clay loam soil induced 1.6% higher proportion of glucuronic acid in the mucilage compared to the German loam soil (Figure 4). Moreover, climate and the interaction of climate and soil significantly affected the proportion of an unknown uronic acid at $\alpha = 0.05$ (Table 2). The Kenyan semi-arid tropical climatic conditions resulted in 1.1% greater proportion of the unknown uronic acid in the mucilage than the German humid temperate climatic conditions (Figure 5). The Kenyan semi-arid tropical climate × Kenyan sandy-clay loam soil and the Kentan semi-arid tropical climate × German loam soil induced 0.8% and 1.5% increase in the proportion of the unknown uronic acid in the mucilage compared to the German humid temperate climate × the German loam soil and the German humid temperate climate × the Kenyan sandy-clay loam soil, respectively (Figure 5).

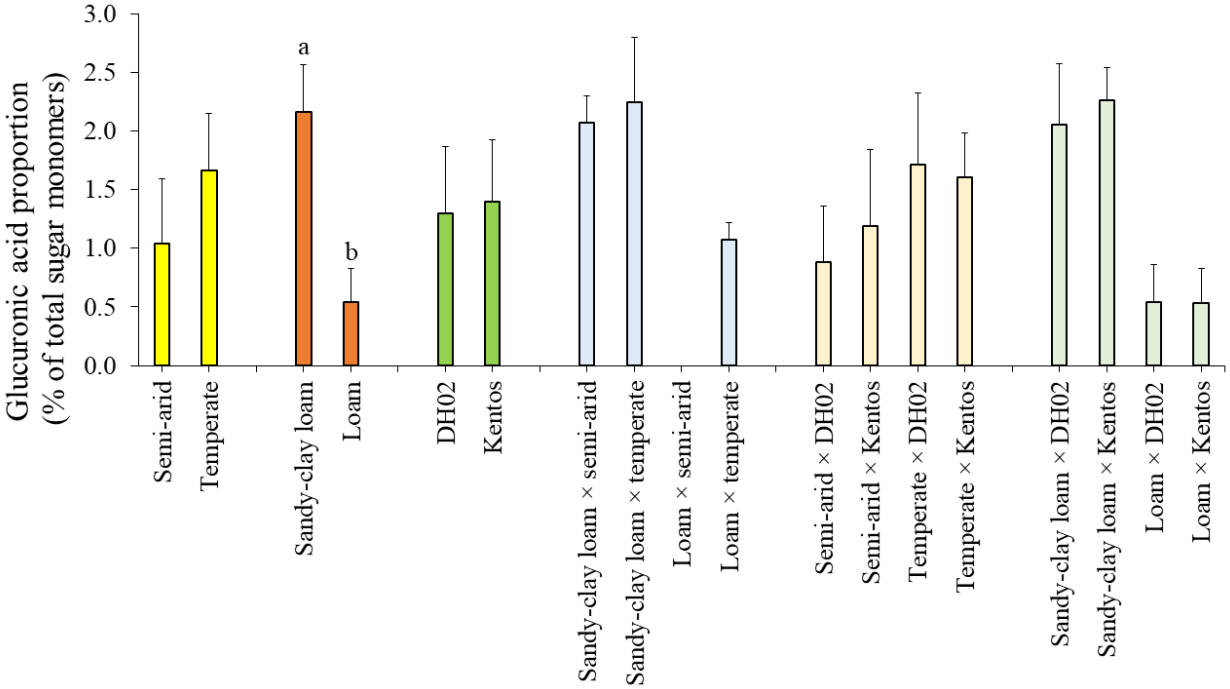


Figure 4. Effect of the factors climate, soil, variety, and their interactions on the proportion of glucuronic acid in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. Semiarid tropical: climate of Kitui, Kenya; humid temperate: climate of Hohenpölz, Germany; sandy-clay loam: infertile soil from Kitui, Kenya; loam: fertile soil from Hohenpölz, Germany; DH02: drought-resistant variety from Kenya; Kentos: drought-susceptible variety from Germany.

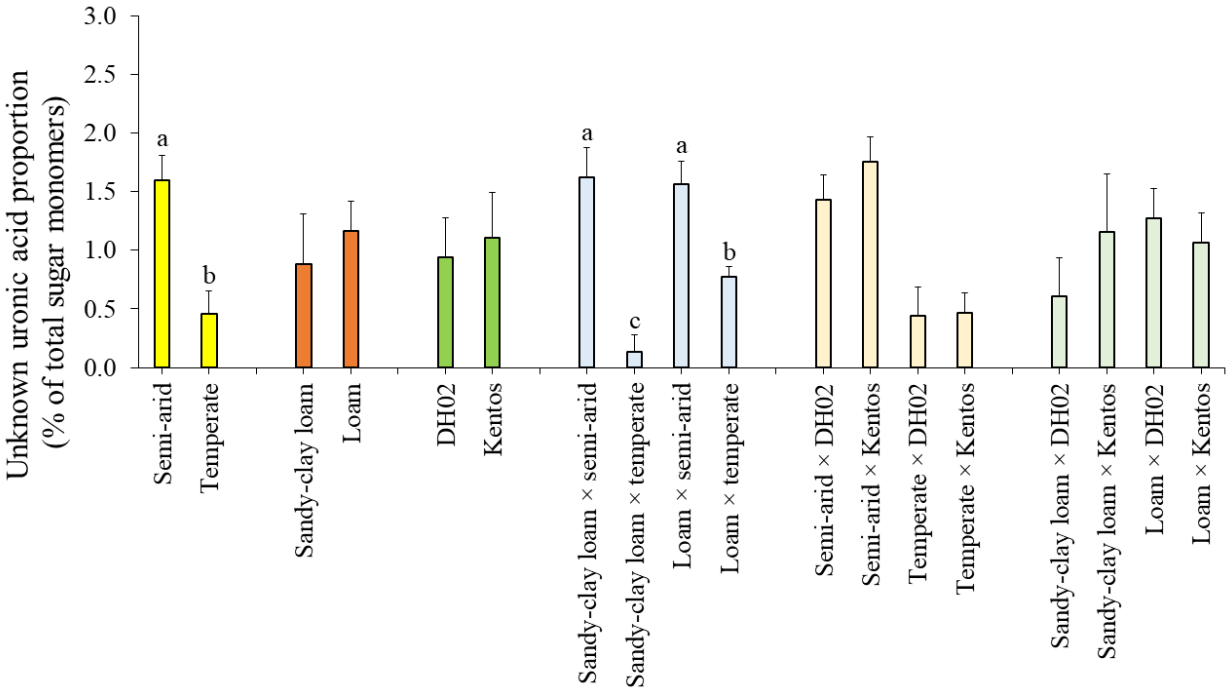


Figure 5. Effect of the factors climate, soil, variety, and their interactions on the proportion of an unkown uronic acid in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. Semi-arid tropical: climate of Kitui, Kenya; humid temperate: climate of Hohenpölz, Germany; sandy-clay loam: infertile soil from Kitui, Kenya; loam: fertile soil from Hohenpölz, Germany; DH02: drought-resistant variety from Kenya; Kentos: drought-susceptible variety from Germany.

Discussion

Mucilage exudation rate of local varieties depends on climate and soil conditions

The Kenyan semi-arid tropical climatic conditions led to 35.8% higher mucilage exudation rate than the German humid temperate climatic conditions (Figure 1). Average relative humidity and cumulative precipitation were similar for the maize growing periods under both climatic conditions. However, their average temperatures and vapor pressure deficits differed considerably, with 19.3 °C and 0.27 kPa and 13.4 °C and 0.15 kPa for the Kenyan semi-arid tropical and German humid temperate climatic conditions, respectively. Therefore, the higher mucilage exudation rate under the Kenyan semi-arid tropical climate compared to the German humid temperate climate could be associated with the increased temperature and vapor pressure deficit (Nazari et al. 2020). Since plants require more water in warmer and drier environments due to a greater loss of water through evapotranspiration (Heckathorn et al. 2013), it was reported that higher temperatures increased the membrane permeability of maize root tip cells for easing water uptake (Ionenko et al. 2010). An increased cell membrane permeability could simultaneously enhance the release of mucilage from the root tip and thereby enhance its exudation rate. By neutron radiography, Carminati (2013) illustrated larger accumulations of mucilage around the root tips of lupin (*Lupinus albus* L.) plants grown under dry conditions compared to those grown under normal

conditions. Carminati (2013) speculated that the observed accumulations were mucilage that increased in response to water stress, which has experimentally been confirmed in the present study, supporting the remarkable role of mucilage in regulating rhizosphere hydraulic processes. This viewpoint is bolstered by the observation that mucilage increases the rhizosphere water content and facilitates root water uptake, delaying the onset of hydraulic failure during conditions with high vapor pressure deficit (Ahmed et al. 2014; Carminati et al. 2010; Naveed et al. 2019; Nazari et al. 2020). Moreover, mucilage is a potential biofilm matrix and protective habitat for the rhizosphere microbiome, with all mucilage sugars functioning as a potential energy source (Bennett et al. 2020; Nazari et al. 2022). Thus, another reason for the higher mucilage exudation rate could be the protection and feeding of a beneficial rhizosphere microbiome for promoting survival despite the increased temperature and vapor pressure deficit. It was indicated that increased temperatures alter those root exudates that boost the plant growth-promoting bacterium *Pseudomonas putida* in order to improve wheat (*Triticum* spp.) survival under heat stress (Zulfikar Ali et al. 2011).

The German loam soil induced 73.7% greater mucilage exudation than the Kenyan sandy-clay loam did (Figure 1). Given that mucilage is released into the soil, microorganisms in the rhizosphere can considerably influence its exudation, but vice versa the presence of mucilage can positively affect microbial growth and abundance (Nazari et al. 2022). Thus, the fivefold higher microbial biomass in the German loam soil (493 μg g^{-1} soil) compared to the Kenyan sandy-clay loam soil (103 μg g^{-1} soil) likely profits from the provision of this carbon source by the root. Other studies demonstrated that microorganisms in the rhizosphere modify the exudation and composition of amino acids, flavonoids, and fatty acids released from the root (Dardanelli et al. 2010; Matilla et al. 2010; Phillips et al. 2004), suggesting that mucilage exudation may also be stimulated by a high microbial abundance in the rhizosphere. Although similar studies have not yet been done on mucilage exudation, it is likely that mucilage plays a key role in the establishment of rhizosphere microbial communities (Nazari et al. 2022).

Furthermore, soil microorganisms prefer to utilize and synthesize hexose sugars (including galactose and glucose) rather than pentoses (xylose and arabinose) (Kögel-Knabner 2002). Hexoses were found to be the main sugar class of maize mucilage in the present study and former studies (Amicucci et al. 2019; Nazari et al. 2020). This underlines the role of mucilage as a carbon source for the rhizosphere microbiome. The potential role of mucilage as a biofilm matrix harboring the rhizosphere microbiome (Nazari et al. 2022) further motivates this perspective. However, more studies are required to identify plant-microbial signaling mechanisms that may control root mucilage exudation and composition.

The Kenyan maize variety DH02 exuded on average 58.2% higher mucilage than the German variety Kentos (Figure 1). In a previous study, it was also found that drought-resistant maize varieties from warm agroecological zones with high vapor pressure deficits (i.e., India and Kenya) exude significantly more mucilage than drought-susceptible varieties from temperate agroecological zones of low vapor pressure deficits (i.e., France and Germany) (Nazari et al.

2020). Vapor pressure deficit is an indicator of water stress for plants (Dai 2013; Grossiord et al. 2020). The Kenyan variety DH02 may exude high amounts of mucilage as an adaptation to the high vapor pressure deficit and evapotranspiration in the region where it was originally bred for. As mucilage plays important roles in maize water uptake under water stress (Ahmed et al. 2018), it is therefore plausible that plant breeders in Kenya unintentionally selected for higher mucilage exudation, while selecting for drought resistance. Carter et al. (2019) also indicated a higher mucilage exudation amount in a drought-resistant barley (*Hordeum vulgare* L.) compared to the drought-susceptible ones, suggesting a similar selection effect.

Another reason for the higher mucilage exudation rate in the Kenyan variety DH02 could be the poor nitrogen status in the soils of Kenya (Mugo et al. 2020; Stewart et al. 2020), on which it was bred. A landrace of maize (Sierra Mixe) originating from a region in Mexico with nitrogen-depleted soils gains most of its needed nitrogen through exudation of high amounts of nodal root mucilage that accommodates nitrogen-fixing bacteria (Amicucci et al. 2019; Van Deynze et al. 2018). This implies that the exudation of high mucilage amounts in the Kenyan variety DH02 could be an adaptive strategy to nitrogen-poor soils of the region where breeding was conducted. A lower mucilage exudation amount from nodal roots of the ancient teosinte maize (*Zea mays* spp.) than its domesticated landrace Sierra Mixe (Van Deynze et al. 2018) is another support for the genetic basis of mucilage exudation. We suggest that maize varieties from warm agroecological zones (e.g., Kenya) or bred in regions with low nitrogen content in soils (i.e., tropical soils) can be precious sources of genetic materials for useful mucilage traits.

Moreover, the greatest mucilage exudation rate (2.7 mg DW root tip^{-1} d^{-1}) was achieved by growing the Kenyan variety DH02 in the German loam soil, induced by the genetic potential of this variety combined with soil conditions promoting high mucilage release by a high relative productivity of the system. This has important implications in regions with relatively fertile soils (i.e., Germany) where the agricultural sector concerns about negative outcomes of climate warming on crop production. Here, the genetic potential regarding drought resistance provided by the high mucilage exudation rate of the Kenyan maize variety may effectively be combined with high-yield traits of commercial temperate zone varieties to open a path for a climate-adapted sustainable agriculture.

Mucilage polysaccharide composition

Overall, hexoses, pentoses, and uronic acids constituted 83.4%, 14.3%, and 2.3% of the mucilage polysaccharides, respectively. These proportions are very similar to those of the mucilage of maize varieties originating from different climatic regions of the world (Nazari et al. 2020; Van Deynze et al. 2018), but different from those of the mucilage of pea (*Pisum sativum* L., hexoses: 49%, pentoses: 38%, uronic acids: 13%) and rice (*Oryza sativa* L., hexoses: 68%, pentoses: 32%, uronic acids: none) (Chaboud and Rougier 1991; Knee et al. 2001). The proportions of hexoses, pentoses, galactose, fucose, mannose, glucose, arabinose, and xylose were very similar in the varieties independent of the climatic and soil conditions in the present study. This suggests that the function

of these sugars may be very generally related to the interaction of the root with the soil, i.e., with its rhizo-microbial communities (Nazari et al. 2020) and, thus, is retained under different environmental conditions. Plant mucilage is a biofilm matrix similar to microbial EPS and all mucilage sugars can most likely be utilized by soil bacteria (Nazari et al. 2022). Many sugars have a backbone function (e.g., galactose, Amicucci et al. 2019) and thus are not supposed to differ significantly even if functional components in the side chains may respond to an environmental factor. Uronic acids are assumed to considerably influence the three-dimensional structure and physico-chemical properties of mucilage (see the next section), and thus are supposed to strongly interact with the environment. In addition, a main function of mucilage hexoses and pentoses is to attract plant-growth-promoting microbial communities, i.e., nitrogen-fixing bacteria (Amicucci et al. 2019). The similarities of the maize varieties in the above-mentioned sugars suggest that the function of these sugars for attracting beneficial microbial communities is retained in varieties bred in different agroecological zones.

Glucuronic acid and an unknown uronic acid were the only uronic acids detected in rather low amounts in the present study. However, other studies found different proportions of glucuronic acid in the mucilage of the landrace maize Sierra Mixe grown under different environmental conditions (i.e., 11.3% in Amicucci et al. 2019; 2.7% in Van Deynze et al. 2018;). In the present study, maize varieties grown under the Kenyan semi-arid tropical climatic conditions had significantly higher proportions of uronic acids and an unknown uronic acid in the mucilage than when they were grown under the German humid temperate climatic conditions. Also, significantly higher uronic acid proportions were detected in maize grown in the Kenyan sandy-clay loam compared to the German loam soil. This indicates that maize responds to soil and climate by altering the uronic acid proportion of its mucilage, implying the plasticity of mucilage uronic acid content. Uronic acids account for the negative charge of mucilage, bridging mainly with divalent Ca^{2+} ions to establish the three-dimensional structure of the mucilage biogel (Brax et al. 2019), although interactions with other divalent or polyvalent cations (Mg^{2+}, $Fe^{3+,}$ Al^{3+}) are also possible (Nazari 2021). Uronic acid-Ca^{2+} interconnections in the mucilage improve soil water retention and liquid-phase connectivity (Aravamudhan et al. 2014; Benard et al. 2019). Thus, it seems that the increased proportion of uronic acids in the exuded mucilage displays a plant strategy against water stress under the Kenyan semi-arid tropical climatic conditions and also in the unfavorable Kenyan sandy-clay loam soil (i.e., water holding capacity = 32.6%; pH = 5.4). The highest uronic acid proportion (3.7%) was observed for the Kenyan semi-arid tropical climatic conditions and Kenyan sandy-clay loam soil, which supports the suggestion that uronic acids increase the soil liquid-phase connectivity through uronic acid-metal ions bridges and may also play a central role in detoxifying free Al^{3+} in the soil solution of strongly weathered soils of the humid and semi-humid tropics (Iijima et al. 2003; Nazari 2021).

Conclusions

Exudation rate and polysaccharide composition of the nodal root mucilage of Kenyan drought-resistant and German drought-susceptible maize varieties grown in the soils and under the climatic

conditions of their agroecological zones of origin, and reciprocally in those of the other variety, were investigated. This study confirms earlier concepts that mucilage exudation rate in maize varieties from warmer agroecological zones is higher than those from temperate agroecological zones, indicating a genetic basis for this trait. However, maize is able to enhance its mucilage exudation rate under warm climatic conditions and in soils of high microbial activity, probably in order to adapt to water stress and support the rhizosphere microbiome. It seems that uronic acids play an important role in maize resistance to water stress, due to their interconnections with Ca^{2+} modifying the mucilage and soil water retention properties. We suggest that plant breeders take an advantage of the agroecological services provided by mucilage, especially considering the ongoing climate warming and increasing climate variability that are exacerbating drought risks for many agroecological zones globally.

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Competing interests

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

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Study 4: Biogels in soils: Plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat

Meisam Nazari[1], Samuel Bickel[2], Pascal Benard[2], Kyle Mason-Jones[3], Andrea Carminati[2] and Michaela A. Dippold[4]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[2]Physics of Soils and Terrestrial Ecosystems, Department of Environmental Systems Science, Institute of Terrestrial Ecosystems, ETH Zürich, Zurich, Switzerland

[3]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands

[4]Geo-Biosphere Interactions, University of Tübingen, Tübingen, Germany

Correspondence: meisam.nazari@forst.uni-goettingen.de

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Abstract

Mucilage is a gelatinous high-molecular-weight substance produced by almost all plants, serving numerous functions for plant and soil. To date, research has mainly focused on hydraulic and physical functions of mucilage in the rhizosphere. Studies on the relevance of mucilage as a microbial habitat are scarce. Extracellular polymeric substances (EPS) are similarly gelatinous high-molecular-weight substances produced by microorganisms. EPS support the establishment of microbial assemblages in soils, mainly through providing a moist environment, a protective barrier, and serving as carbon and nutrient sources. We propose that mucilage shares physical and chemical properties with EPS, functioning similarly as a biofilm matrix covering a large extent of the rhizosphere. Our analyses found no evidence of consistent differences in viscosity and surface tension between EPS and mucilage, these being important physical properties. With regard to chemical composition, polysaccharide, protein, neutral monosaccharide, and uronic acid composition also showed no consistent differences between these biogels. Our analyses and literature review suggest that all major functions known for EPS and required for biofilm formation are also provided by mucilage, offering a protected habitat optimized for nutrient mobilization. Mucilage enables high rhizomicrobial abundance and activity by functioning as carbon and nutrient source. We suggest that the role of mucilage as a biofilm matrix has been underestimated, and should be considered in conceptual models of the rhizosphere.

Keywords: biofilm, EPS, microorganism, mucilage, rhizosphere, root

Introduction

Plant roots are the major organs responsible for water and nutrient uptake from soil. Methodological difficulties in sampling belowground traits result in a much more detailed understanding of above- than belowground plant ecophysiology (Oburger and Schmidt, 2016; McCormack et al., 2017). Roots exude a diverse set of compounds into the rhizosphere, including sugars, amino acids, and secondary metabolites, which regulate rhizosphere functions (Walker et al., 2003; Dutta et al., 2013). Mucilage is a gelatinous high-molecular-weight substance produced by almost all plants, comprising approximately half of root exudates (Chaboud, 1983). The mucilage backbone is built of polysaccharides, but proteins, minerals, and lipids are also part of the biogel (Nazari, 2021). So far, mucilage has mainly been recognized to have hydraulic, mechanical, and physical functions in the rhizosphere. For instance, mucilage increases the rhizosphere water content, improves plant water uptake under drought, reduces friction against the growing root, and stabilizes soil aggregates (Young, 1995; Czarnes et al., 2000; Iijima et al., 2003; Carminati et al., 2010; Ahmed et al., 2015). However, only a few studies have investigated the relevance of mucilage for microbial processes. For example, it has been indicated that microorganisms utilize mucilage as an energy source and habitat (Mary et al., 1993; Ahmed et al., 2018a,b). It has been reported that maize (*Zea mays* L.) crown root mucilage harbors nitrogen-fixing bacteria, which contribute to the fixation of a considerable amount of the plant's nitrogen requirement (Van Deynze et al., 2018; Amicucci et al., 2019). Mucilage likely plays a central role in mediating plant-microbe interactions in the rhizosphere, but the magnitude of its relevance remains unclear.

Microorganisms can live planktonically, in suspended aggregates, and in attached biofilms (Flemming and Wuertz, 2019). Extracellular polymeric substances (EPS) produced by microorganisms are a three-dimensional matrix accounting for more than 90% of the dry mass of microbial biofilms (Flemming and Wingender, 2010). EPS are mainly composed of polysaccharides, but also contain proteins, nucleic acids, lipids, and minerals (Flemming and Wingender, 2010). EPS are formed upon the attachment of microorganisms to surfaces in order to establish biofilms (Fong and Yildiz, 2015; Jamal et al., 2018). It has been shown that EPS enhance the liquid phase viscosity compared to water and create an interconnected network (Stoodley et al., 2002; Flemming and Wingender, 2010; Volk et al., 2016). EPS improve soil water retention and liquid-phase connectivity (Rosenzweig et al., 2012; Benard et al., 2019), due to uronic acid-Ca^{2+} binding in their chemical structure (Aravamudhan et al., 2014). Alginate is an anionic polysaccharide found in EPS, consisting of only uronic acids such as glucuronic acid, galacturonic acid, and mannuronic acid (Sutherland, 2001; Van Hullebusch et al., 2004; Flemming and Wingender, 2010). Alginate participates in the formation of microcolonies at the beginning of the biofilm formation process, increases EPS hydration, and assists in trapping cations such as Ca^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} (Wuertz et al., 2001; Van Hullebusch et al., 2004; Flemming and Wingender, 2010).

EPS constitute an important part of the carbon pool in soils that plays key roles in soil microbial ecology (Flemming and Wingender, 2010). However, a main function of EPS is to protect microorganisms against environmental stresses such as drought, acidity, or salinity (Kumar et al., 2007; Vardharajula and Sk, 2014). EPS improve soil moisture status in microbial hotspots like the rhizosphere (Kuzyakov and Blagodatskaya, 2015). EPS are capable of absorbing 15–20 times more water than their dry weight and thus strongly increase the water holding capacity of soils (Chenu, 1993; Adessi et al., 2018). EPS strongly influence interactions between bacteria and their viruses (bacteriophages) by binding virus particles and slowing down their movement (Vidakovic et al., 2018). EPS also facilitate chemical communications between microorganisms within the biofilm, leading to increased microbial turnover and element cycling (Joubert et al., 2006; Flemming et al., 2007). Furthermore, EPS in soil can enhance the exchange of genetic material between microorganisms, trap nutrients, protect microorganisms against antimicrobial factors, and act as a carbon source for microorganisms, but they are also a key component involved in soil aggregate formation and thus in the formation of further soil micro-habitats (Costa et al., 2018).

Plants may produce mucilage not merely for improving hydraulic, mechanical, and physical functions in the rhizosphere, but potentially also to function as a biofilm matrix and support a rapid establishment of dense symbiont microbial communities and high microbial activity in the rhizosphere. Mucilage has a high potential to function as a biofilm matrix by providing a moist environment, protective barrier, and carbon and nutrient source for microbial communities. This study analyzes and reviews existing evidence to determine whether plant mucilage and microbial EPS have comparable physical and chemical properties, leading to analogous biophysical and biochemical features. This indicates similar microbial habitat properties of both biogels. To support this perspective, viscosity and surface tension as important physical properties and total polysaccharide, total protein, neutral monosaccharide, and uronic acid proportions as important chemical properties of mucilage and EPS are compared. Furthermore, to assess its quantitative relevance, we estimate the extent of the "mucilage biofilm" along the root axis, including bioenergetic viewpoints of microbial advantages living in a "plant-provided" biofilm and discuss the implications of this biofilm matrix as a key prerequisite for the high microbial activity in the rhizosphere.

Methodology

Data collection and standardization

In total, 376 datasets were collected from 83 related papers published between 1974 and 2019. The online tool WebPlotDigitizer was used to extract data from the charts (https://automeris.io/WebPlotDigitizer). The viscosity and surface tension data were considered physical indices for the comparison of mucilage and EPS. To evaluate chemical properties of mucilage and EPS, total polysaccharide, total protein, neutral monosaccharide, and uronic acid proportions were compared. The investigated neutral monosaccharides included galactose, fucose,

glucose, mannose, arabinose, rhamnose, and xylose, and uronic acids included glucuronic and galacturonic acid.

We considered some criteria for selection of the data. For the physical properties, the viscosity values had been measured at a solute concentration of 0.5 mg ml^{-1}, shear rate of 0.5 s^{-1}, and temperature of 20–25 ◦C, and the surface tension values had been measured at a solute concentration of 0.5 mg ml^{-1} and temperature of 20–25 ◦C. Only root and seed mucilage, which has rhizospheric relevance, were considered. All data related to the chemical properties were derived from pure mucilage and EPS. Data not fulfilling these criteria were excluded. SI units were used to standardize the viscosity (Pa s) and surface tension (N m^{-1}) values. A list of plant and microbial species that produced the biogel, accompanied by their references, has been provided in Supplementary Material 1.

Calculations of spatial and temporal distribution of mucilage and bacterial cell abundances around growing roots

To calculate the mucilage spatial and temporal distribution at a given exudation rate around a growing model root, we assumed a root-soil system with the following parameters: Soil porosity = 50%; crown root diameter = 3.3 mm (unpublished data of maize); mucilage exudation rate = 1.41 mg dry weight per day and root tip (unpublished data of the same maize plants); maximum hydration ratio of mucilage = 425:1 (wet mass: dry mass, unpublished data of the same maize plants), assuming a 39% water saturation upon exudation at the root tip and a rapid saturation to 100% within 6 h (Sealey et al., 1995); root elongation rate = 30 mm per day (Schmidt et al., 2013); and a maximum decomposition rate of the mucilage = 50% in 7 days (Ahmed et al., 2018a). Note that the diffusion of mucilage was neglected. Detailed equations and explanations of the mucilage spatial and temporal distribution model have been provided in Supplementary Material 2.

Considering bacteria as main mucilage consumers (Ahmed et al., 2018b), bacterial abundance in the rhizosphere was estimated. The mucilage available to bacterial degradation (C_{in}) was assumed to produce bacterial biomass under carbon limitation. The growth and biomass yield of several rhizosphere bacteria using glucose are comparable to the growth achieved by mucilage as a sole carbon source (Knee et al., 2001). Here, we considered the carbohydrate fraction of mucilage obtained in this study (f_c = 0.77) to be available for bacterial consumption (considering the mass-fraction of carbon for simple sugars; e.g., glucose w_c = 0.4). The upper bound on mucilage-derived carbon (C_{in}) that can be allocated to produce bacterial cell biomass (M_B) was obtained by considering an average carbon use efficiency (CUE) for a range of carbon sources ($M_B = fc \times w_c \times$ CUE $\times C_{in}$). We assumed that maintenance costs were negligible and all cell biomass could be produced within a day. For the calculation presented here, we used an average CUE of 0.6 based on genome scale metabolic predictions (Saifuddin et al., 2019). To estimate the number of cells (N_{cell}) that could feed on degraded mucilage, we divided the cell biomass carbon by an average bacterial cell carbon mass (M_{cell}) of 10 fg C per cell ($N_{cell} = M_B/M_{cell}$) (Khachikyan et al., 2019).

Furthermore, we estimated the EPS produced (M_{EPS}) by a given bacterial abundance by using an EPS yield per unit of cellular biomass ($M_{EPS} = M_B \times Y_{EPS}$ with Y_{EPS} = 10 mg g^{-1}) (Shene et al., 2008). Our model calculation assumed that all cells produce EPS.

Statistical analyses

All data were analyzed by IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, United States). The data were tested for homogeneity of variance and normality by Levene's test and Shapiro–Wilk test, respectively, and transformed logarithmically if they did not fulfill these prerequisites. The Independent Samples *t*-test was used to test for significant differences between mucilage and EPS in terms of the investigated properties at the significance level of 0.05. All charts were designed using SigmaPlot 14.0 (Systat, San José, CA, United States).

Results

Viscosity and surface tension

The viscosity of mucilage and EPS did not differ significantly (Figure 1A). Average viscosities for mucilage and EPS were 0.27 Pa s and 0.43 Pa s, respectively. There was also no significant difference between the surface tension of mucilage and EPS (Figure 1B). Average surface tensions for mucilage and EPS were 0.053 N m^{-1} and 0.051 N m^{-1}, respectively.

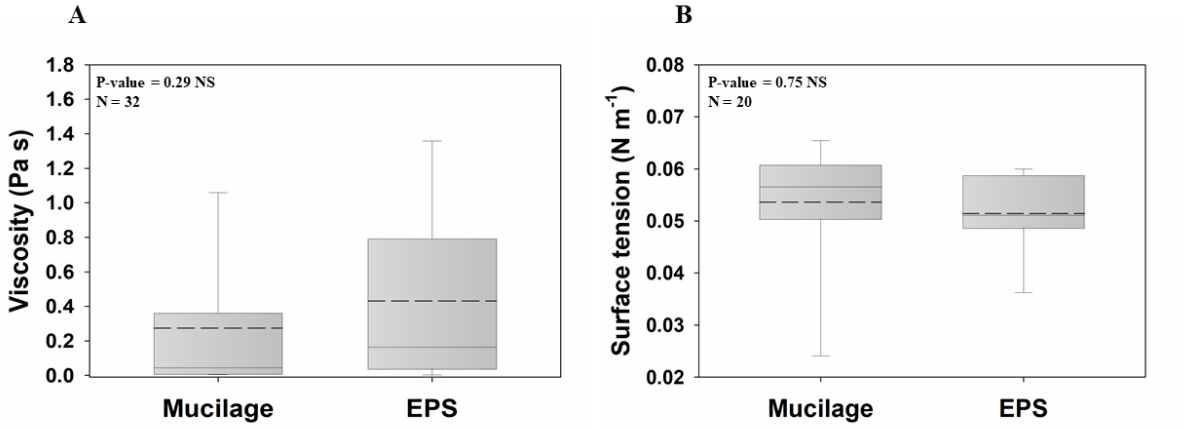

Figure 1. Comparison of mucilage and EPS viscosity (A) and surface tension (B), using Independent Samples *t*-test at the significance level of 0.05. The dashed and solid lines on each box indicate the arithmetic mean and median, respectively. The box defines the 25th and 75th percentiles. NS, non-significant; N, number of data points. The viscosity values had been measured at a solute concentration of 0.5 mg ml^{-1}, shear rate of 0.5 s^{-1}, and temperature of 20–25 ◦C, and the surface tension values had been measured at a solute concentration of 0.5 mg ml^{-1} and temperature of 20–25 ◦C. Viscosity of water at 25 ◦C = 0.00089 Pa s; Surface tension of water at 25 ◦C = 0.072 N m^{-1}.

Total polysaccharide and protein

Polysaccharides were the major chemical constituent of both biogels (77.4% and 74.6% for mucilage and EPS, respectively) and did not significantly differ between them (Figure 2A). The same was true for the total protein proportions of both biogels, being on average 5.8% and 7.7% in mucilage and EPS, respectively (Figure 2B).

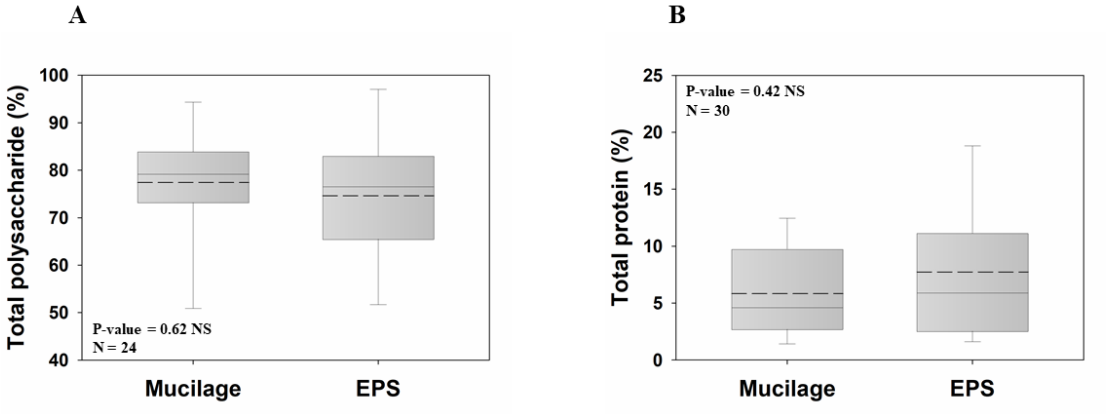

Figure 2. Comparison of mucilage and EPS total polysaccharide (A) and total protein (B), using Independent Samples *t*-test at the significance level of 0.05. The dashed and solid lines on each box indicate the arithmetic mean and median, respectively. The box defines the 25th and 75th percentiles. NS, non-significant; N, number of data points.

Neutral monosaccharide and uronic acid composition

Six out of nine studied monomers of the biogels' polysaccharide backbone did not significantly differ in proportion between mucilage and EPS, namely galactose (mucilage = 23.8%; EPS = 22.8%), fucose (13.9%; 9.9%), glucose (16.7%; 28.7%), rhamnose (12.4%; 15%), xylose (13.4%; 8.1%), and glucuronic acid (8%; 12.8%). In contrast, mannose (3.9%; 18.6%) was significantly higher in EPS than in mucilage (nearly fivefold higher), whereas arabinose (16.3%; 4.8%) and galacturonic acid (27.3%; 7.8%) had higher proportions (3.4-fold and 3.5-fold higher, respectively) in mucilage than in EPS (Figures 3A–I).

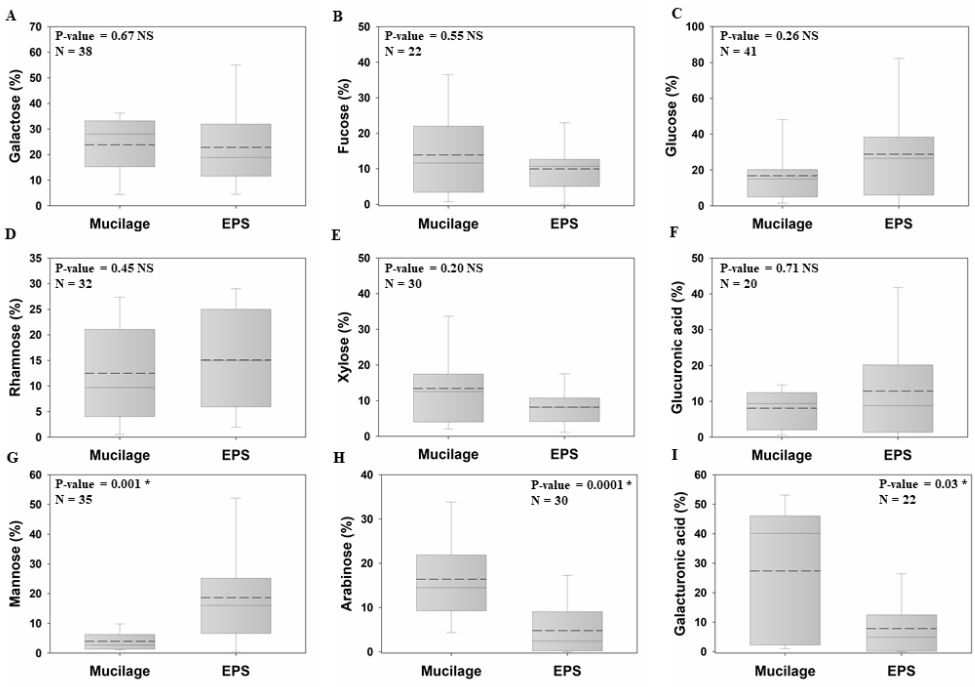

Figure 3. Comparison of the proportion of mucilage and EPS galactose (A), fucose (B), glucose (C), rhamnose (D), xylose (E), glucuronic acid (F), mannose (G), arabinose (H), and galacturonic acid (I), using Independent Samples at the significance level of 0.05. The dashed and solid lines on each box indicate the arithmetic mean and median, respectively. The box defines the 25th and 75th percentiles. NS and * indicate a non-significant and significant difference, respectively. N: number of data points.

Spatial and temporal organization of mucilage around growing roots

Based on simplified assumptions of root growth, exudation rate, decomposition rate, hydration ratio, and mucilage expansion into the soil, a simple model for the size and extent of a potential "mucilage biofilm" was developed (Figure 4). The axial rhizosphere extent directly at the root tip was 1.12 mm with a mucilage content of 1.89 mg g^{-1} soil. The kinetic of water saturation and swelling is rapid with an average 6 h until the mucilage of root tips reaches its constant volume. Therefore, 9.2 mm above the root tip, the mucilage is fully hydrated, reaching its final radial extent of 2.05 mm. By swelling, the content decreases to 0.8 mg g^{-1} soil, a value hardly changing by decomposition along the daily grown segment of 30 mm. Assuming a linear decomposition rate of 50% in 7 days, mucilage is only half decayed at a distance 21 cm above the root tip.

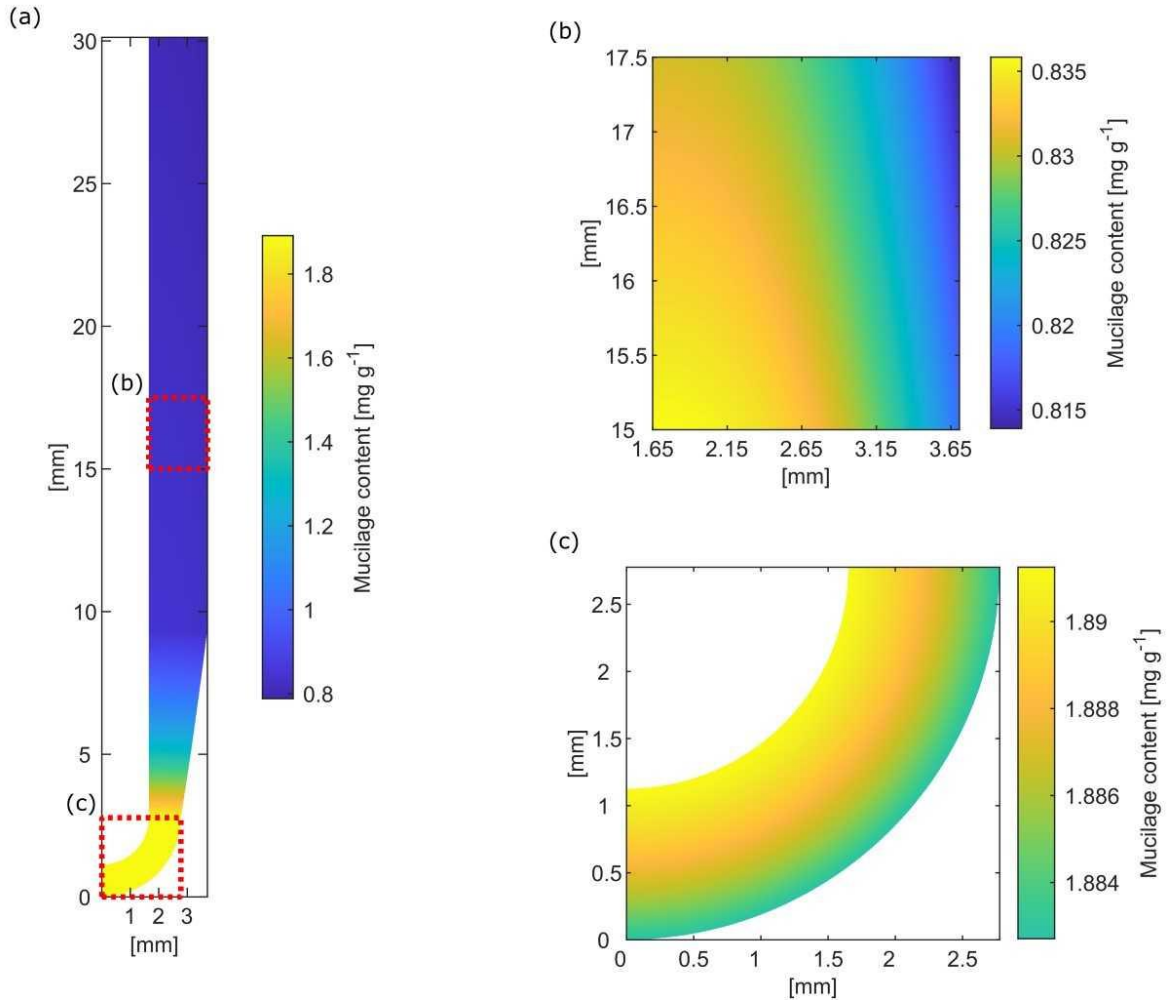

Figure 4. Spatial and temporal distribution of mucilage around a root segment grown within 1 day (= 30 mm) [(A) along the root; (B) lateral; (C) at the root tip]. Mucilage-affected soil is indicated by yellow to blue color along the root and by yellow to green at the root tip, reflecting the increasing radial extent by swelling as well as the decreasing content of mucilage by decomposition.

Microbial abundance and extracellular polymeric substances production in the mucilage matrix

We consider that two different zones around the root receive degradable carbon at varying rates: (i) the zone within 1.65 mm from the root tip where the estimated total mucilage C exudation rate (C_{in_rc}) is 5 mg d^{-1}, and (ii) the 28.35 mm root zone above the root tip (grown within 1 day) where the mucilage concentration is reduced by gel swelling and degradation (C_{inez} = 122 mg d^{-1}). The high C concentrations at the root tip allow for a maximum number of bacterial cells growing on the basis of mucilage C consumption, which is in the order of 10^9 cells per day. In contrast, lower C concentrations at the 28.35 mm zone above the root tip resulted in only 3×10^{10} bacterial cells grown per day. Despite its 25 times larger volume, the 28.35 mm root above the root tip can only host around 10 times more bacterial cells than the small soil volume surrounding the 1.65 mm of the root tip. The bacterial cells could potentially produce around 0.01 mg EPS per day at the root

tip and 0.29 mg EPS per day within the 28.35 mm zone of daily growth. Assuming the above given mucilage exudation of 1.41 mg d^{-1} and root tip with a 50% decomposition in 7 days (i.e., 7.1% decomposition per day), 101 mg of mucilage covering the root gets decomposed and replaced by 0.3 mg of EPS per day, assuming mucilage as the sole C source. This leads to a relatively small but continuous modification and thinning of the biofilm along the root axis.

Discussion

Although individual plant and microbial species and their physiological conditions crucially affect the biogels produced, our study revealed an overall high degree of similarity in the physical and chemical properties of EPS and mucilage. The selected physical and chemical properties control many of the beneficial attributes of EPS, such as the maintenance of hydraulic connectivity, the formation of aggregates or the reduction of enzyme, carbon and nutrient losses. Therefore, the similarity in these physical and chemical properties suggests that mucilage can also function as a biofilm matrix.

Although our study found wide variability among the investigated plant and microbial species, the physical and chemical properties of EPS and mucilage varied only within a moderate range. Hence, both biogels offer similar soil microbial habitats, shaped by vegetation type and soil conditions. In the following, we discuss how the physical and chemical characteristics of mucilage provide three substantial prerequisites for biofilm formation: a moist environment, a protective barrier, and carbon and nutrient provision (Flemming and Wingender, 2010; Velmourougane et al., 2017).

Biogels as microbial habitats

Our results demonstrate that microbial EPS have high viscosity and low surface tension. These two key physical properties of EPS can play important roles in the formation and persistence of microbial biofilms in soils (Lieleg et al., 2011; Benard et al., 2019). High viscosity and low surface tension facilitate adhesion and cohesion of biofilms to mineral or organic surfaces in the soil, bridging microbial cells for biofilm development, and aggregating soil particles (Flemming and Wingender, 2010; Costa et al., 2018). The physical properties of EPS imply several protecting functions against antibiotics, disinfectants, heavy metals, and even against harmful effects of oxygen, by reducing the diffusion of these compounds toward the microbial cells (Flemming and Wingender, 2010). Furthermore, the enhanced soil water retention and liquid-phase connectivity provided by EPS protect microorganisms against drought but also against deep frost (Bore et al., 2017; Benard et al., 2019). Last but not least, viscos EPS can protect against grazing protozoa by adhering to their cilia and blocking their feeding apparatus (Liu and Buskey, 2000; Flemming and Wingender, 2010).

The results of our analyses showed that the viscosity and surface tension of mucilage and EPS are not significantly different. The similarity of mucilage to EPS in terms of these physical characteristics implies that mucilage can provide a biofilm-like habitat to support the life and survival of microorganisms in soils, specifically in the rhizosphere. Numerous studies have

confirmed that mucilage provides a moist environment and protective barrier against abiotic and biotic stresses. Mucilage increases the water content of the rhizosphere, connects soil particles, increases the soil liquidphase connectivity, and facilitates root water uptake under drought, due to its high viscosity and low surface tension (Young, 1995; Carminati et al., 2010; Ahmed et al., 2015; Benard et al., 2019; Zarebanadkouki et al., 2019). Mucilage absorbs 27–589 times more water than its dry weight (McCully and Boyer, 1997; Huang and Gutterman, 1999; Capitani et al., 2013; Nazari et al., 2020), which is considerably higher than the amount of water absorbed by a similar quantity of EPS. The binding of negatively charged uronic acids to Ca2C governs hydrationdehydration dynamics in biogels (Dean et al., 2007; North et al., 2014; Brax et al., 2019). The results of our study indicate rather similar proportions of glucuronic acid in mucilage and EPS but significantly higher galacturonic acid in mucilage. This high proportion of galacturonic acid in mucilage is likely one of the major reasons for the higher water absorption capacity of mucilage than EPS. In addition to hydraulic functions, mucilage is cohesive and adhesive to surfaces and therefore, similar to EPS, improves soil aggregation in the rhizosphere through strengthening bonds between soil particles (Czarnes et al., 2000).

Mucilage ameliorates heavy metals toxicity in the rhizosphere, protects roots against salinity, and functions as a barrier against harmful effects of oxygen (Horst, 1995; Zarebanadkouki et al., 2019). It also traps pathogenic and herbivorous insects and protects microbial symbionts (Haughn and Western, 2012; Galloway et al., 2020). Moreover, an 8 mm layer of crude maize mucilage maintained very low oxygen levels (below 5%) (Van Deynze et al., 2018), which is very similar to oxygen levels in bacterial biofilms (Wessel et al., 2014; Wang et al., 2017). The low oxygen levels in the mucilage can support a microaerobic environment but can also promote crucial functions like nitrogenase activity (Van Deynze et al., 2018; Bennett et al., 2020). It can be deduced that mucilage, like EPS, provides a moist environment and a protective barrier for soil microorganisms in order to form biofilms.

Association of chemical composition and biogel functions

Generally, polysaccharides and proteins are major components of EPS and mucilage (Chaboud and Rougier, 1991; Wingender et al., 2001; Flemming and Wingender, 2010; Lembre et al., 2012; Behbahani et al., 2017; Pandit et al., 2020), and our analyses reveal a similar proportional contribution of these components to both biogels. The combination of EPS polysaccharides and proteins is important for the formation, organization, and stability of the biofilm (Flemming and Wingender, 2010; Fong and Yildiz, 2015; Limoli et al., 2015; Shukla and Rao, 2017). Both substance classes likely jointly contribute to the high viscosity of EPS and mucilage and to their low surface tension (Benard et al., 2019). Our analyses also show similar proportions of polysaccharides and proteins, with similar viscosities and surface tensions. This suggests that mucilage can also act in biofilm formation, organization, and stability.

Furthermore, EPS and mucilage have other common properties such as enzymes, extracellular DNA (eDNA), and lipids. However, the proportion of these constituents is low (Nazari, 2021).

EPS contain enzymes and eDNA produced by microorganisms inhabiting the biofilm. EPS enzymes can degrade matrix biopolymers such as polysaccharides and proteins in order to provide microorganisms with carbon and energy, a process occurring in EPS mainly under carbon starvation (Costa et al., 2018). This can become a central process of microbial C supply in a plant-provided mucilage biofilm matrix. In the case of the mucilage biofilm, biogel producing and consuming organisms are different and the "biofilm producer" is an autotrophic organism generally not suffering from low C supply. Like EPS, mucilage was also shown to contain several enzyme classes active in the degradation of major mucilage polysaccharides, releasing monosaccharides such as galactose, mannose, fucose, xylose, and arabinose (Pozzo et al., 2018; Voiniciuc et al., 2018; Bennett et al., 2020), supporting the concept of mucilage as a microbial C source.

Extracellular polymeric substances eDNA increases the structural stability of biofilms, functions as an important agent of microbial aggregation, and acts as an intercellular connector (Molin and Tolker-Nielsen, 2003; Yang et al., 2007; Flemming and Wingender, 2010). Mucilage also comprises eDNA that increases the stability of mucilage and protects root tips against pathogenic infection (Wen et al., 2009; Hawes et al., 2016; Ropitaux et al., 2020).

Lipids play a part in the hydrophobicity of EPS and help microorganisms adhere to waxy, plastic (e.g., Teflon), and pyrite surfaces (Neu and Poralla, 1988; Neu et al., 1992). Similar functions of lipids were described for mucilage, e.g., they control mucilage hydrophobicity and thus the interaction of mucilage with soil solids, water, and transported ions (Read et al., 2003; Chen and Arye, 2017; Nazari, 2021). Mucilage turns hydrophobic upon drying (Ahmed et al., 2016), a process that causes water repellency in the rhizosphere and prevents hydraulic failure in the rhizosphere under drought (Carminati, 2013; Zickenrott et al., 2016). This may be an important mechanism protecting the rhizosphere microbiome from drought effects and maintaining their activity even under water limitation (Ahmed et al., 2018a).

Our study also analyzed the monomer composition of polysaccharides, which are the quantitatively dominant fraction in both biogels. Our results indicated that EPS and mucilage have similar proportions of galactose, fucose, glucose, rhamnose, xylose, and glucuronic acid, while the proportions of mannose, arabinose, and galacturonic acid significantly differed. EPS galactose, fucose, and arabinose play an important role in the enhancement, dispersion, and stability of biofilms (Imberty et al., 2004; Tielker et al., 2005; Diggle et al., 2006; Johansson et al., 2008; Byrd et al., 2009; Ma et al., 2009). Pel and Psl are two major polysaccharides generally present in EPS, which play essential roles in biofilm establishment (Vu et al., 2009; Flemming and Wingender, 2010). Pel is mainly composed of glucose, while Psl is rich in mannose, glucose and rhamnose (Byrd et al., 2009). Thus, the higher mannose content of EPS than mucilage can presumably be explained by the higher proportion of Psl in soil biofilms. Pel and Psl are also necessary for the formation, adherence, and attachment of biofilms to abiotic and biotic surfaces and also for the stability of biofilm architecture (Byrd et al., 2009; Ma et al., 2009; Franklin et al., 2011; Zhurina et al., 2014). Pel and Psl often display high functional redundancy, which suggests that the relative

proportions of glucose vs. mannose and rhamnose have rather minor functional implications in EPS (Colvin et al., 2011). This suggests that also variations in these sugars between mucilage and EPS may be of minor functional relevance. In contrast to Psl and Pel, alginate is an anionic polysaccharide of EPS responsible for trapping of cations, a decisive process in biofilm establishment. For instance, calcium functions like a bridge between alginate molecules, leading to thick and compact biofilms with enhanced mechanical stability (Körstgens et al., 2001). The significantly higher proportion of galacturonic acid in mucilage than in EPS suggests that the alginate-related features of EPS are more pronounced and essential in mucilage. Most relevant here is the subsequent increase in extrinsic Ca^{2+} bridges in mucilage, which connect the uronic acids and therefore increase the mucilage stability (Moore and Fondren, 1988; Brax et al., 2019). Thus, this study suggests that the mechanical stability of biogels may differ as a result of the higher galacturonic acid proportion in mucilage than in EPS.

Moreover, uronic acids, being present in the form of their carboxylate anions in soils, may also function as buffering agents in the rhizosphere under extremely acidic conditions. Although carboxylate anions (oxalate, citrate, malate, etc.) released at the zone of maximal root exudation also buffer the proton exudation from the root, these compounds are of low-molecular-weight and are consequently rapidly decomposed. In contrast, galacturonic acid with a logarithmic acid dissociation constant (pKa) of 3.5 might therefore more efficiently and over a larger zone of the mucilage-covered rhizosphere fulfill this buffering function, at least in very acidic soils. This might be a mechanism contributing to avoiding a very strong rhizosphere acidification and thus a limitation of microbial life and activity (Malik et al., 2018).

In summary, we found a remarkably similar chemical composition of mucilage and EPS, which supports the contention that mucilage can function as a biofilm matrix. Although the individual role of each chemical mucilage component and its impact on microbial life has not yet been unraveled, it is unlikely that the limited deviation in chemical composition between mucilage and EPS would significantly diminish the potential of mucilage to function as a biofilm matrix.

Mucilage as a nutrient and carbon source for microorganisms

All EPS components are a potential source of nutrients (Flemming and Wingender, 2010). Since EPS and mucilage share many compositional similarities, mucilage can also be decomposed and consumed by microorganisms. Enzymatic release of highly abundant sugars in mucilage such as galactose, fucose, and arabinose can feed microorganisms residing in the mucilage (Bennett et al., 2020). The presence of endogenous glycosyl hydrolase enzymes in mucilage, which release the terminal fucose and arabinose residues, further augments this claim (Pozzo et al., 2018). Other studies also reported that microorganisms utilize mucilage as an energy source (Mary et al., 1993; Ahmed et al., 2018a,b; Veelen et al., 2018), with average times of 7–15 days for the consumption of 50% of the mucilage carbon added to the soil (Ahmed et al., 2018a). The high protein content of mucilage leads to a C:N ratio of approximately 16:1 (Mary et al., 1993), which is approximately double the C:N ratio of microorganisms (between 7:1 and 8.6:1) (Cleveland and Liptzin, 2007; Xu

et al., 2013). Thus, considering that 50% of the C is utilized *via* catabolism and oxidized to gain energy (Manzoni et al., 2012), mucilage has the ideal composition to function as a sole energy, C and N source for microorganisms. Consequently, microorganisms solely need to be supplied with mineral nutrients (P, K, Ca, Mg, etc.)—a common interest shared with their mucilage-providing plants. Our analyses strongly support the claim that mucilage is used as a source of nutrients covering the C and N demand of microorganisms and enabling high growth rates in the mucilagecovered rhizosphere. It is important to note that mucilage can provide a moist environment and protective barrier for EPS-producing but also for non-EPS-producing rhizosphere microorganisms, which can attach to solid surfaces but without formation of biofilms. Similarly, a study in bulk soil revealed that non-EPS-producing microorganisms can also benefit from the biogel produced by EPS-producing microorganisms (Chew and Yang, 2017). However, EPS production can consume large proportions of the available energy of a microbial cell. It can thus be considered as a bioenergetically "expensive" process for microorganisms. Plants, as photoautotrophs, are (partly) in the soil and yet have access to photosynthetically fixed C and thus can invest in extracellular biogels more easily than heterotrophic microorganisms. Mucilage can synergistically support EPS-producing as well as non-EPS-producing microorganisms in the rhizosphere and even overlapping biogel production of microbial EPS and mucilage can occur (Carminati and Vetterlein, 2013). This mucilage-EPS interaction can further boost the formation and modification of biofilms in the rhizosphere with advantageous functions for microorganisms and the plant.

Spatial and temporal implications of mucilage matrix for microbial life around the root

Holz et al. (2018) measured the mucilage distribution of approximately 1 mm around the root. Considering the influence of porosity on the radial extent of mucilage, our estimated maximum mucilage distribution of 2.05 mm around the root is well in agreement with the measured mucilage distribution of Holz et al. (2018). The quantitative relevance of mucilage as a biofilm matrix is defined by the radial and axial extent around the root, and the latter is largely defined by its decomposition kinetics. The maximal decomposition of 50% mucilage within 7 days of incubation under optimal conditions (Ahmed et al., 2018a), assuming a linear decomposition rate, suggests that only 7.14% of the daily mucilage production gets decomposed per day. Therefore, the axial extent of mucilage can reach several decimeters above the root tip without substantial thinning of the mucilage by decomposition (Figure 5).

The contribution of EPS to the root-covering biogel was already suggested by Carminati and Vetterlein (2013), but experimental or analytical studies quantifying the contribution of both biofilm matrices to the rhizosphere biofilm are still lacking. Our estimation of maximal EPS production capacity suggests that only a minor proportion (0.3%) of the decomposed mucilage is replaced by EPS, assuming mucilage as the sole C source (Figure 5). However, the proportion of EPS producers as well as their EPS production rate might be underestimated by our input data derived from pure culture isolates, and excluding fungal EPS. However, especially in the root elongation zone, a few millimeters above the root tip, the exudation of low-molecular-weight

substances provides an additional carbon source to be potentially utilized for EPS production (Yang and Crowley, 2000; Sasse et al., 2018; Rüger et al., 2021). Averaging the scarce data available on root exudation rates or amounts suggest low-molecular-weight exudate quantities in the range of 2.4×10^{-7} g d^{-1} cm^{-1} (Oburger et al., 2013; Gunina and Yakov, 2015). Even assuming all of this C is readily available for microbial utilization (Sasse et al., 2018; Rüger et al., 2021), this daily release of C is still a magnitude lower than the C provided by the mucilage decomposition (8×10^{-6} g C d^{-1} cm^{-1}). This suggests that low-molecular-weight exudates may play a minor role as C substrate for EPS production, but more importantly, that their function as a microbial C source might have been overestimated compared to mucilage C.

An approximation of the maximal number of bacterial cells growing on the decomposed mucilage C resulted in 10^{10} cells per cm^3 mucilage-affected rhizosphere volume, or 10^9 bacterial cells per g mucilage-affected rhizosphere soil (Figure 5). Although only a limited number of studies have quantified absolute bacterial abundance in the rhizosphere, most of them through gene copy numbers gained by qPCR, 10^9 is a realistic number for bacterial abundance (Zhu et al., 2016). This suggests that even under the assumption of only moderate decomposition (∼7% per day), mucilage C can function as a major C source supporting a high bacterial abundance in the rhizosphere, potentially without losing its function as a biofilm matrix for several decimeters along the root axis.

The production of EPS requires cellular resources and may be costly for microorganisms (Jayathilake et al., 2017). Hence, their fitness and competitiveness are reduced compared to nonEPS-forming microorganisms if no further environmental stress provides EPS producers with ecological advantage (Vardharajula and Sk, 2014). Therefore, an EPS-based biofilm with the extent of the mucilage-covered rhizosphere volume is impossible for heterotrophic EPS producers in soils. Compared to EPS production by heterotrophic soil microorganisms, mucilage as a biogel does not exhaust soil C sources, but is formed from the photosynthetically fixed C of the autotrophic plant. Considering the bioeconomy of the plant-microbe system, the direct production of the biogel by the autotrophic organism is more efficient than exuding low-molecular-weight C resources for heterotrophic organisms, of which only a minor proportion will be invested in biogel biosynthesis. Consequently, plant mucilage production is an efficient C investment in the context of the whole plant-soil continuum providing (a) a stable habitat for the establishment of the rhizo-microbial community; and (b) a major C source supporting the observed abundances of microbial cells in the rhizosphere zone (Sasse et al., 2018; Rüger et al., 2021). Both properties are “services” provided by the autotrophic plant to boost the rhizosphere microbiome toward high cell densities and highly active microorganisms. Consequently, the microbiological features of the immediate rhizosphere can be linked to mucilage, providing a biofilm matrix for the rhizosphere microbiome. Nonetheless, EPS are likely still important in the rhizosphere, because rhizosphere bacteria capable of producing EPS are associated with better root colonization (Costa et al., 2018; Knights et al., 2021).

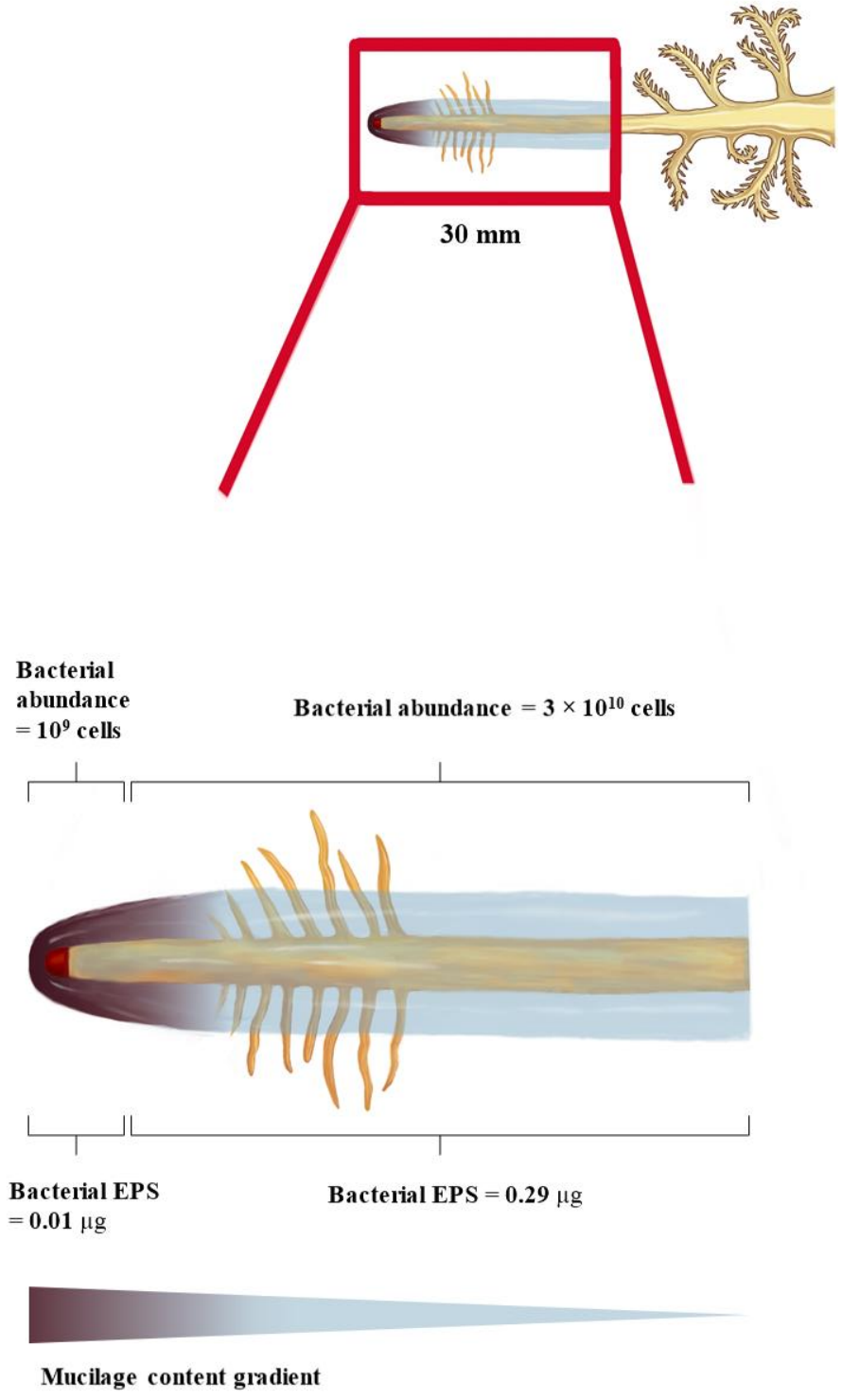

Figure 5. Spatial and temporal model of mucilage biofilm and its bacterial abundance and EPS production along a maize root segment grown within 1 day (= 30 mm). Mucilage content decreases away from the root tip due to bacterial decomposition. Mucilage extent increases away from the root tip due to swelling.

Conclusion

Our analyses revealed similar chemical composition and physical properties in plant mucilage and microbial EPS. This suggests that many functions of mucilage and EPS are comparable and consequently supports the potential of plant mucilage to function as a biofilm matrix similar to EPS. However, in contrast to an EPS-based biofilm, the high rhizosphere C investment required to form the biofilm matrix does not need to be covered by heterotrophic soil microorganisms. Instead, this functional C is provided directly by the autotrophic plant. As autotrophic organisms with substantially higher biomass, the proportional investment of plants in mucilage C is magnitudes lower than for an EPS-producing microbial colony that produce their own biofilm matrix. Therefore, mucilage exudation may be a major contributor to soil biogels, forming large volumes of stable microbial habitats around plant roots. The rhizosphere microbiome is protected against environmental stresses like drought besides being supplied with moderately available C that supports a high rhizosphere microbial abundance. The rather slow decomposition of mucilage compared to the rapid growth of roots leads to axial root segments in the decimeter range surrounded by this unique mucilage-based microhabitat. Therefore, we recommend a reconsideration of mucilage not only as a physical matrix that affects rhizosphere hydraulics, but as a biofilm matrix that supports the rhizosphere microbiome and its resistance to environmental stresses.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

MN and MD proposed the idea. MN performed the literature review and comparative analyses, and wrote the manuscript draft. SB developed the bacterial abundance and EPS production model. PB and AC developed the mucilage exudation model. KM-J shared ideas and scientifically enriched the text. MD supervised the project. All authors read the manuscript draft, commented on it, and confirmed it before submission.

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Supplementary material

Supplementary material 1

Supplementary table. Investigated physical properties of the biogels produced by different plant and microbial species.

Species	Biogel source	Biogel type	Composition	References
Actinidin chinensis	Root	Mucilage	Polysaccharides	Redgwell 1983
Linum usitatissimum L.	Seed	Mucilage	Polysaccharides	Barbary et al 2009
Lallemantia royleana	Seed	Mucilage	Polysaccharides	Alizadeh Behbahani et al 2018
Zea mays L.	Root	Mucilage	Polysaccharides	Chaboud and Rougier 1991
Linum usitatissimum L.	Seed	Mucilage	Polysaccharides	Paynel et al 2013
Plantago major	Seed	Mucilage	Polysaccharides	Alizadeh Behbahani et al 2017
Eruca sativa	Seed	Mucilage	Polysaccharides	Koocheki et al 2012
Lepidium perfoliatum	Seed	Mucilage	Polysaccharides	Koocheki et al 2013
Lepidium sativum	Seed	Mucilage	Polysaccharides	Karazhiyan et al 2011
Ocimum basilicum L.	Seed	Mucilage	Polysaccharides	Razavi et al 2009
Cydonia oblonga	Seed	Mucilage	Polysaccharides	Jouki et al 2014
Salvia macrosiphon	Seed	Mucilage	Polysaccharides	Farahnaky et al 2013
Actinidin chinensis	Root	Mucilage	Proteins	Redgwell 1983
Linum usitatissimum L.	Seed	Mucilage	Proteins	Barbary et al 2009
Lallemantia royleana	Seed	Mucilage	Proteins	Behbahani et al 2018
Zea mays L.	Root	Mucilage	Proteins	Chaboud and Rougier 1991
Linum usitatissimum L.	Seed	Mucilage	Proteins	Uschapovsky et al 2015
Citrus jambheri Lush	Seed	Mucilage	Proteins	Naqvi et al 2011
Sachtion citrumelo	Seed	Mucilage	Proteins	Naqvi et al 2011
Yuma citrange	Seed	Mucilage	Proteins	Naqvi et al 2011
Zea mays L.	Root	Mucilage	Proteins	Gould and Northcote 1986
Plantago major	Seed	Mucilage	Proteins	Alizadeh Behbahani et al 2017
Eruca sativa	Seed	Mucilage	Proteins	Koocheki et al 2012
Lepidium perfoliatum	Seed	Mucilage	Proteins	Koocheki et al 2013
Lepidium sativum	Seed	Mucilage	Proteins	Karazhiyan et al 2011
Ocimum basilicum L.	Seed	Mucilage	Proteins	Razavi et al 2009
Cydonia oblonga	Seed	Mucilage	Proteins	Jouki et al 2014
Salvia macrosiphon	Seed	Mucilage	Proteins	Farahnaky et al 2013
Pisum sativum L.	Root	Mucilage	Sugars	Knee et al 2001
Arabidopsis thaliana L.	Seed	Mucilage	Sugars	Rautengarten et al 2008
Glossostemon bruguieri	Seed	Mucilage	Sugars	Ibrahim et al 1997
Glossostemon bruguieri	Root	Mucilage	Sugars	Ibrahim et al 1997
Kosteletzkya virginica	Root	Mucilage	Sugars	Ghanem et al 2010
Oryza sativa L.	Root	Mucilage	Sugars	Chaboud and Rougier 1984
Lepidium sativum	Root	Mucilage	Sugars	Ray et al 1998
Linum usitatissimum L.	Seed	Mucilage	Sugars	Fedeniuk and Biliaderis 1994
Actinidin chinensis	Root	Mucilage	Sugars	Redgwell 1983
Linum usitatissimum L.	Seed	Mucilage	Sugars	Barbary et al 2009
Plantago spp.	Seed	Mucilage	Sugars	Phan et al 2016
Zea mays L.	Root	Mucilage	Sugars	Amicucci et al 2019
Zea mays L.	Root	Mucilage	Sugars	Van Deynze et al 2018
Zea mays L.	Root	Mucilage	Sugars	Osborn et al 1999
Linum usitatissimum L.	Seed	Mucilage	Sugars	Wannerberger et al 1991
Coffea arabica L.	Seed	Mucilage	Sugars	Avallone et al 2000
Lallemantia royleana	Seed	Mucilage	Sugars	Alizadeh Behbahani et al 2018
Zea mays L.	Root	Mucilage	Sugars	Chaboud 1983
Zea mays L.	Root	Mucilage	Sugars	Bacic et al 1986
Zea mays L.	Root	Mucilage	Sugars	Gould and Northcote 1986

Supplementary table. Continued.

Species	Biogel source	Biogel type	Composition	References
Pseudomonas fluorescens	Bacterial	EPS	Polysaccharides	Xu et al 2011
Pseudomonas fluorescens	Bacterial	EPS	Polysaccharides	Xu et al 2011
Klebsiella pneumoniae	Bacterial	EPS	Polysaccharides	Verhoef et al 2005
Rahnella aquatilis	Bacterial	EPS	Polysaccharides	Verhoef et al 2005
Brevundimonas vesicularis	Bacterial	EPS	Polysaccharides	Verhoef et al 2005
Geitlerinema spp.	Cyanobacterial	EPS	Polysaccharides	Richert et al 2005
Plectonema cf. battersii	Cyanobacterial	EPS	Polysaccharides	Richert et al 2005
Chroococcus submarinus	Cyanobacterial	EPS	Polysaccharides	Richert et al 2005
Rhabdoderma cf. rubrum	Cyanobacterial	EPS	Polysaccharides	Richert et al 2005
Breundimonas esicularis sp.	Bacterial	EPS	Polysaccharides	Verhoef et al 2002
Cryptococcus flavus	Fungal	EPS	Polysaccharides	Pavlova et al 2009
Bacillus licheniformis	Bacterial	EPS	Polysaccharides	Spano et al 2013
Pseudomonas fluorescens	Bacterial	EPS	Proteins	Xu et al 2011
Pseudomonas fluorescens	Bacterial	EPS	Proteins	Xu et al 2011
Sagittula stellata	Bacterial	EPS	Proteins	Xu et al 2011
Sagittula stellata	Bacterial	EPS	Proteins	Xu et al 2011
Geitlerinema spp.	Cyanobacterial	EPS	Proteins	Richert et al 2005
Plectonema cf. battersii	Cyanobacterial	EPS	Proteins	Richert et al 2005
Chroococcus submarinus	Cyanobacterial	EPS	Proteins	Richert et al 2005
Rhabdoderma cf. rubrum	Cyanobacterial	EPS	Proteins	Richert et al 2005
Breundimonas esicularis sp.	Bacterial	EPS	Proteins	Verhoef et al 2002
Microcystis spp.	Cyanobacterial	EPS	Proteins	Li et al 2009
Volcaniella eurihalina	Bacterial	EPS	Proteins	Bejar et al 1996
Pseudomonas fluorescens	Bacterial	EPS	Proteins	Ceyhan and Ozdemir 2008
Cryptococcus flavus	Fungal	EPS	Proteins	Pavlova et al 2009
Pseudoalteromonas spp.	Bacterial	EPS	Proteins	Nichols et al 2005
Bacillus licheniformis	Bacterial	EPS	Proteins	Spano et al 2013
Schizothrix spp.	Cyanobacterial	EPS	Proteins	Kawaguchi and Decho 2002
Acidophilic Spp.	Bacterial	EPS	Sugars	Jiao et al 2010
Klebsiella pneumoniae	Bacterial	EPS	Sugars	Verhoef et al 2005
Rahnella aquatilis	Bacterial	EPS	Sugars	Verhoef et al 2005
Brevundimonas vesicularis	Bacterial	EPS	Sugars	Verhoef et al 2005
Synechocystis sp.	Cyanobacterial	EPS	Sugars	Kawaguchi and Decho 2000
Oscillatoria sp.	Cyanobacterial	EPS	Sugars	Kawaguchi and Decho 2000
Breundimonas vesicularis sp.	Bacterial	EPS	Sugars	Verhoef et al 2002
Volcaniella eurihalina	Bacterial	EPS	Sugars	Bejar et al 1996
Synechocystis sp.	Cyanobacterial	EPS	Sugars	Ozturk and Aslim 2010
Synechocystis sp.	Cyanobacterial	EPS	Sugars	Ozturk and Aslim 2010
methanogenic granules	Bacterial	EPS	Sugars	Veiga et al 1997
Methanobacterium formicicum	Bacterial	EPS	Sugars	Veiga et al 1997
Methanobacterium formicicum	Bacterial	EPS	Sugars	Veiga et al 1997
Bacillus coagulans	Bacterial	EPS	Sugars	Kodali et al 2000
Thalassiosira sp.	Algal	EPS	Sugars	Giroldo et al 2003
Lactobacillus pentosus	Bacterial	EPS	Sugars	Sanchez et al 2006
Lactobacillus casei	Bacterial	EPS	Sugars	Kojic et al 1992
Pseudomonas aeruginosa	Bacterial	EPS	Sugars	Onbasli and Aslim 2009
Pseudomonas aeruginosa	Bacterial	EPS	Sugars	Onbasli and Aslim 2009
Pseudomonas stutzeri	Bacterial	EPS	Sugars	Onbasli and Aslim 2009
Achnanthes longipes	Algal	EPS	Sugars	Wustman et al 1997
Amphora coffeaeformis	Algal	EPS	Sugars	Wustman et al 1997

Cymbella cistula	Algal	EPS	Sugars	Wustman et al 1997
Burkholderia cepacia	Bacterial	EPS	Sugars	Ceyhan and Ozdemir 2008
Burkholderia gladioli	Bacterial	EPS	Sugars	Ceyhan and Ozdemir 2008
Pseudomonas fluorescens	Bacterial	EPS	Sugars	Ceyhan and Ozdemir 2008
Cryptococcus flavus	Fungal	EPS	Sugars	Pavlova et al 2009
Pseudoalteromonas spp.	Bacterial	EPS	Sugars	Nichols et al 2005

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Supplementary material 2

Estimation of mucilage extent and content along a root segment

Exudation $rate = 1.41\ mg\ day^{-1}$

Exudation rate is the dry weight of mucilage exuded from the root tip per day.

Growth rate = 30 mm day^{-1}

The increase in root length per day.

Degradation of mucilage to 0.5 of its initial mass: 168 hours (7 days)

*Fraction of mucilage degraded by microbial activity in one week.*Root diameter $= 3.3$ mm

Soil porosity $= 0.5$

Soil mineral density $= 2.65\ \frac{\text{mg}}{\text{mm}^3}$

Density of the soil solid phase used to derive mucilage content from liquid mucilage concentration and porosity.

$$\text{Density of hydrated mucilage} = 1\ \frac{\text{mm}^3}{\text{mg}}$$

Since the ratio of dry to wet weight of mucilage is on the order of mg per g we neglect the impact of mucilage concentration on liquid density.

$$\text{Initial ratio of wet to dry weight of exuded mucilage} = \frac{166}{1}$$

Initial ratio of wet to dry weight of mucilage exuded from the root tip.

$$\text{Initial volume of mucilage exuded in one day} = 1.41\ \text{mg} * \frac{166}{1} * 1\ \frac{\text{mm}^3}{\text{mg}} = 234\ \text{mm}^3$$

$$\text{Ratio of wet to dry weight of fully hydrated mucilage} = \frac{425}{1}$$

Final ratio of wet to dry weight of mucilage after full hydration i.e., maximum volumetric extent.

$$\text{Time until full hydration after exudation} = 6\ \text{hours}$$

We assume full hydration of exuded mucilage in 6 hours.

The following calculations and approximations were done using MATLAB (R2020a). The geometry of a root was assumed as a cylinder as root axis and a semi-spherical cap as root tip. Exudation is assumed at the root tip into the volume of a spherical cap of soil at a constant rate. Mucilage hydration and degradation are derived from an initially uniform distribution of a given volume of mucilage exuded in one day i.e., with the same extent from any point of the root surface.

For the uniform distribution of mucilage and given input parameters, the time to fill the semi-spherical soil volume at the root tip is 100 minutes hence the average age of mucilage in this volume is 50 minutes. Since we assume a linear hydration i.e., swelling of mucilage over time and a constant exudation rate, the hydrated volume of mucilage is derived in this region for its average residence time of 50 minutes. In this time, mucilage swells from its initial wet weight to dry weight ratio of 166/1 to 203/1 (i.e., 16.5 to 20.1 mm^3). The gradient in mucilage content from the semi-spherical root tip is derived for given degradation rate as a function of distance (which is a function of time) from the root surface.

In the second step, this profile of partly hydrated mucilage extent and mucilage content at the root tip are extended along the root axis. First, mucilage hydration and extent are derived for the average residence time of mucilage along the root axis which is function of growth rate. Then, the gradient in mucilage content from the root surface is calculated for this updated radial extent. Finally, the mucilage content is updated for mucilage age as function of distance from the root surface.

MATLAB. 2020. The MathWorks, Inc. Version 9.8.0.1396136 (R2020a) Update 3

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Study 5: Root mucilage improves plant nitrogen uptake from dry soil

Meisam Nazari[1], Andrea Carminati[2], Mohsen Zarebanadkouki[3]; Nataliya Bilyera [4], Rosepiah Munene[1], Kyle Mason-Jones[5], Timothy Beissinger[6, 7], Michaela A. Dippold[1, 8]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[2]Physics of Soils and Terrestrial Ecosystems, Department of Environmental Systems Science, Institute of Terrestrial Ecosystems, ETH Zürich, Zurich, Switzerland

[3]Chair of Soil Physics, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

[4]Department of Soil Science, Institute for Plant Nutrition and Soil Science, Christian-Albrechts-University of Kiel, 24118, Kiel

[5]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands

[6]Division of Plant Breeding Methodology, Department of Crop Sciences, University of Goettingen, Goettingen, Germany

[7]Center for Integrated Breeding Research, University of Goettingen, Goettingen, Germany

[8]Geo-Biosphere Interactions, University of Tübingen, Tübingen, Germany

*Correspondence: Meisam.nazari@forst.uni-goettingen.de; Meisam.nazari1991@gmail.com

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Abstract

Many studies have highlighted the potential function of root mucilage for improving plant water and nutrient uptake from dry soils. However, there is no experimental evidence that mucilage really does this, because the performed experiments used artificial conditions in which mucilage was mixed with soil in the absence of plant. For the first time, this study investigated the influence of root mucilage on plant water and nitrogen uptake in a dry soil using drought-resistant (DH02 and DH04 from Kenya) and drought-susceptible (Kentos and Keops from Germany) maize varieties differing in mucilage quantity and quality. A pot experiment was performed as a randomized complete block design consisting of six replicates in a plant growth chamber. The variety DH02 exuded 186% more mucilage than the variety Kentos; and the variety Keops exuded 264% and 160% more mucilage than the varieties Kentos and DH04, respectively. The lowest mucilage water-holding capacities belonged to Keops (291 times its dry weight) and Kentos (599 times its dry weight). The highest leaf nitrogen contents were observed in DH02 (35.1 SPAD) and DH04 (38.3 SPAD). The leaf nitrogen content was positively correlated with the mucilage water-holding capacity ($r = 0.56$), indicating the function of mucilage water-holding capacity to improve the plant nitrogen uptake from dry soils. The leaf stomatal conductance and plant transpiration rate were not significantly correlated with the mucilage properties, suggesting no role for mucilage in improving plant water uptake from dry soils. In conclusion, mucilage improves plant nutrient uptake but there is no evidence of its role in water uptake from dry soils.

Keywords: drought**,** nutrient, rhizosphere, root exudates, stomata, Zea mays L.

Introduction

Increased temperatures and irregular precipitation patterns are expected to enhance the risk of drought and water scarcity worldwide in the future decades, which can lead to decreased crop yield and food insecurity (Food and Agriculture Organization 2012). Drought causes soil drying and restricts water availability to plant roots and therefore reduces water uptake by the plant (Passioura, 1980; Draye et al., 2010). To avoid too negative water potentials, plants gradually close their stomata and reduce transpiration (Meyer and Green, 1980; Comstock, 2002; Sinclair et al., 2005). Although stomatal closure supports plant survival under drought, the costs are reduced photosynthesis and biomass production. Soil drying also limits plant nutrient uptake through decreasing the availability and diffusion of nutrients (i.e., nitrogen and phosphorus) into the roots (Darrah, 1993; Moldrup et al., 2001; Chou et al., 2012; Moyano et al., 2013). Overall, reduced plant water and nutrient uptake under drought is detrimental to agricultural production globally and especially in drought-prone regions.

Understanding which plant traits and plant-soil processes support soil water and nutrient availability to plants is crucial for sustainable agricultural production under drought. A well-known plant strategy to tolerate drought is to grow deep roots for acquiring water and nutrients from deep soil layers. Another important plant strategy is to modify properties of the soil around their roots, the rhizosphere, by secreting a suit of substances from their roots called rhizodeposits. Rhizodeposits refer to a group of substances including sugars, organic acids, amino acids, and mucilage (Walker et al., 2003; Jones et al., 2009; Oburger and Jones, 2018; Nazari, 2021). Mucilage is a viscoelastic high-molecular-weight substance exuded by root tips, containing mainly polysaccharides but also proteins, minerals, and lipids (Nazari, 2021) and its role in plant water and nutrient uptake under drought has recently received substantial attention (Carminati and Vetterlein, 2013; Ahmed et al., 2015; Zarebanadkouki et al., 2019).

Together with other root exudates and root hairs, mucilage plays a part in the formation of rhizosheath in plants, improving the root-soil connection, and facilitating water and nutrient uptake (Watt et al., 1993; McCully, 1999; Dakora and Phillips, 2002; Sasse et al., 2018). The high viscosity of mucilage enhances the soil liquid-phase connectivity, increases water content in the rhizosphere, and facilitates root water and nutrient uptake under drought (Young 1995; Carminati et al., 2010; Carminati et al., 2011; Carminati and Vetterlein, 2013; Ahmed et al., 2015; Benard et al., 2018; Benard et al., 2019; Zarebanadkouki et al., 2019). When saturated, mucilage holds between 25 and 600 times its dry weight water, increasing the water-holding capacity of the rhizosphere and preventing a steep decline in hydraulic conductivity in the drying soil (McCully and Boyer, 1997; Huang and Gutterman, 1999; Capitani et al., 2013; Kroener et al., 2014; Carminati et al., 2017; Nazari et al., 2020). Mucilage can also considerably reduce the surface tension of water easing the drainage of small pores (Read et al., 2003; Naveed et al., 2017).

Further, mucilage improves plant nutrient availability and uptake through promoting plant-microbe symbiosis and nutrient cycling in the rhizosphere (Walker, 2003; Hinsinger et al., 2009;

Kuzyakov, 2010; Nazari et al., 2022). Mucilage contains several enzymes capable of degrading soil organic matter and improving nutrient mobilization in the rhizosphere (Ma et al., 2010; Pozzo et al., 2018; Nazari, 2021). Moreover, microorganisms inhabiting the crown root mucilage of a Mexican landrace of maize Sierra Mixe (*Zea mays* Y.) can provide 29-82% of the plant's nitrogen nutrition through biological nitrogen fixation (Van Deynze et al., 2018; Amicucci et al., 2019; Bennet et al., 2020). However, an important question is whether mucilage in modern hybrid maize (*Zea mays* L.) varieties can also contribute to the nitrogen fixation of the plant.

Although the studies on mucilage highlight its role in improving plant water and nutrient uptake under drought, there is no experimental evidence that mucilage really facilitates plant water and nutrient uptake, because these experiments used artificial conditions in which mucilage was mixed with soil in the absence of plant. Recently, studies showed that the drought-resistant maize and barley (*Hordeum vulgare* L.) varieties exude higher amounts of mucilage than the drought-susceptible ones (Carter et al., 2019; Nazari et al., 2020). The identification of such varieties provides us with a great opportunity to realistically test the impact of mucilage on plant water and nutrient uptake under drought conditions. For the first time, this study aimed to provide experimental evidence of the function of mucilage for plant water and nitrogen uptake in a dry soil using four drought-resistant and drought-susceptible maize varieties differing in mucilage quantity and quality.

Materials and methods

Soil and plant preparation

A pot experiment was performed as a randomized complete block design (RCBD) consisting of six replicates in a plant growth chamber. Two drought-resistant maize varieties from Kenya (DH02 and DH04) and two drought-susceptible maize varieties from Germany (Kentos and Keops) were used. DH02 and DH04 are varieties developed by the Kenya Seed company (Kitale, Kenya) for cultivation in arid and semi-arid regions of Kenya. Kentos and Keops are high-yielding maize varieties developed by the KWS SAAT company (Einbeck, Germany) for cultivation in humid temperate regions of Germany. The soil was a loam Luvisol collected from 0-25 cm depth of an agricultural farm located in Hohenpölz, Bavaria, Germany. The soil had a total organic carbon of 1.77%, total nitrogen of 0.19%, microbial biomass carbon of 493 µg g^{-1}, water-holding capacity of 63.3%, and pH of 6.4.

The pots of 15 cm height and 15 cm diameter were uniformly filled with 2-mm sieved dry soil. Seeds of the maize varieties were pre-germinated on wet filter paper for three days and then one seedling was placed in each pot at a depth of 3 cm. The plants were grown in a growth chamber with a photoperiod of 12 hours day and 12 hours night. The average temperature and relative humidity in the growth chamber were 26 °C ± 1 and 60% ± 5. The light sources in the chamber were 243W light-emitting diode (LED) with a near-daylight spectral composition (Kind LED Growth Lights, California, USA). Drought was simulated by setting the soil water content to 30% water-holding capacity for a week from the growth stage beginning of stem elongation (BBCH 30)

to the growth stage nine or more nodes visible (BBCH 39). The soil water content was kept at 70% water-holding capacity for about two weeks from sowing to BBCH 30.

Sampling of mucilage

Mucilage was sampled from nodal roots of the maize varieties at the growth stage nine or more nodes visible (BBCH 39) according to Ahmed et al (2015). Briefly, emerged nodal roots were immersed in distilled water for 24 hours to get maximum mucilage hydration. Then, the hydrated mucilage was harvested from the nodal root tips using a 5 ml pipettor. Forceps was used to sample any small amount of mucilage remaining on the nodal root. The mucilage samples were collected in 50 ml vials, weighed, and freeze-dried (Beta 1-8 LSCplus, Christ, Osterode, Germany). Mucilage exudation rate was expressed as the dry weight (DW) of freeze-dried mucilage per nodal root tip per day. Mucilage water-holding capacity was calculated as the weight of fully hydrated mucilage – the weight of freeze-dried mucilage / the weight of freeze-dried mucilage and expressed as times its dry weight.

Measurement of plant water and nitrogen uptake

Leaf stomatal conductance and plant transpiration rate were used as measures of plant water uptake. Stomatal conductance was measured by a leaf porometer (Decagon Devices, USA) at the abaxial surface of the center of the fully developed sixth leaf. Before measurement, the porometer device was calibrated according to the company guide. For measuring plant transpiration rate, the soil of each pot was covered with polyethylene bags to prevent evaporation. Then, transpiration rate was measured by weighing the pots expressed as the weight of lost water per hour. Stomatal conductance and transpiration rate were measured at the growth stage nine or more nodes visible (BBCH 39).

For measuring plant nitrogen uptake, a portable hand-held SPAD (Single-Photon Avalanche Diode) 502 Plus device (Konica Minolta, Japan) was used at the center of the fully developed sixth leaf. The SPAD meter can very accurately measure the nitrogen status of maize at different phenological stages (Piekielek and Fox, 1992; Blackmer et al., 1994; Blackmer and Schepers, 1995; Rorie et al., 2011; Makarian et al., 2016). The SPAD measurements were done at the growth stage nine or more nodes visible (BBCH 39).

Measurement of leaf stomatal density

For measuring leaf stomatal density, pieces of plant leaves were bleached in a 10% sodium hypochlorite solution for 24 hours to remove the mesophyll. The abaxial surface of the center of the fully developed sixth leaf of each plant was observed using a light microscope (Olympus BX40, Olympus Optical Co., Ltd., Tokyo, Japan) in order to count stomata.

Measurement of mucilage nitrogen fixation

For measuring nitrogen fixation in mucilage, the ^{15}N natural abundance (expressed as delta units, ‰) of freeze-dried mucilage was analyzed. Kjeldahl digestion was used to determine the content

of total organic nitrogen followed by steam distillation. ^{15}N natural abundance was determined by the Delta XP isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany). Plants that acquire nitrogen from the atmosphere demonstrate low and negative ^{15}N natural abundance values and plants that derive nitrogen from soil exhibit high and positive values (Boddey et al., 2001; Van Deynze et al., 2018). The ^{15}N natural abundance analysis was implemented at the Centre for Stable Isotope Research and Analysis of the Georg-August University of Göttingen.

Statistical analysis

Data analysis was done by IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). The data were checked for normality and the homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. The data not meeting these assumptions were transformed into square roots. One-way analysis of variance (ANOVA) was used to test for significant effects of the maize varieties at the significance level (α) of 0.05. Tukey's HSD (Honestly Significant Difference) test was used for pair-wise comparison of the means between the significant factors at $\alpha = 0.05$. The data of mucilage exudation rate and stomatal conductance did not fulfill the homogeneity of variances assumption even after transformation, for which Welch's test of unequal variances was used. Pearson's correlation test was used to detect significant relationships between the measured variables at $\alpha = 0.01$. The boxplots were designed using SigmaPlot 14.0 (Systat, San José, CA, United States). All results presented are arithmetic mean.

Results

Mucilage exudation rate and water-holding capacity

Mucilage exudation rate and water-holding capacity significantly differed between the maize varieties at $\alpha = 0.05$ (Table 1). The variety DH02 exuded 186% more mucilage than the variety Kentos (Figure 1A). Moreover, the variety Keops exuded 264% and 160% more mucilage than the varieties Kentos and DH04, respectively. Mucilage exuded by the varieties Keops and Kentos had 84.9% and 68.9% lower water-holding capacity than mucilage exuded by the variety DH04, respectively (Figure 1B).

Table 1. Analysis of variance (ANOVA) results for the investigated variables affected by the maize varieties (at $\alpha = 0.05$; $n = 6$).

Source of variation	df	Sum of squares	Mean square	F-value	P-value
Mucilage exudation rate	3	0.30	0.10	5.76	0.02 *
Mucilage water-holding capacity	3	2482.64	827.58	7.25	0.002 *
Leaf nitrogen content	3	447.51	149.17	19.38	<0.0001 *
^{15}N natural abundance	3	116.74	38.91	5.90	0.005 *
Stomatal conductance	3	7131.99	2377.99	6.43	0.01 *
Transpiration rate	3	0.37	0.12	0.61	0.61 NS
Stomatal density	3	38.88	12.96	0.04	0.98 NS

*: significant effect; NS: non-significant effect

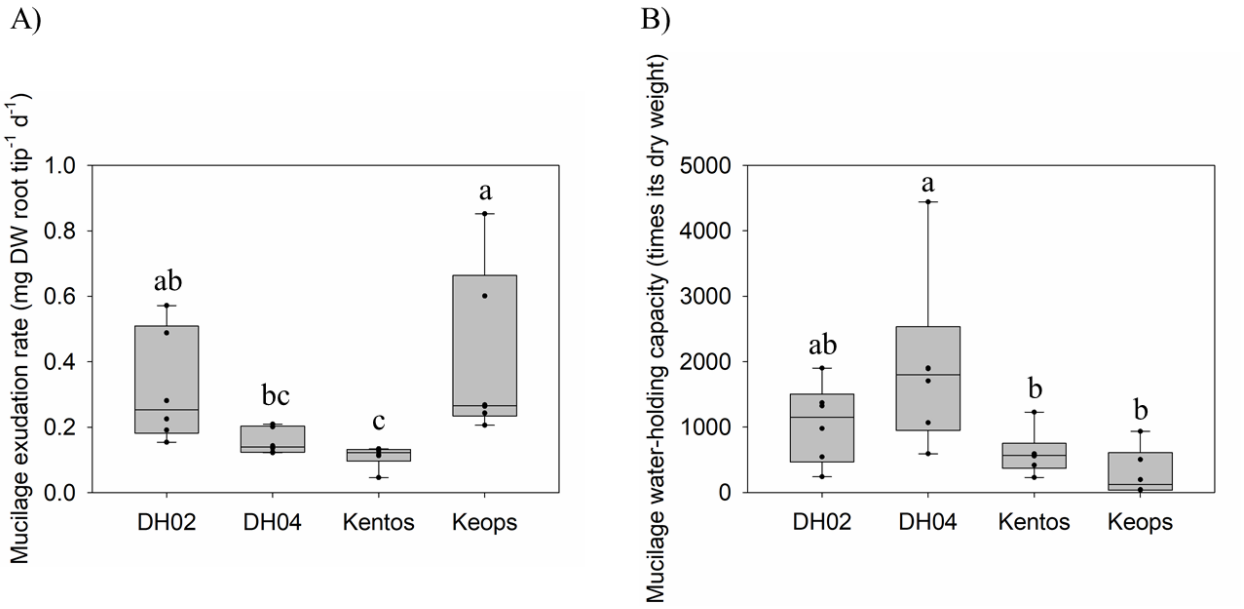

Figure 1. Mucilage exudation rate (A) and mucilage water-holding capacity (B) of nodal roots of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box defines the 25th and 75th percentiles.

Leaf nitrogen content and mucilage ^{15}N natural abundance

The varieties had significantly different leaf nitrogen contents (Table 1). The leaf nitrogen contents of the varieties DH02 and DH04 were 28.5% and 40.3% higher compared to the leaf nitrogen content of the variety Kentos and 18.1% and 28.9% higher than the leaf nitrogen content of the variety Keops, respectively (Figure 2A). There was also a significant difference between the varieties regarding the mucilage ^{15}N natural abundance (Table 1). The lowest ^{15}N natural abundance (- 1.25 ‰) belonged to the variety Kentos, which was significantly different from the ^{15}N natural abundance of the other varieties ranging from 3.70 ‰ to 3.92 ‰ (Figure 2B).

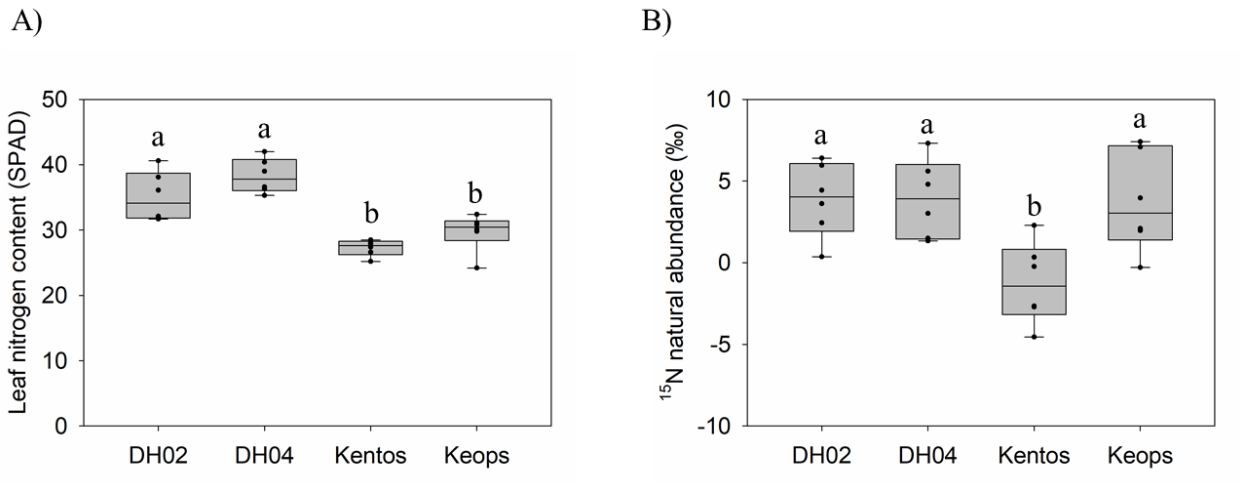

Figure 2. Leaf nitrogen content (A) and mucilage ^{15}N natural abundance (B) of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, n = 6). The box defines the 25th and 75th percentiles.

Leaf stomatal conductance and plant transpiration rate

Leaf stomatal conductance significantly differed between the varieties (Table 1). The highest stomatal conductance (80.1 mmol m^{-2} s^{-1}) belonged to the variety DH02 and significantly differed from that of the other varieties (Figure 3A). Plant transpiration rate did not significantly differ between the varieties (Table 1) and ranged from 2 to 2.3 g H_2O h^{-1} (Figure 3B).

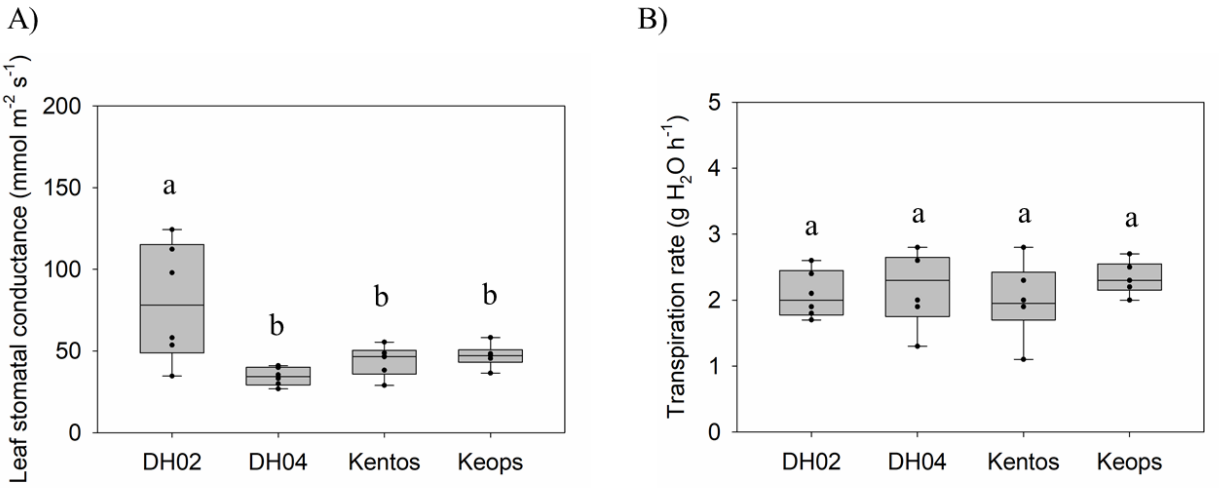

Figure 3. Leaf stomatal conductance (A) and plant transpiration rate (B) of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box defines the 25th and 75th percentiles.

Leaf stomatal density

Leaf stomatal density did not significantly differ between the varieties (Table 1) and ranged from 104 to 108 stomata mm^{-2} (Figure 4). Moreover, it was visually inspected that the leaf stomata of the maize varieties were almost closed at the time of sampling (Figure 5).

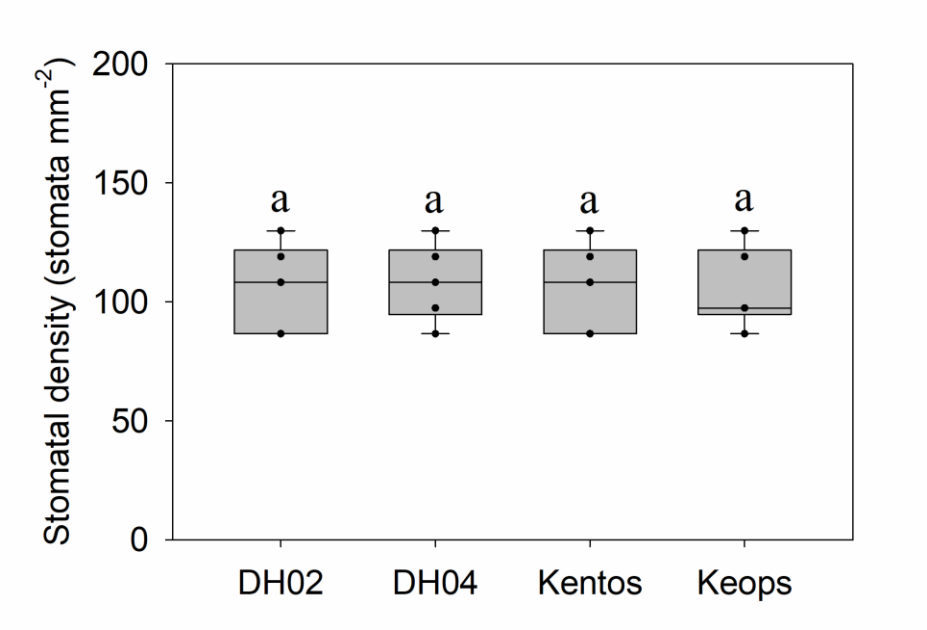

Figure 4. Abaxial leaf stomatal density of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box defines the 25th and 75th percentiles.

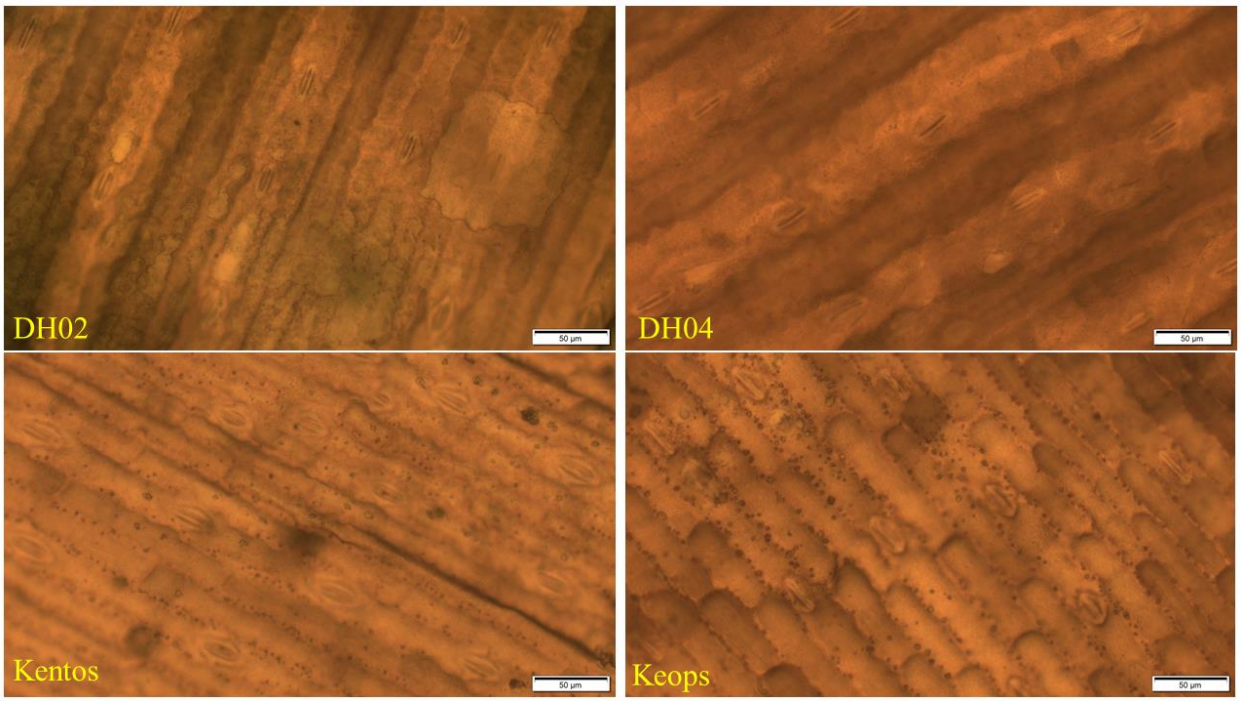

Figure 5. Abaxial leaf stomata of the maize varieties from Kenya (drought-resistant DH02 and DH04) and Germany (drought-susceptible Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39).

Correlations between the measured variables

The mucilage exudation rate had a negative correlation with the mucilage water-holding capacity (r = - 0.45) but a positive correlation with the mucilage ^{15}N natural abundance (r = 0.45) (Table 2). The leaf nitrogen content was positively correlated with the mucilage water-holding capacity (r = 0.56) and ^{15}N natural abundance (r = 0.54).

Table 2. Correlation matrix of the investigated variables affected by the maize varieties (Pearson's correlation, n = 48).

	MER	**MWHC**	**LNC**	**^{15}N NA**	**SC**	**T**	**SD**
MER							
MWHC	- 0.45 *						
LNC	0.03	0.56 **					
^{15}N NA	0.45 *	0.10	0.54 **				
SC	0.12	- 0.11	0.28	0.21			
T	0.24	0.01	0.03	0.35	- 0.11		
SD	0.07	- 0.16	0.07	- 0.24	0.18	- 0.24	

MER: mucilage exudation rate; MWHC: mucilage water-holding capacity; LNC: leaf nitrogen content; ^{15}N NA: ^{15}N natural abundance; SC: stomatal conductance; T: transpiration rate; SD: stomatal density

The values are Pearson's correlation coefficient.

*: significant correlation at $\alpha = 0.05$.

**: significant correlation at $\alpha = 0.01$.

Discussion

Mucilage exudation rate and water-holding capacity differ between the maize varieties

The drought-resistant maize variety DH02 exuded significantly more mucilage than the drought-susceptible variety Kentos, which verifies the results of a former study on these varieties in a field experiment (Nazari et al., 2020). Furthermore, Carter et al (2019) found that a drought-resistant barley variety exudes higher amounts of mucilage from its roots than a drought-susceptible one. Breeding in dry conditions of Kenya for drought resistance could have unintentionally selected for the high mucilage exudation rate in DH02, because of the putative function of mucilage during drought. For example, mucilage sugars (i.e., galactose, fucose, glucose) provide rhizosphere microorganisms with energy for the production of extracellular polymeric substances (EPS) against drought (Nazari et al., 2022). Mucilage, similar to some other root exudates, could also boost plant-growth-promoting bacteria (e.g., *Pseudomonas* putida) to improve the plant survival in dry conditions (Zulfikar Ali et al. 2011). The membrane permeability of maize root tip cells is increased in dry conditions for easing water uptake (Ionenko et al. 2010). Therefore, another reason for the higher mucilage exudation rate in DH02 might have been the enhanced membrane permeability of the root tip and thereby facilitation of mucilage exudation as the results of breeding in dry conditions. Unlike our expectation, the drought-susceptible variety Keops had a significantly higher mucilage exudation rate than DH04 and a similar mucilage exudation rate to

DH02. This indicates that other factors besides drought may influence mucilage exudation rate. Since mucilage is a biofilm matrix and source of energy and nutrients for rhizosphere microorganisms (Van Deynze et al., 2018; Bennet et al., 2020; Nazari et al., 2022), we speculate that the breeding of Keops in microbially rich soils has led to its high mucilage exudation rate. In a recent study, it was indicated that the maize varieties grown in a microbially rich soil from Germany had higher mucilage exudation rates compared to the same varieties grown in a microbially poor soil from Kenya (Nazari et al., under review). Overall, it seems that breeding in dry conditions and microbially rich soils have led to the development of the maize varieties with high mucilage exudation rates.

Mucilage exuded by the varieties Keops and Kentos had significantly lower water-holding capacity than mucilage exuded by the variety DH04. Mucilage water-holding capacity is a function of uronic acid-calcium ion interconnections in the mucilage (Brax et al., 2019; Nazari et al., 2021). Thus, the lower mucilage water-holding capacities could have been due to low uronic acid composition or calcium content in the mucilage of Keops and Kentos. Moreover, the mucilage exudation rate negatively correlated with the mucilage water-holding capacity ($r = -0.45$). It conveys that the varieties sacrificed the mucilage quantity (exudation rate) for its quality (water-holding capacity) in the dry soil. The significantly higher mucilage water-holding capacity in DH04 and a higher trend in DH02 than Keops and Kentos indicate its potential functional importance for maize resistance against drought. Enhanced mucilage water-holding capacity is a plant strategy to increase the rhizosphere water content and hydraulic conductivity in dry conditions (Carminati et al., 2010; Kroener et al., 2014; Benard et al., 2019), probably to keep the root-soil contact and plant survival by preventing the destruction of root tissues and hairs as well as root shrinkage. Considering the function of mucilage as a biofilm matrix (Nazari et al., 2022), a well hydrated mucilage covers more soil particles and microorganisms and provides wet conditions in the rhizosphere for the favor of the plant and microorganisms in the dry soil.

Association of mucilage with plant nitrogen uptake

Leaf nitrogen content was significantly higher in the drought-resistant varieties than in the drought-susceptible ones. The positive correlation between the leaf nitrogen content and mucilage water-holding capacity ($r = 0.56$) indicates that the exudation of a mucilage capable of absorbing huge amounts of water is a plant strategy to take up more nitrogen from dry soil. This could have happened through four major processes: 1. The high mucilage water-holding capacity has kept the rhizosphere wet at the low water potential of the dry soil (McCully and Boyer, 1997; Carminati et al., 2010), decreased the average diffusion path of nitrogen to the root surface (tortuosity), and thereby increased the diffusive transport of nitrogen into the roots. 2. Mucilage has increased the liquid phase connectivity of the rhizosphere soil through its high water-holding capacity, high viscosity, and low surface tension (Carminat et al., 2017; Benard et al., 2019; Nazari et al., 2022). 3. Since the plant-absorbable forms of nitrogen in soil namely nitrate and ammonium need to be solubilized in water, the high mucilage water-holding capacity could have eased their solubilization and uptake by the roots. 4. Mucilage is a biofilm matrix providing rhizosphere

microorganisms with habitat, energy, and nutrients and its high water-holding capacity enhances the extent of the biofilm (Nazari et al., 2022), increasing the microbial activity and mineralization of organic nitrogen compounds in the rhizosphere. Each of these four processes could have contributed an unknown part to the increased leaf nitrogen contents in the drought-resistant varieties. Through an artificial setup mixing chia seed mucilage with soil, Zarebanadkouki et al (2019) indicated that the mucilage facilitated nutrient diffusion in the drying soil, which is verified by the results of the present study.

The leaf nitrogen content did not have a significant correlation with the mucilage exudation rate but had a positive correlation with the mucilage ^{15}N natural abundance ($r = 0.54$), indicating that the pathway of nitrogen has been from the leaf (source) to the mucilage (sink). The generally large values of the mucilage ^{15}N natural abundance (- 1.25 to 3.92) imply that nitrogen in the mucilage has come from the soil (Boddey et al., 2001; Van Deynze et al., 2018), although the mucilage exudation rate was positively correlated with the mucilage ^{15}N natural abundance ($r = 0.45$). A plausible reason for the lack of nitrogen fixation could have been the high soil nitrogen content in which the plants have preferred not to fix nitrogen from the atmosphere, because it is an energy-expensive way of attaining nitrogen and have instead taken it up from the soil through the above-mentioned processes. Van Deynze et al (2018) demonstrated that the crown root mucilage of the Sierra Mixe maize landrace provides 29-82% of the plant's nitrogen need when cultivated in the nitrogen-poor soil of the plant's region of origin. It would be interesting to evaluate the nitrogen fixation ability of modern hybrid maize varieties in soils with low nitrogen content in prospective studies.

Association of mucilage with plant water uptake

The highest leaf stomatal conductance (80.1 mmol m^{-2} s^{-1}) belonged to the variety DH02. Plant transpiration rate did not significantly differ between the varieties. Since the stomatal conductance and plant transpiration rate were not significantly correlated with the mucilage properties, other factors than mucilage could have controlled the plant hydraulics in the dry soil. For example, the high stomatal conductance of DH02 may have been caused by long roots and/or a high density of root hairs, which have assisted the plant to acquire more water. However, the similar transpiration rates of the varieties indicate that the higher stomatal conductance in DH02 compared to the other varieties could have been temporary and decreased later. It might have been a temporary strategy for the plant to cool itself down as a reaction to the dry soil.

The leaf stomatal densities were similar between the varieties but not correlated with the plant hydraulics, suggesting that the leaf stomatal density of maize does not change over breeding periods in different environmental conditions and also that stomatal density does not play any key role in water uptake by maize from dry soils. The visual inspection of the leaf stomata indicates that they have been almost closed at the time of sampling (Figure 5). It seems that the varieties have closed their stomata to prevent water loss in response to the loss of hydraulic conductivity in the dry soil (Carminati and Javaux, 2020).

Unlike the results of modelling studies with artificial setups (mixing mucilage with soil) and artificial roots on the function of mucilage for root water uptake in drying soils (e.g., Young 1995; Carminati et al., 2010; Ahmed et al., 2015), our results could not prove such a function. Why could not mucilage facilitate root water uptake in the dry soil? To answer this question, we should look at the root water uptake process, which is through the water potential gradient in the rhizosphere. In a wet soil, water moves from the soil to the roots, due to the higher water potential in the soil than in the roots. In the dry soil, this may not have happened, because the water potential in the bulk soil is lower than the water potential in the rhizosphere. In fact, there has not been that much water in the dry soil to move toward the roots based on the water potential gradient law. Even though the mucilage could have enhanced the water content, liquid phase connectivity, and water-holding capacity of the drying soil (Carminati et al., 2011; Carminati and Vetterlein, 2013; Kroener et al., 2014; Benard et al., 2019), it may not necessarily mean that more water would be taken up by the roots and plant. However, to be more conclusive, stomatal conductance and transpiration rate should be measured at several steps during soil drying. For instance, the 30% soil water-holding capacity in the present study might have not been enough to induce a mucilage-associated plant response to the dry soil. The performance of similar studies but with gradually drying soils (i.e., using the root pressure chamber method) could further reveal the function of mucilage for plant hydraulics under various soil water potentials. Furthermore, more maize varieties and also other plant species (i.e., C_3 plants) have to be used in future studies, due to the variability of other traits (i.e., root length, root hairs, etc.).

Conclusions

The exudation rate and water-holding capacity of root mucilage differed between the drought-resistant and drought-susceptible maize varieties, indicating the potential genetic basis of these traits. Nonetheless, we recommend the use of more varieties grown in different environmental conditions to assess the heritability of these traits. Based on the already discovered functions of mucilage, breeding in dry conditions and also in microbially rich soils seem to induce a high mucilage exudation rate in maize. The high mucilage water-holding capacities of the drought-resistant maize varieties imply the specific role of mucilage water-holding capacity in drought resistance, i.e., in keeping the rhizosphere wet for the survival of the rhizosphere microorganisms and plant under drought.

In contrast to the results of the studies performed with artificial setups mixing mucilage and soil (without plant), our results do not support the idea that mucilage facilitates plant water uptake from dry soils. However, plants capable of exuding a mucilage that holds huge amounts of water can take up more nitrogen from dry soils. This implies the importance of mucilage water-holding capacity as a belowground trait for maintaining the uptake of nutrients from drying soils. Thus, we recommend that plant breeders take root mucilage water-holding capacity into account in their breeding programs for sustaining plant nutrient uptake and productivity in dry environments.

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III Other independent publications during the Ph.D. studies

Study 6: Impacts of logging-associated compaction on forest soils: A meta-analysis

Meisam Nazari[1], Mohammad Eteghadipour[2], Mohsen Zarebanadkouki[3], Mohammad Ghorbani[4], Michaela A. Dippold[1], Nataliya Bilyera[5,6] and Kazem Zamanian[7,8]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[2]Department of Water and Soil, Faculty of Agricultural Engineering, Shahrood University of Technology, Shahrood, Iran,

[3]Chair of Soil Physics, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany,

[4]Department of Agroecosystems, Faculty of Agriculture, University of South Bohemia, Ceske Budejovice, Czechia

[5]Department of Soil and Plant Microbiome, Institute of Phytopathology, Christian-Albrechts University of Kiel, Kiel, Germany,

[6]Department of Soil Science, Institute for Plant Nutrition and Soil Science, Christian-Albrechts University Kiel, Kiel, Germany,

[7]Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany,

[8]School of Geographical Sciences, Nanjing University of Information Science and Technology, Nanjing, China

Correspondence: meisam.nazari@forst.uni-goettingen.de, meisam.nazari1991@gmail.com.

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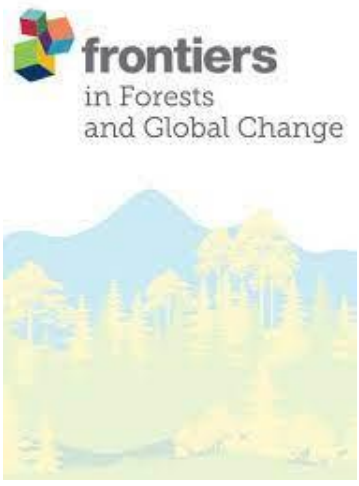

Abstract

Soil compaction associated with mechanized wood harvesting can long-lastingly disturb forest soils, ecosystem function, and productivity. Sustainable forest management requires precise and deep knowledge of logging operation impacts on forest soils, which can be attained by meta-analysis studies covering representative forest datasets. We performed a meta-analysis on the impact of logging-associated compaction on forest soils microbial biomass carbon (MBC), bulk density, total porosity, and saturated hydraulic conductivity (Ksat) affected by two management factors (machine weight and passage frequency), two soil factors (texture and depth), and the time passed since the compaction event. Compaction significantly decreased soil MBC by -29.5% only in subsoils (>30 cm). Overall, compaction increased soil bulk density by 8.9% and reduced total porosity and Ksat by -10.1 and -40.2%, respectively. The most striking finding of this meta-analysis is that the greatest disturbance to soil bulk density, total porosity, and Ksat occurs after very frequent (>20) machine passages. This contradicts the existing claims that most damage to forest soils happens after a few machine passages. Furthermore, the analyzed physical variables did not recover to the normal level within a period of 3–6 years. Thus, altering these physical properties can disturb forest ecosystem function and productivity, because they play important roles in water and air supply as well as in biogeochemical cycling in forest ecosystems. To minimize the impact, we recommend the selection of suitable logging machines and decreasing the frequency of machine passages as well as logging out of rainy seasons especially in clayey soils. It is also very important to minimize total skid trail coverage for sustainable forest management.

Keywords: soil compaction, forest soils, logging, microbial biomass carbon, soil physical properties

Study 7: Spatial and temporal resolution improvement of actual evapotranspiration maps using Landsat and MODIS data fusion

Hamid Salehi[1], Ali Shamsoddini[2], Seyed Majid Mirlatifi[1], Behnam Mirgol[3] and Meisam Nazari[4]

[1]Department of Water Engineering, Tarbiat Modares University, Tehran, Iran

[2]Department of Remote Sensing and GIS, Tarbiat Modares University, Tehran, Iran

[3]Center for Agroecology, Water and Resilience, Coventry University, Coventry, United Kingdom

[4]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

Correspondence: meisam.nazari@forst.uni-goettingen.de

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Abstract

Producing daily actual evapotranspiration (ET_a) maps with high spatial resolution has always been a challenge for remote sensing research. This study assessed the feasibility of producing daily ET_a maps with a high spatial resolution (30 m) for the sugarcane farmlands of Amir Kabir Sugarcane Agro-industry (Khuzestan, Iran) using three different scenarios. In the first scenario, the reflectance bands of Landsat 8 were predicted from the moderate resolution imaging spectroradiometer (MODIS) imagery using the spatial and temporal adaptive reflectance fusion model (STARFM) algorithm. Also, the thermal bands of Landsat 8 were predicted by the spatiotemporal adaptive data fusion algorithm for temperature mapping (SADFAT). Then, ET_a amounts were calculated employing such bands and the surface energy balance algorithm for land (SEBAL). In the second scenario, the input data needed by SEBAL were downscaled using the MODIS images and different methods. Then, using the downscaled data and SEBAL, daily ET_a amounts with a spatial resolution of 30 m were calculated. In the third scenario, ET_a data acquired by MODIS were downscaled to the scale of Landsat 8. In the second and third scenarios, downscaling of the data was carried out by the ratio, regression, and neural networks methods with two different approaches. In the first approach, the Landsat image on day 1 and the relationship between the two MODIS images on day 1 and the other days were used. In the second approach, the simulated image on the previous day and the relationship between the two consecutive images of MODIS were used. Comparing the simulated ET_a amounts with the ET_a amounts derived from Landsat 8, the first scenario had the best result with an RMSE (root mean square error) of 0.68 mm day^{-1}. The neural networks method used in the third scenario with the second approach had the worst result with an RMSE of 2.25 mm day^{-1}, which was however a better result than the ET_a amounts derived from MODIS with an RMSE of 3.19 mm day^{-1}. The method developed in this study offers an efficient and inexpensive way to produce daily ET_a maps with a high spatial resolution. Furthermore, we suggest that STARFM and SADFAT algorithms have acceptable accuracies in the simulation of reflectance and thermal bands of Landsat 8 images for homogeneous areas.

Keywords: data fusion, evapotranspiration, Landsat, MODIS, remote sensing

Study 8: Climate change impact assessment and adaptation strategies for rainfed wheat in contrasting climatic regions of Iran

Meisam Nazari[1], Behnam Mirgol[2] and Hamid Salehi[3]

[1]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[2]Center for Agroecology, Water and Resilience, Coventry University, Coventry, United Kingdom

[3]Department of Water Engineering, Tarbiat Modares University, Tehran, Iran

Correspondence: meisam.nazari@forst.uni-goettingen.de, meisam.nazari1991@gmail.com.

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Abstract

This is the first large-scale study to assess the climate change impact on the grain yield of rainfed wheat for three provinces of contrasting climatic conditions (temperate, cold semi-arid, and hot arid) in Iran. Five integrative climate change scenarios including +0.5◦C temperature plus-5% precipitation, +1◦C plus-10%, +1.5◦C plus-15%, +2◦C plus-20%, and +2.5◦C plus-25% were used and evaluated. Nitrogen fertilizer and shifting planting dates were tested for their suitability as adaptive strategies for rainfed wheat against the changing climate. The climate change scenarios reduced the grain yield by -6.9 to -44.8% in the temperate province Mazandaran and by -7.3 to -54.4% in the hot arid province Khuzestan but increased it by +16.7% in the cold semi-arid province Eastern Azarbaijan. The additional application of +15, +30, +45, and +60 kg ha^{-1} nitrogen fertilizer as urea at sowing could not, in most cases, compensate for the grain yield reductions under the climate change scenarios. Instead, late planting dates in November, December, and January enhanced the grain yield by +6 to +70.6% in Mazandaran under all climate change scenarios and by +94 to +271% in Khuzestan under all climate change scenarios except under the scenario +2.5◦C temperature plus-25% precipitation which led to a grain yield reduction of -85.5%. It is concluded that rainfed wheat production in regions with cold climates can benefit from the climate change, but it can be impaired in temperate regions and especially in vulnerable hot regions like Khuzestan. Shifting planting date can be regarded as an efficient yield-compensating and environmentally friendly adaptive strategy of rainfed wheat against the climate change in temperate and hot arid regions.

Keywords: climate change, nitrogen, planting date, precipitation, rainfed wheat, temperature

Study 9: Past and future drought trends, duration, and frequency in the semi-arid Urmia Lake Basin under a changing climate

Behnam Mirgol[1], Meisam Nazari[2], and Hadi Ramezani Etedali[1], Kazem Zamanian[3]

[1]Department of Water Engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran

[2]Division of Biogeochemistry of Agroecosystems, Georg-August University of Göttingen, Göttingen, Germany

[3]Department of Soil Science of Temperate Ecosystems, Georg-August University of Göttingen, Göttingen, Germany

Correspondence: meisam.nazari@forst.uni-goettingen.de

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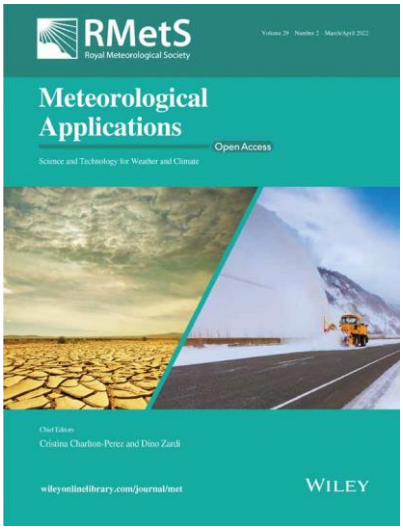

Abstract

Although the Urmia Lake has undergone remarkable drought conditions within the past decades mainly due to climate change, drought studies covering the entire Urmia Lake Basin and all drought aspects are lacking. The present study investigated the spatial and temporal drought conditions in the Urmia Lake Basin for the past (1988–2017) and future (2021–2050 and 2051–2080) periods using five general circulation models (GCMs) under the IPCC (Intergovernmental Panel on Climate Change) scenarios RCP 2.6, RCP 4.5, and RCP 8.5. The standardized precipitation index (SPI) and standardized precipitation and evapotranspiration index (SPEI) were compared. The SPEI predicted more drought events than the SPI, and it seemed to be a more suitable drought index than the SPI for the basin. In general, the future periods would encounter less drought conditions in terms of significant drought trends and duration than the observed period under all scenarios, but the frequency of quarterly severe drought events in the future periods would be higher than in the observed period. Furthermore, the stations Urmia (western bank) and Tabriz and Maragheh (eastern banks) would face the highest frequency of different types of quarterly drought events in the future periods compared with the observed period. The predicted high frequencies of drought events for the future periods can intensify the current low water level situation of the Urmia Lake, which seriously threatens all types of ecosystems in the basin. Therefore, serious actions need to be taken into account for efficient ecosystem and water resources management in the basin.

Keywords: drought, LARS-WG, precipitation, SPEI, SPI, temperature

Study 10: Soil organic matter mobilization by re-compaction of old forest skid trails

Meisam Nazari[a,b], Malte Horvat[a], Rainer Georg Joergensen[b], Stephan Peth[a]

[a]Soil Science, University of Kassel, Nordbahnhofstr. 1a, 37213, Witzenhausen, Germany

[b]Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213, Witzenhausen, Germany

Correspondence: malte.horvat@uni-kassel.de

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Abstract

Wood harvesting is restricted to a system of permanent skid trails in order to minimize the soil disturbance and damage. Therefore, it is not the compaction of previously undisturbed forest soil, but the re-compaction of already existing skid trails that is of practical relevance when investigating machinery-induced wood harvest effects on soil properties. This study investigated the effects of machinery-induced re-compaction on soil physical, chemical, and microbiological properties of an old skid trail in a spruce forest by wheeling 8 times with a maximum total load of 32.2 Mg, using a genuine old skid trail as a control. Re-compaction significantly increased the bulk density and reduced the porosity, whereas the soil organic carbon (SOC) and total nitrogen (N) contents were not significantly affected. However, re-compaction reduced the SOC/total N ratio by 10%, suggesting considerable SOC mineralization after re-compaction. K2SO4 extractable C contents were increased by 94% at 0–3 cm and 67% at 7–10 cm depth after re-compaction. This led to 20% and 90% increased microbial biomass C/soil organic C (MBC/SOC) ratios at 0–3 cm and at 7–10 cm depth, respectively. In contrast, the ergosterol/MBC ratio was significantly decreased by 10% at 0–3 cm and by about 30% at 7–10 cm depth by re-compaction, apparently due to the promotion of bacteria and mobilization of soil organic matter.

Keywords: soil compaction, porosity, extractable C, microbial biomass, ergosterol, skid trail

Curriculum Vitae

PERSONAL DETAILS:

Date of birth: 19.11.1991 Tel: 0049-01781758092

Gender: Male E-mail: meisam.nazari@forst.uni-goettingen.de

Marital Status: Single Postal Address: Gosslerstrasse 13/24, 37073 Göttingen, Germany

Nationality: Iranian

EDUCATION:

M.Sc. in Sustainable Agriculture (Specialization: Organic Agriculture), October 2016-February 2019, Georg-August University of Göttingen-University of Kassel, joint degree, Germany, GPA: 2.3 (good)

Thesis: Effect of tillage and compost on physical properties and water fluxes of a loess soil in Neu-Eichenberg, Germany, Grade: 2.3 (good)

Supervisor: Prof. Dr. Stephen Peth Advisor: Assistant Prof. Dr. Daniel Uteau

B.Sc. Agricultural Engineering (Specialization: Agronomy), October 2010–July 2014, Shahrood University of Technology, Iran, GPA: 14.97/20

Thesis: Breaking the seed dormancy of important rangeland plants by ultrasonic waves, Grade: 19.50/20

Supervisor: Associate Prof. Dr. Hamid Reza Asghari

EMPLOYMENT HISTORY:

October-2019 to November 2022, Department of Biogeochemistry of Agroecosystems, Faculty of Agricultural Sciences, Georg-August University of Göttingen, Göttingen, Germany

Ph.D. student

Main activities: Conducting research on the biogeochemical functions of maize mucilage in the rhizosphere

July-2019 to October-2019, Department of Biogeochemistry of Agroecosystems, Faculty of Agricultural Sciences, Georg-August University of Göttingen, Göttingen, Germany

Research assistant

Main activities: Conducting research on the polysaccharide and phospholipid composition of maize mucilage

November-2018 to January-2019, Department of Research and Development, KWS SAAT, Germany

Researcher

Main activities: Testing ultrasound treatment for pathogenic disinfection and dormancy breaking of sugar beet seeds

March-2018 to October-2018, Department of Grassland Science and Renewable Plant Resources, Faculty of Organic Agricultural Sciences, University of Kassel, Witzenhausen, Germany

Student research assistant

Main activities: Digitalizing and classifying the satellite images of agricultural lands of Bangalore (India) by the QGIS software for evaluating the urbanization impacts

March-2017 to March-2018, Department of Soil Science, Faculty of Organic Agricultural Sciences, University of Kassel, Witzenhausen, Germany

Student research assistant

Main activities: Conducting research on the soil trafficability of old skid trails in a spruce forest (assessing the soil physical, hydrological, and microbiological properties)

December-2016 to March-2017, Department of Organic Plant Production and Agroecosystems Research, Faculty of Organic Agricultural Sciences, University of Kassel, Witzenhausen, Germany

Student research assistant

Main activities: Conducting research on the dormancy breaking of wild yam species by ultrasonic waves

RESEARCH INTERESTS:

Soil-plant interactions/ rhizosphere/ soil physics/ soil and plant ecology/ drought resistance

CITATION STATISTICS:

Researchgate: https://www.researchgate.net/profile/Meisam_Nazari4

Google Scholar: https://scholar.google.com/citations?user=v2Q7KL0AAAAJ&hl=en

Publons: https://publons.com/researcher/3019633/meisam-nazari

ORCID .https://orcid.org/0000-0003-0122-3065

THE MOST IMPORTANT PUBLICATIONS:

Nazari, M.*, Bickel, S., Benard, P., Mason-Jones, K., Carminati, A., Dippold, M.A. (2022), "Biogels in soils: plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat'', Frontiers in Plant Science, 12, 798992.

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Nazari, M., Sharififar, A., Asghari, H.R. (2014), "Medicago Scutellata seed dormancy breaking by ultrasonic waves", Plant Breeding and Seed Science, Vol. 69, No. 1, pp. 15-24.

CONFERENCE PAPERS/ABSTRACTS:

Nazari, M.*, Bickel, S., Benard, P., Mason-Jones, K., Carminati, A., and Dippold, M. A. (2022), "Biogels in the rhizosphere: Plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat", EGU General Assembly, 23–27 May, Vienna, Austria.

Nazari, M.*, Sadeghianfar, P., Canaani, Z., Eteghadipour, M. (2017), "Water absorption behavior of barley seed cells is affected by ultrasonic waves", 16th Euro Global Summit on Food and Beverages, 02-04 March, Amsterdam, Netherlands.

REFEREE FOR INTERNATIONAL JOURNALS:

Land Degradation and Development/ Rhizosphere/ Agronomy/ Agriculture

GRANTS AND FELLOWSHIPS:

2019 to 2022, German Federal Environmental Foundation (DBU) Ph.D. scholarship, Germany (scholarship and consumables)

COMPUTER SKILLS:

Microsoft Office/ R/ SPSS/ SigmaPlot/ Hydrus 1-D/ APSIM/ GIS/ Meta-data Analysis

LANGUAGE PROFICIENCY:

Fluent: English, Persian, Azerbaijani Fair: German, Turkish Basic: Arabic

PROFESSIONAL MEMBERSHIPS:

European Geosciences Union (EGU)

Declaration

I hereby declare, to the best of my knowledge and belief, that this thesis contains no material previously published or written by another person, except where due reference has been made in the text of the thesis. This thesis contains no material which has been accepted or definitely rejected for the award of any other doctoral degree at any university.

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