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RESEARCH ARTICLE

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

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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

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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 drought-susceptible 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.

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)

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

TOC total organic carbon, *TN* total nitrogen, *MBC* microbial biomass carbon, *WHC* water-holding capacity

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 (Fig. 1). On average, the German loam soil led to 73.7% higher mucilage exudation rate than the Kenyan sandy-clay loam soil (Fig. 1). The Kenyan maize variety DH02 exuded 58.2% more mucilage compared to the German variety Kentos (Fig. 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 (Fig. 1).

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 (Fig. 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 (Fig. 2B). The proportions of hexoses, pentoses, galactose, fucose, mannose, glucose, arabinose, and

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 (%)

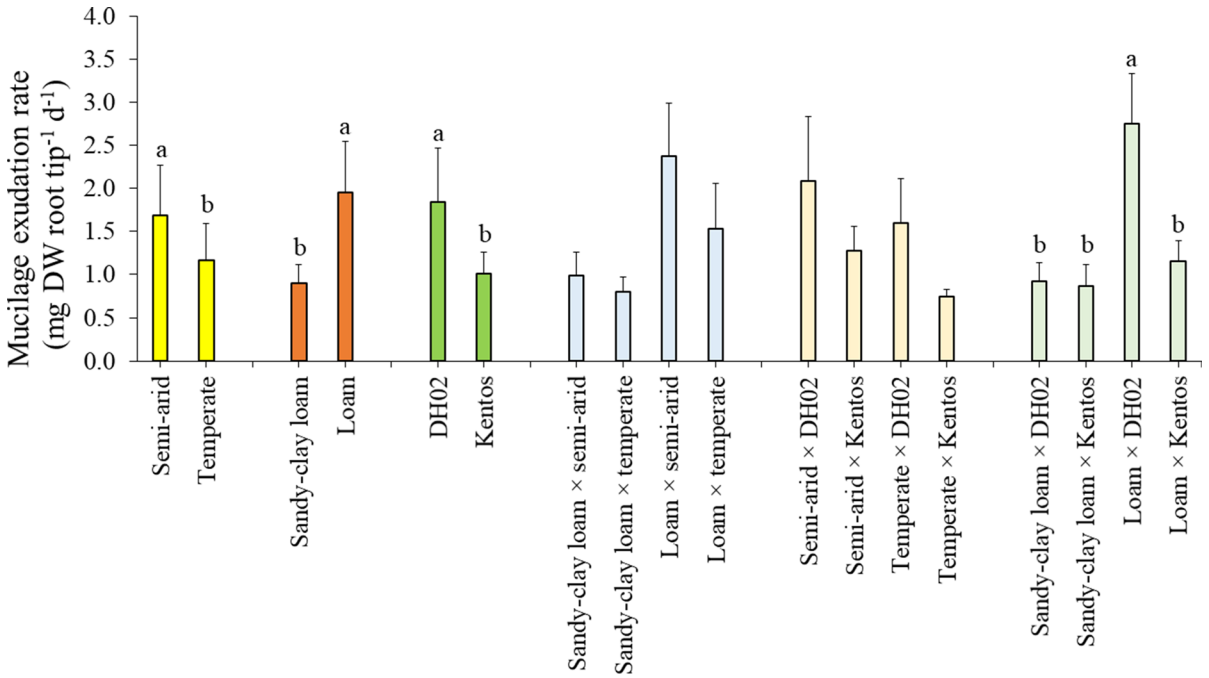

Fig. 1 Effect of the factors climate, soil, variety, and their interactions on the mucilage exudation rate of maize aerial nodal roots. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. 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

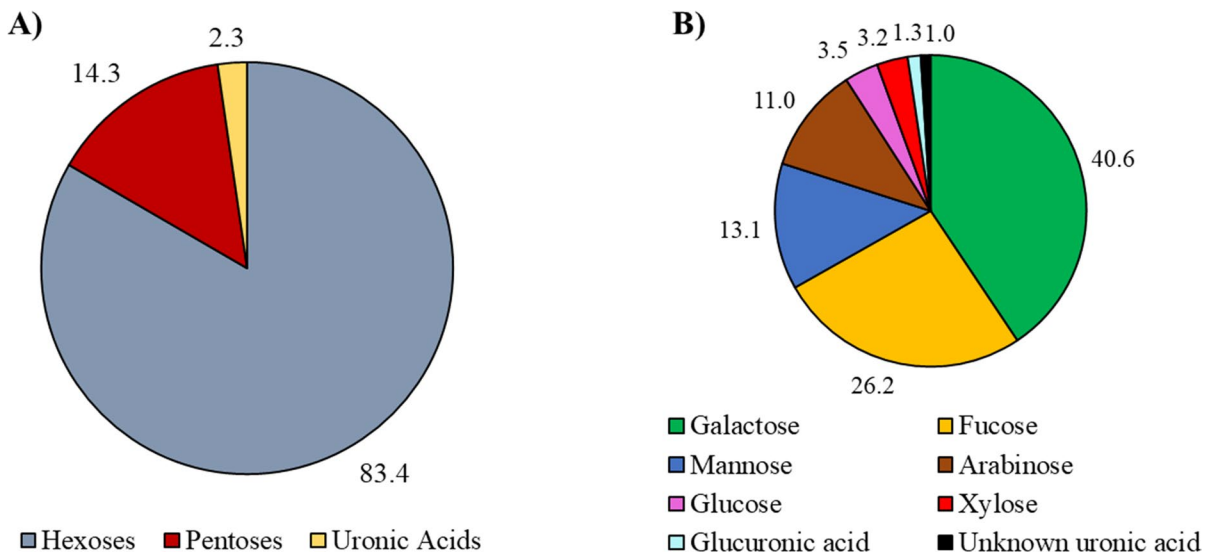

Fig. 2 Average proportion (in %) of the hexoses, pentoses, uronic acids (**A**) and each monomer (**B**) in the nodal root mucilage of the maize varieties DH02 and Kentos ($n = 32$)

xylose were neither significantly affected by climate, soil, and variety nor by their interactions (Table 2).

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 semi-arid tropical climatic conditions resulted in 0.51% greater uronic acid proportion in the exuded mucilage than the German humid temperate climatic conditions (Fig. 3). The Kenyan sandy-clay loam soil induced 1.3% more uronic acids in the mucilage in comparison with the German loam soil (Fig. 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 (Fig. 3).

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 (Fig. 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 (Fig. 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 (Fig. 5).

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

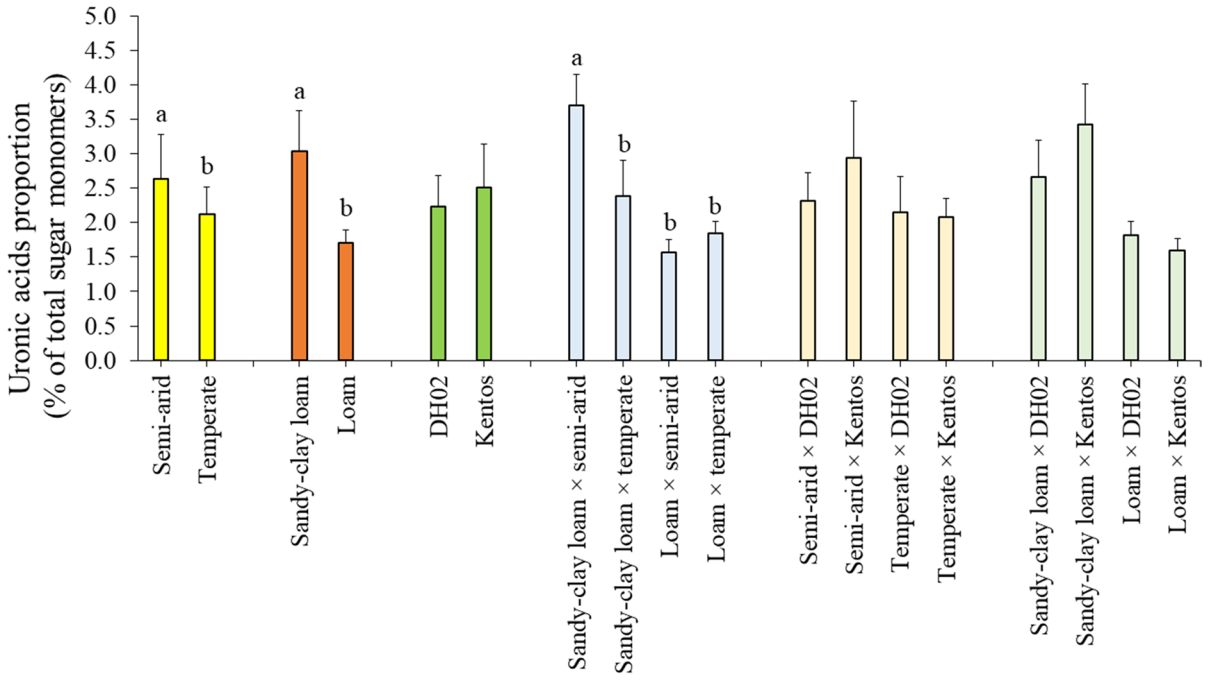

Fig. 3 Effect of the factors climate, soil, variety, and their interactions on the proportion of uronic acids in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error 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

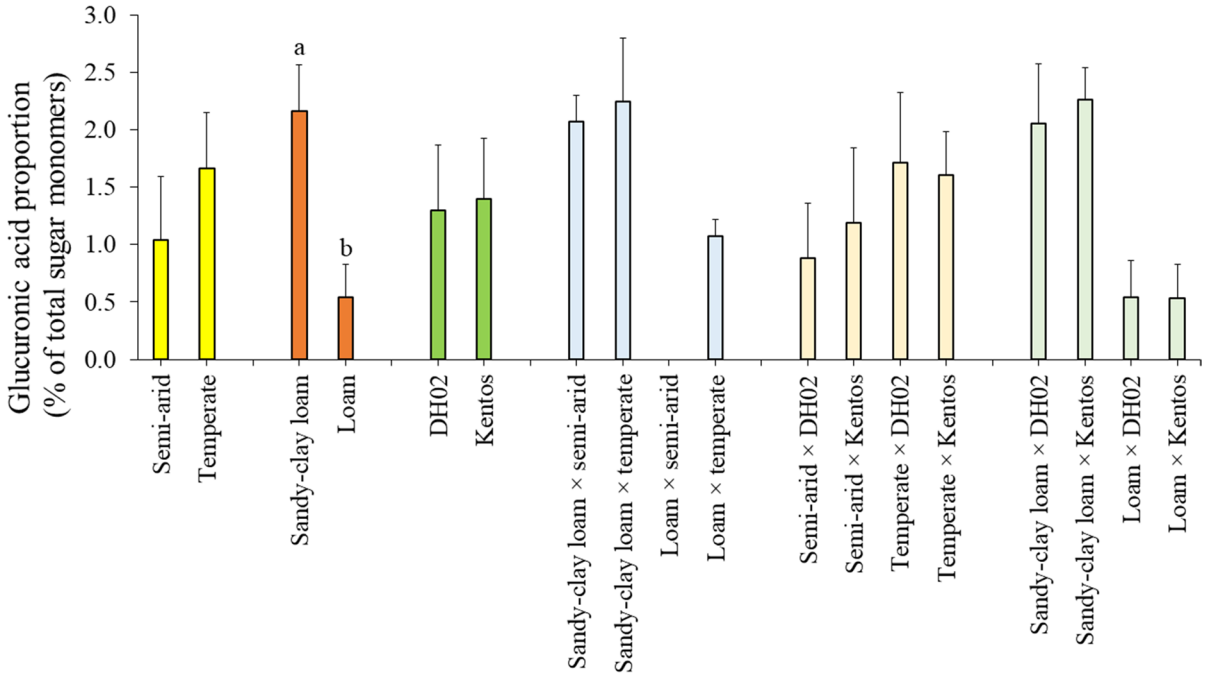

Fig. 4 Effect of the factors climate, soil, variety, and their interactions on the proportion of glucuronic acid in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard error of the mean. 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

(Fig. 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

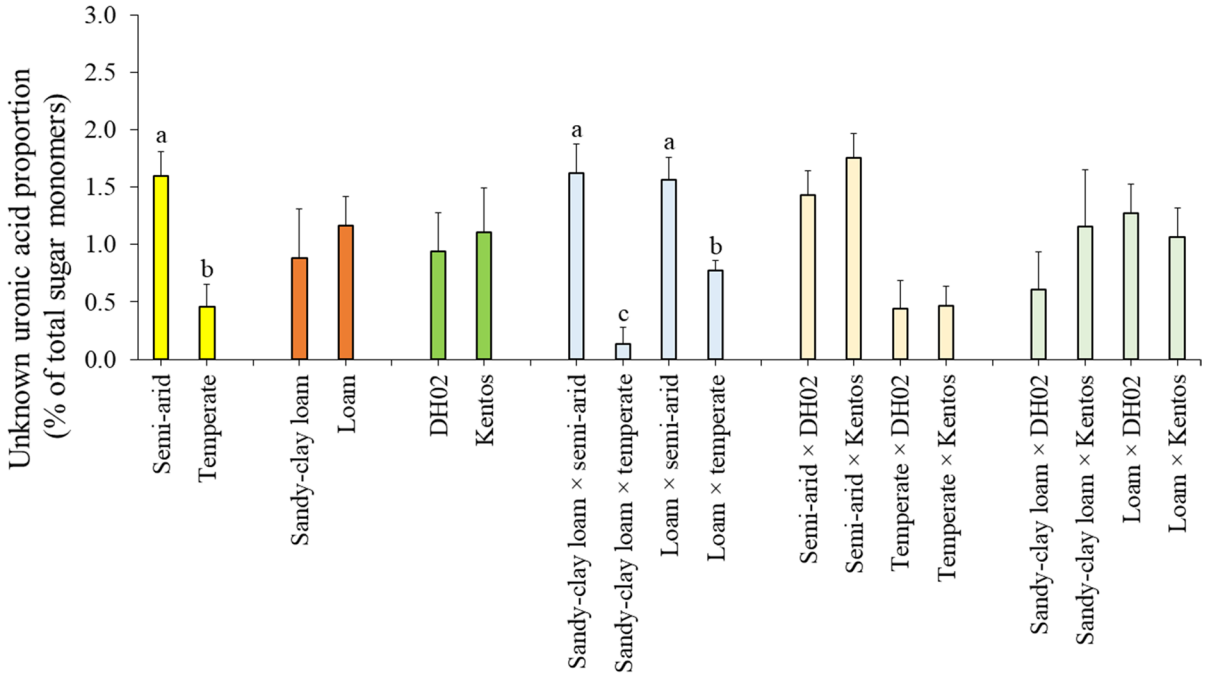

Fig. 5 Effect of the factors climate, soil, variety, and their interactions on the proportion of an unkown uronic acid in maize aerial nodal root mucilage. Different letters on each bar indicate a statistically significant difference (Tukey's HSD, at $\alpha = 0.05$, $n = 4$). Error bars show the standard 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

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 (Fig. 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 (Fig. 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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Declarations

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