

Rhizosphere engineering: Innovative improvement of root environment



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ABSTRACT

The ability of roots to extract water and nutrients from soil depends on the biophysical properties of the rhizosphere, which are strongly influenced by mucilage secretion. The aim of this study was to introduce the concept of rhizoligands to engineer the biophysical properties of the rhizosphere. A rhizoligand is defined as an additive that increases the wettability of the rhizosphere and links the mucilage network to main intimate contact with the root surface. We hypothesize that rhizoligands: i) facilitate the rewetting of the rhizosphere during repeated drying and wetting cycles; ii) enhance rhizosheath formation; iii) increase enzyme activities in the rhizosphere; and iv) increase plant biomass.

A commercial surfactant was selected as the prototype rhizoligand to test the effect on the rhizosphere biophysical properties of white lupin grown in quartz sand and subjected to six drying-rewetting cycles. Half of the plants were irrigated with water and the other half with the rhizoligand solution. When plants were 50 days old, we measured: i) soil water content; ii) rhizosheath mass; iii) activity of selected enzymes; iv) carbon content in the rhizosphere; and v) plant biomass.

Rhizoligand increased rewetting rate of the rhizosphere after drying and subsequent rewetting, resulting in a greater soil water content. Rhizosheath formation was improved in plants irrigated with rhizoligand and sand particles attached to the roots increased by 1.64 times compared to plants irrigated with water. Activity of the enzymes chitinase, sulfatase, and β -glucosidase were 4, 7.9, and 1.5 times greater in the rhizosphere of plants irrigated with rhizoligand than in the rhizosphere of plants irrigated with water. Plant biomass was 1.2 fold greater in samples irrigated with rhizoligand solution than in samples irrigated with water.

We conclude that application of rhizoligand improves plant performance by influencing the water dynamics in the rhizosphere and the plant, increasing the mechanical stability of the rhizosheaths and increasing the enzyme activities in the rhizosphere. Such effects are probably triggered by the interaction between mucilage and the applied rhizoligand, which reduces mucilage swelling (possibly by cross-linking mucilage polymers) and thus by increasing its viscosity keeps the mucilage close to the root surface. We propose the rhizoligand concept as a strategy to engineer the rhizosphere properties and to improve plant tolerance to water shortage.

1. Introduction

Water shortage has strong direct and indirect adverse effects on plant growth and crop yield. As soils dry, the transport of water and nutrients to the roots becomes limited by the low hydraulic conductivity. As the soil dries further, roots shrink and air-filled gaps form between soil and roots consequently limiting water and nutrient flow toward the root surface (Nobel and Cui, 1992; McCully 1995; Carminati

et al., 2009).

During soil drying microbial activity decreases (Austin et al., 2004; Sanaullah et al., 2011). Since plant–microbial interactions play a central role in nutrient availability, soil drying has a further negative impacts on the nutrient availability and uptake by plants (Hamilton and Frank, 2001; Hermans et al., 2006; Landi et al., 2006).

Increasing evidence suggests that plants modify their surrounding soil environment, the rhizosphere, to better exploit water and nutrient

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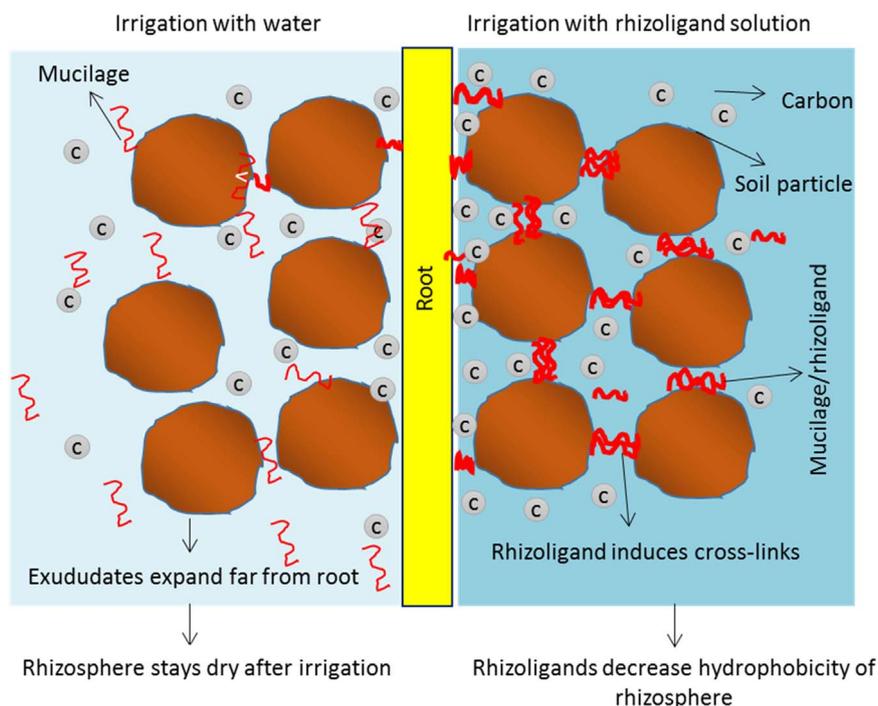


Fig. 1. Conceptual model of rhizoligand interactions with mucilaginous compounds secreted by roots in the rhizosphere. The interactions between rhizoligand and hydrophobic mucilage groups reduce mucilage swelling and increase its viscosity. The high viscosity of mucilage increases the binding between soil particles and the root surface. The right side of the root in the figure illustrates the effect of rhizoligand on linking mucilage polymers, whereas the left figure indicates the case of a root not treated with rhizoligands.

resources (Hinsinger et al., 2009; Spohn and Kuzyakov, 2013). Plants release a significant portion of their photosynthetic products into the soil as root exudates through the process of rhizodeposition (Jones et al., 2009; Pausch et al., 2013). The constituents of these exudates include low-molecular-weight compounds, such as sugars, amino acids, and organic acids (Fischer et al., 2010), and high-molecular-weight compounds, such as mucilage a biopolymer.

Mucilage alters the physical properties of the rhizosphere (Watt et al., 1994; Young, 1995; Carminati et al., 2010; Moradi et al., 2011) and nutrient availability (Richardson et al., 2001; Belfort et al., 2007; Miransari, 2013). Upon secretion from the root tip, mucilage penetrates into the soil pore spaces and coats the soil particles. As the soil dries, mucilage partly dehydrates, increasing its viscosity and binds the soil particles together keeping them in contact with the roots (Albalasmeh and Ghezzehei, 2014). This biopolymer formed layer of soil particles adhering to the roots is commonly called the rhizosheath (Watt et al., 1994). Rhizosheath forms at the root surface of many plant including many grasses and agriculturally important crops such as maize, sorghum, wheat, and barley (McCully, 1999; Delhaize et al., 2012; George et al., 2014). Rhizosheath formation is strongly correlated to length and density of root hairs (Delhaize et al., 2012). The volume and stability of the rhizosheath depends on the plant species, the number of drying and rewetting cycles and sources of secreted mucilage (Nambiar, 1976; Watt et al., 1993, 1994; George et al., 2014). Rhizosheaths of grasses formed under dry conditions are larger, more coherent, and more strongly bound to the roots than those formed in wet soils (Watt et al., 1994, 1993). Under dry conditions, mass of rhizosheath adhered to the roots of certain grasses was approximately three times bigger than the rhizosheath adhered to the roots of same plants grown in wet conditions (Watt et al., 1994, 1993). The authors hypothesized that drying and wetting cycles i) may stabilize mucilage close to the root surface by inducing new cross links in its network ii) and may also alter the quantity and quality of root and microbial exudates (Watt et al., 1994; McCully, 1999).

Several important functions for water and nutrient uptake have been attributed to the rhizosheath, particularly under water stress

condition, including: i) maintaining the contact between roots and soil during drying (Watt et al., 1993; Young, 1995; North and Nobel, 1997); ii) keeping soil next to the roots wetter than the bulk soil, possibly a means of maintaining the hydraulic connection between the roots and soil (Watt et al., 1993; Young, 1995; Carminati et al., 2010, 2011; Moradi et al., 2011); and iii) providing a favorable habitat for microbial activity due to larger soil moisture and carbon content around the roots (Drenovsky et al., 2004; Pausch and Kuzyakov, 2011; Kuzyakov and Blagodatskaya, 2015).

Moradi et al. (2012) found that the contact angle was higher in the rhizosheath of lupin compared to the adjacent bulk soil. This corroborates neutron radiography experiments which showed that the rhizosphere becomes water repellent upon drying (Carminati et al., 2010; Zarebanadkouki et al., 2016). The development of hydrophobicity is probably a consequence of lipids in mucilage (Read et al., 2003). Hydrophobicity in the rhizosphere has been demonstrated to limit fluxes of water across the rhizosphere thus reducing root water uptake (Zarebanadkouki and Carminati, 2014).

Over the last decade, surfactants have been used to improve irrigation efficiency in hydrophobic soils and to increase water infiltration in the root zone (Franklin, 2007; Chaichi et al., 2015; Daneshnia et al., 2015; Jafarian et al., 2015). Density and quality of the turf grass exposed to water stress improved after soil treatment with a surfactant (Franklin, 2007). Although the application of surfactants to facilitate the rewetting of water repellent soils is a well-established practice, the mechanisms of surfactants' interactions with root exudates and mucilage have not been explored.

The aim of this study was to evaluate if rhizosphere properties could be engineered to optimize water and nutrient transport as well as microbial activity at the root-soil interface. To test this concept two areas were explored: i) is it possible to stabilize and maintain mucilage and other root exudates in the vicinity of the roots? and ii) does this engineered biopolymer facilitate the rewetting of the rhizosphere upon drying and wetting cycles? We tested whether a selected commercial surfactant (ACA1820, Aquatrols Corporation of America, Paulsboro, New Jersey, U.S.A) could act as a rhizoligand.

We define rhizoligand a substance that: i) decreases mucilage swelling and ii) facilitates the rewetting of the rhizosphere.

We hypothesize that the application of rhizoligands stabilizes mucilage at the root surface and stimulates rhizosheath formation. The underlying hypothesis is that by decreasing mucilage swelling, rhizoligands maintain mucilage close to the roots increasing the strength of the bonds between the root surface and the soil particles, enhancing rhizosheath formation. Furthermore, we hypothesize that the higher water content in the rhizosphere of plants treated with rhizoligands enhances microbial activity in the rhizosphere under drought.

Fig. 1 shows our conceptual model of the mode of action of rhizoligands. Rhizoligand and mucilage have hydrophilic and hydrophobic functional groups. In an aqueous environment, the hydrophilic heads of rhizoligand link to water molecules, whereas the hydrophobic heads associate with the hydrophobic mucilage groups. Such a concept is based on experiments with surfactants and polymeric gels having hydrophobic groups (Goddard, 1994; Hansson and Lindman, 1996). The interactions between hydrophobic and hydrophilic groups of rhizoligands and mucilage form additional bridges between the mucilage polymers limiting mucilage swelling and increasing its stability. After mixing with rhizoligand, mucilage in soil becomes more viscous and remains at greater concentration in the vicinity of the roots. According to Albalasmeh and Ghezzehei (2014) mucilage starts to bind soil particles when its viscosity is sufficiently high. By increasing mucilage viscosity, rhizoligand application is expected to improve binding of soil particles and therefore rhizosheath formation.

To test our concept, we first investigated the capability of a surfactant to reduce the swelling of mucilage. Then, the effects of rhizoligand on biophysical properties of the rhizosphere of lupins grown in quartz sand were evaluated. The plants were subjected to repeated drying and rewetting cycles and the following parameters were measured: i) the soil water content after rewetting; ii) the rhizosheath formation; iii) the enzyme activities and carbon content in the rhizosphere; and iv) plant biomass.

2. Materials and methods

2.1. Mucilage swelling

To test our conceptual model, we first measured mucilage swelling. We used mucilage from chia seeds (*Salvia hispanica*), which showed a physical behavior similar to that of mucilage from maize and lupin: it forms a gel upon immersion in water and turns hydrophobic upon drying (Kroener et al., 2014). However, mucilage from chia seed might differ from root mucilage. The physicochemical properties of root mucilage might depend on many factors, such as age and growing conditions, and they are likely to show large variations among plant species (Zickenrott et al., 2016).

Chia seeds were mixed with water at a ratio of 1–10 (g seeds/g water) and the mixture was stirred using a magnetic stirrer for 2 h. The mixture was passed through a series of sieves with size of 0.5 and then 0.2 mm by applying a suction of -800 hPa. Afterwards, 200 g of the extracted wet mucilage were placed in a large petri dish (20 cm in diameter) and they were let dry in a ventilated oven at a temperature of 40 °C. The initial concentration of mucilage was calculated as the dry mass of mucilage (oven dry) divided by the wet mass of mucilage and it was estimated to be 0.6%. The petri dish was covered with a thin layer of paraffin enabling us to easily remove the thin layer of dried mucilage. This procedure resulted in a relatively uniform layer of dried mucilage (with respect to the thickness). A small piece of dried mucilage (1 cm \times 2 cm) was weighted and immersed in water (control treatment) and in a selected rhizoligand solution (ACA1820, Aquatrols Corporation of America, Paulsboro, New Jersey, U.S.A) at a concentration of 1 mL of surfactant per liter of water. This prototype rhizoligand was used in all experiments. Mucilage adsorbed water and swelled until

it reached its maximum swelling capacity (which took approximately 2 days). The excess water was gently removed (i.e. it was poured through a coarse sieve) the swollen mucilage was collected and its water content was determined gravimetrically. We have replicated the measurements five times.

2.2. Plant and soil preparation

Seeds of white lupin (*Lupinus albus* L. cv. Feodora) were soaked in 10% H_2O_2 solution for 5 min and then they were thoroughly washed. The seeds were subsequently germinated in the darkness on moist filter papers for 2 days. The seedlings were planted in thin aluminum containers (28 cm width, 30 cm height, and 1 cm thickness) filled with quartz sand (particle size ranging from 50 to 250 μ m). The quartz sand was packed to a bulk density of 1.4 g cm^{-3} . The containers were filled homogeneously, while they were laid horizontally and the sand was passed through a sieve with mesh size of 2 mm in order to reduce soil layering. The germinated seeds were planted at a depth of 1 cm into the containers (one seed per container) and plants were transferred to a climate chamber under controlled conditions: a daily light cycle of 14 h and 10 h of darkness, a light intensity of 500 μ mol $m^{-2} s^{-1}$, day: night temperature of 24: 19 °C, and relative humidity of 60%.

During the first three days after planting, seedlings were irrigated daily from the top. After the shoots emerged, we covered the soil surface with 1 cm layer of gravel (ca. 2 mm in diameter) to minimize evaporation. The plants were subsequently irrigated by capillary rise on every fourth day. Containers were slowly immersed in 15 cm water table for one hour. The containers were then gently lifted, allowing free drainage of excessive water through the holes at the bottom of the container. This procedure resulted in an average soil water content (volume of water divided by the total soil volume) of 0.25 – 0.30 cm^3 cm^{-3} after irrigation.

When the plants were two weeks old and the sand had a water content of 0.27 – 0.30 cm^3 cm^{-3} , the first drying cycle was started. The irrigation was stopped and plants were let dry until reaching a water content of 0.04 – 0.05 cm^3 cm^{-3} (near wilting point). The plants were then divided into two groups: one group was irrigated with water and the second group with water containing rhizoligand (ACA1820) at a concentration of 0.05 g L^{-1} . The drying and rewetting cycles were repeated six times, with the plants being rewetted by capillary rise when they reached a water content of 0.04 – 0.05 cm^3 cm^{-3} . To determine soil water content during each cycle of drying, the containers were weighted every 12 h. The two treatments (water versus rhizoligand) were replicated four times.

2.3. Analysis of rhizosheath properties

After the six drying/wetting cycles, when the plants were approximately 50 days old, we let the plants dry until the sand reached a water content of 0.04 – 0.05 cm^3 cm^{-3} . Thereafter, we laid the containers horizontally and opened the detachable plate of each container. The whole root system and the sand adhering to the roots were removed from the sand and shaken gently to remove any loose sand. The sand adhering to the roots after shaking is referred to as rhizosheath.

We cautiously removed most of the roots from the upper part of the containers and placed them immediately in plastic bags to minimize evaporation and shrinkage of the roots. The root segments were spread on an A3 plexiglass tray of the WinRhizo flatbed scanner (Epson STD 4800) equipped with a double light source to avoid root overlapping. The images were acquired using the TWAIN interface at 800 dpi resolution.

Length, radius and volume of roots and rhizosheath were analyzed using the software WinRhizo 2008a image analysis system (Reagent Instruments Inc., Canada). The average radius of the rhizosheath plus root was calculated in treatments with and without rhizoligand. Note that we independently determined the thickness of roots after removing

the attached sand in water and water containing rhizoligand samples. For quantification of scanned images by WinRhizo, we first selected the root segments that had no lateral roots and applied a threshold filter to distinguish the root-rhizosheath from their backgrounds. Then, the segmented roots were skeletonized and the length of each root segment was calculated (Zarebanadkouki et al., 2016). WinRhizo gave the total surface area in each root-rhizosheath segment, A [cm^2]. Assuming that the root-rhizosheath had a cylindrical shape, the average thickness of the root-rhizosheath was calculated as

$$A = 2\pi rL \quad (1)$$

where L is length of the skeletonized root-rhizosheath segment [mm] and r is the radius of the segmented root-rhizosheath [mm].

In parallel, the mass of roots and rhizosheaths were gravimetrically determined as following: A soft brush was used to remove adhering sand particles (rhizosheath). Two grams of these collected rhizosheaths were used to assess carbon content and enzyme activity. Thereafter, to remove any remaining sand particles from root surface, roots were immersed in distilled water for 24 h and then adhering sand particles were removed by brushing them away. The sand particles were removed from roots and dried in an oven for 48 h at 104 °C. Then, their mass was gravimetrically determined and normalized for the dry mass of roots (Watt et al., 1994).

2.4. Carbon content analysis

The carbon content in one gram of the rhizosheath and bulk soil was analyzed by VarioMax CNS apparatus (VarioMax CNS, Elementar, Germany) according to the Dumas combustion method. The bulk soil was defined as the sand remaining in the sample containers after removal of the roots – i.e. the not-adhering sand (Chimento et al., 2016).

2.5. Enzyme assays

We measured the enzyme activity in the rhizosheath and in the bulk soil. The method to sample the rhizosheath is explained above. Extracellular enzyme activities were assayed using fluorogenically labeled substrates (Marx et al., 2005; Razavi et al., 2015). Four enzymatic activities were analyzed: (1) β -glucosidase, which is involved in carbon cycle; (2) Chitinase, which is involved in carbon and nitrogen cycle; (3) acid phosphatase, which is involved in phosphorus cycle; and (4) sulfatase, which involved in sulfur cycle. In order to assess enzyme activities in rhizosheath and bulk soil, four types of fluorogenic substrates based on 4-methylumbelliferone (MUF) were used (Table 1), (Stemmer et al., 1998; Koch et al., 2007). The MUF-substrates were dissolved in 2-methoxyethanol. Saturation concentrations of fluorogenic substrates were determined in preliminary experiments (Razavi et al., 2015). Pre-dissolved MUF substrates were further diluted with sterile universal buffer [MES ($\text{C}_6\text{H}_{13}\text{NO}_4\text{SNa}_{0.5}$)].

One gram of sand was mixed in 50 ml water for two minutes using a

Table 1
Description of the substrates for estimation of enzyme activities in the rhizosheath and bulk soil.

Enzyme	Substrate	Buffer
C-cycle enzymes		
β -glucosidase	4-methylumbiliferyl- β -D-glucopyranoside	MES ^a
N-cycle enzymes		
Chitinase	4-methylumbiliferyl-N-acetylglucosaminide	MES
P-cycle enzyme		
Acid phosphatase	4-methylumbiliferyl phosphate	MES
S-cycle enzyme		
Sulfatase	4-methylumbiliferyl sulfate potassium salt	Sodium acetate

^a MES: ($\text{C}_6\text{H}_{13}\text{NO}_4\text{SNa}_{0.5}$).

magnetic stirrer and low-energy sonication (40 J S^{-1} output energy) for two minutes (Stemmer et al., 1998; Koch et al., 2007). Subsequently, 50 μl of sand suspension was added to 150 μl of each test substrate solution (containing either 50 μl universal buffer) in a 96-well microplate (Puregrade, Germany) and incubated for 2 h. Fluorescence was measured in microplates at excitation wavelength of 355 nm, emission wavelength of 460 nm, slit width of 25 nm, with a Victor³1420-050 Multilabel Counter (PerkinElmer, USA).

Each enzyme was assayed in triplicate for each sample (bulk soil and rhizosheath of lupin). All assays were run at 20 °C. Enzyme activities were expressed as MUF release in nmol per g dry soil per hour ($\text{nmol MUF g}^{-1} \text{ soil h}^{-1}$), (Razavi et al., 2015).

2.6. Plant biomass measurement

At the end of the drying/wetting experiments, when the plants were approximately 50 days old, we collected roots and shoots. The dry weight of roots and shoots were determined gravimetrically. The roots were separated from the shoots and carefully all the soil particles attached to the roots were removed as previously described prior to weighing. Both roots and shoots were dried in oven for 24 h at 105 °C and then were weighted individually.

2.7. Statistical analysis

To evaluate statistical differences between two samples, *t*-test in the software R (version 3.3.2) was applied. The replicates were compared to determine if differences were statistically significant between plants irrigated with water and rhizoligand solution. Differences were reported to be significant at an error probability level of $p < 0.05$.

3. Results

3.1. Mucilage swelling

Maximum swelling of chia mucilage significantly decreased with the rhizoligand addition ($P < 0.05$) (Fig. 2). Rhizoligand reduced the final swelling of chia mucilage by a factor of 1.89 in comparison to water. One gram of dry mucilage adsorbed $272 \pm 18 \text{ g}$ of water and $144 \pm 14 \text{ g}$ of rhizoligand solution.

3.2. Wetting and drying cycle

The average soil water content shortly after irrigation was greater in the plants irrigated with the rhizoligand solution compared to water (Fig. 3). Irrigation via capillary rise resulted in an average soil water content of 0.26 ± 0.01 and $0.23 \pm 0.01 \text{ cm}^3 \text{ cm}^{-3}$ in plants irrigated with and without rhizoligand, respectively. The differences resulted mainly from the fact that the rhizoligand increased the wettability of the rhizosphere, as shown in Ahmed et al. (2017) using the same quartz sand, plant variety and rhizoligand. Note that the drying cycles for plants irrigated with rhizoligand solution were one to two days longer than the plants irrigated with water, confirming results previously attributed to lower transpiration rates (Ahmed et al., 2017).

3.3. Rhizosheath development

The rhizosheath of plants treated with rhizoligand were much thicker than those of the plants irrigated with water (Fig. 4). For better illustration of the differences, two roots with and without cluster roots are shown at higher resolution (Fig. 5). These figures were digitalized by WinRhizo scanner. The average radius of root and rhizosheath for the roots without cluster was $0.42 \pm 0.09 \text{ mm}$ in plants irrigated with water and $0.65 \pm 0.12 \text{ mm}$ in plants treated with the rhizoligand (Fig. 6a). The average thickness of roots and their rhizosheaths of cluster roots were $0.38 \pm 0.08 \text{ mm}$ and $0.63 \pm 0.16 \text{ mm}$, in plants

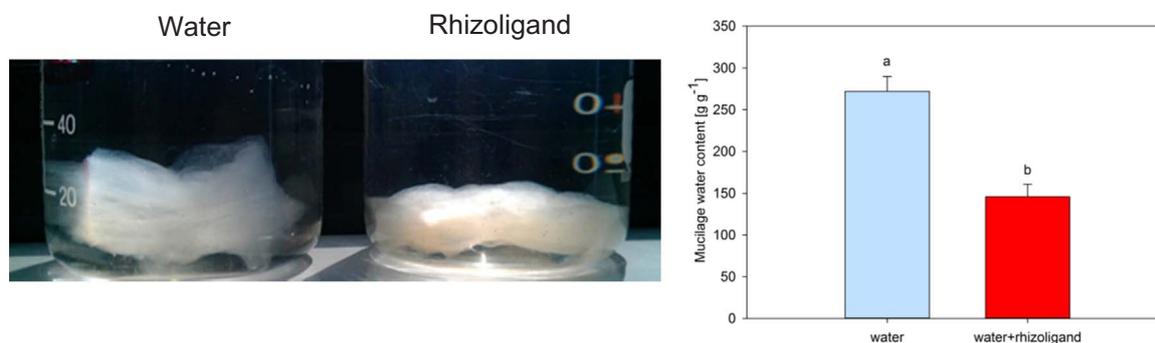


Fig. 2. Left: swelling of dried chia mucilage in water and rhizoligand solution. The mucilage was let hydrate for 48 h. Right: maximum swelling of dried mucilage in water and rhizoligand solution. Each value is the average of 5 replications. The rhizoligand decreased the maximum swelling of mucilage by a factor 1.9. Letters indicate significant difference at $P < 0.05$.

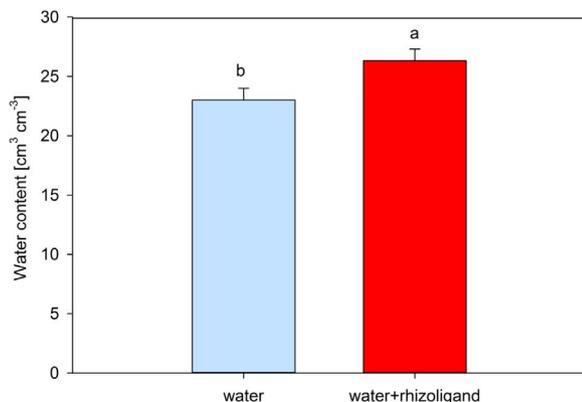


Fig. 3. Soil water content of a 30-day-old lupin shortly after irrigation with water and rhizoligand solution. The soil water content was measured gravimetrically by weighing. Each value is the average of four plants. Letters indicate significant difference at $P < 0.05$.

irrigated with water and rhizoligand, respectively (Fig. 6b). The root radius remains the same independent on rhizoligand addition: 0.31 ± 0.18 mm irrigated with water and 0.31 ± 0.14 mm irrigated with rhizoligand. In line with these, the mass of the rhizosheath per dry mass of roots was 11 ± 1.54 g g^{-1} in the water treatment and 18 ± 0.8 g g^{-1} in the rhizoligand treatment (Fig. 6c).

3.4. Carbon content and enzyme activities in the rhizosheath

The average carbon content in the rhizosheath of samples irrigated with water was 0.35 ± 0.04 mg g^{-1} and it was 0.47 ± 0.16 mg g^{-1} for the samples irrigated with the rhizoligand solution (Fig. 7a). The difference was not significant. The carbon content in bulk soil was significantly lower: it was 0.1 ± 0.01 mg g^{-1} in the bulk soil of samples irrigated with water and 0.13 ± 0.01 mg g^{-1} in the samples irrigated with the rhizoligand solution, respectively (Fig. 7a).

While average carbon content per gram of rhizosheath was not statistically different, plants in soils treated with rhizoligand produced a greater rhizosheath mass than plants irrigated with water. The total carbon content in the rhizosheath, calculated by multiplying the carbon content by the dry mass of rhizosheath, was 3.93 ± 1.04 mg C/g root in the samples irrigated with water while, it was 7.10 ± 0.92 mg C/g in plants irrigated with rhizoligand. Total carbon content accumulated in the rhizosheath of plants irrigated with the rhizoligand solution was 1.80 times greater ($P < 0.05$) than in the rhizosheath of plants irrigated with water (Fig. 7b).

Differences in the activity of three out of four enzymes in the rhizosheath were found in plants irrigated with rhizoligand as compared to those irrigated with water. Rhizosheath activity of three enzymes (chitinase, sulfatase and β -glucosidase) was significantly greater ($P < 0.05$) in the rhizosheath of plants irrigated with the rhizoligand solution than in plants irrigated only with water. In

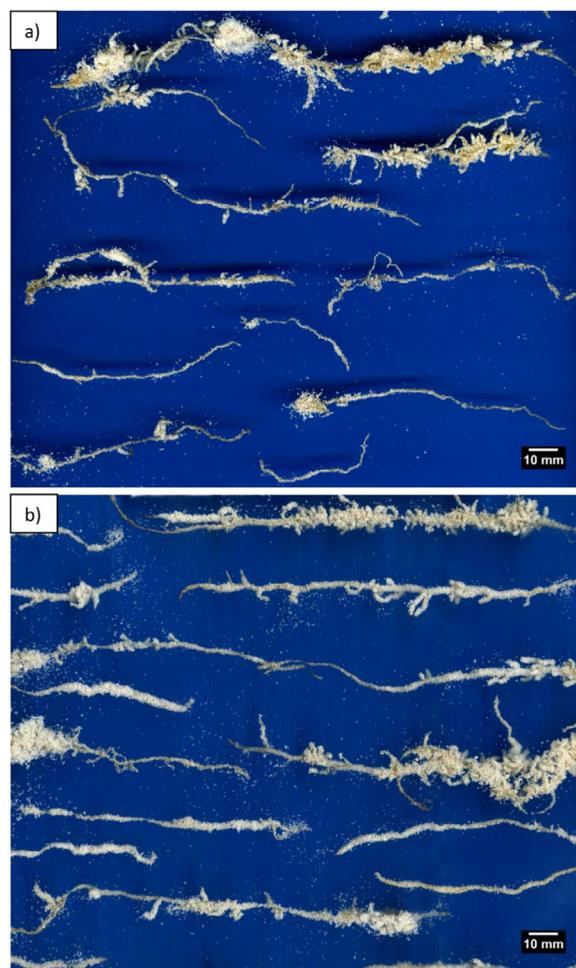


Fig. 4. Roots and surrounding rhizosheath scanned with WinRhizo for the plants irrigated with water (a) and water treated with rhizoligand (b). Thickness of rhizosheath in both selected roots with and without cluster roots was greater in plants irrigated with rhizoligand than with water.

contrast, the application of rhizoligand did not affected the activity of phosphatase in the rhizosphere (Fig. 8).

3.5. Plant biomass

The average shoot dry weight was 2.33 ± 0.23 g and 2.43 ± 0.16 g in plants irrigated with water and with rhizoligand solution, respectively (Fig. 9a). The average root dry weight was 3.36 ± 0.64 g and 4.33 ± 0.22 g in plants irrigated with water and with rhizoligand solution, respectively (Fig. 9a). Shoot dry weight did not appear to be influenced by rhizoligand treatment, however, rhizoligand treatment

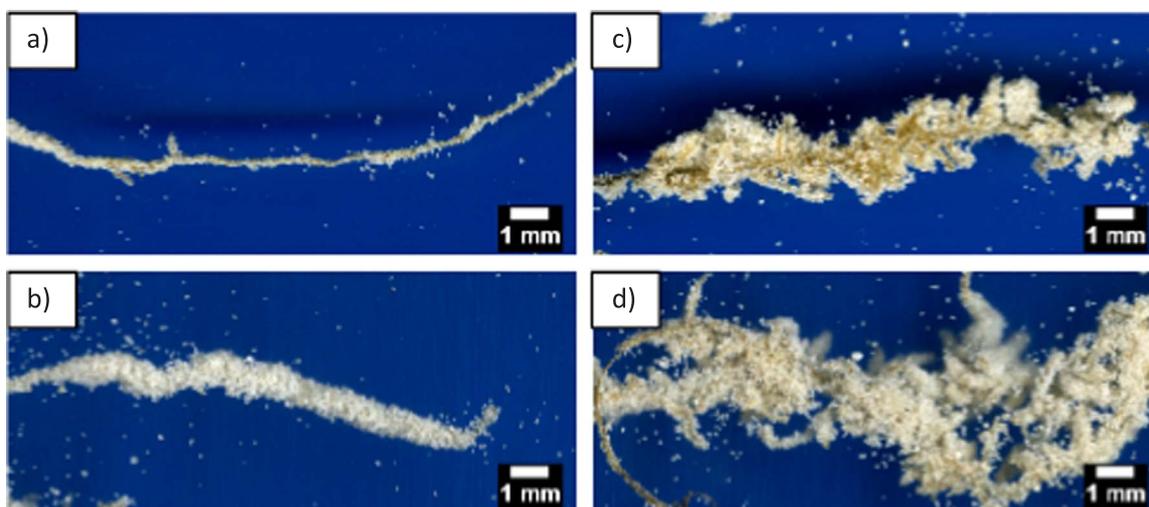


Fig. 5. Selected roots and surroundings rhizosheath scanned with WinRhizo for the plants irrigated with water (a, c) and with the rhizoligand solution (b, d). The figures show greater radius of rhizosheath in bare root (b) and cluster root (d) of plants irrigated with rhizoligand.

resulted in statistically significant increases in root dry weight ($P < 0.05$). Total plant biomass was 5.70 ± 0.46 g and 6.76 ± 0.23 g in samples irrigated with water and rhizoligand solution, respectively. Total plant biomass was 1.18 fold greater in the plants irrigated the rhizoligand treatment than in plants irrigated with water ($P < 0.05$) (Fig. 9b).

4. Discussion

The selected surfactant (ACA1820) acted as a rhizoligand: increasing the wettability of the rhizosphere and reducing mucilage swelling (according to the rhizoligand definition). These effects also resulted in thicker rhizosheaths, greater activity of selected enzymes and plant

biomass, particularly root mass.

The tested rhizoligand increased the soil water content in the rhizosphere after irrigation of dry samples (Fig. 3). Previous studies have shown that the rhizosphere of lupins grown in sandy soils becomes hydrophobic and as a consequence delays rewetting after drying (Moradi et al., 2012; Zarebanadkouki et al., 2016). Application of rhizoligand rewets the water repellent rhizosphere of lupins, as shown in Ahmed et al. (2017). As a consequence, the application of rhizoligand provides a greater volume of water available to the plants during repeated drying/wetting cycles. Interestingly, despite of the greater water content of rhizosphere, plants irrigated with rhizoligands transpired less and had a greater water use efficiency (Ahmed et al., 2017). Similar results were reported on water use efficiency by Jafarian et al.

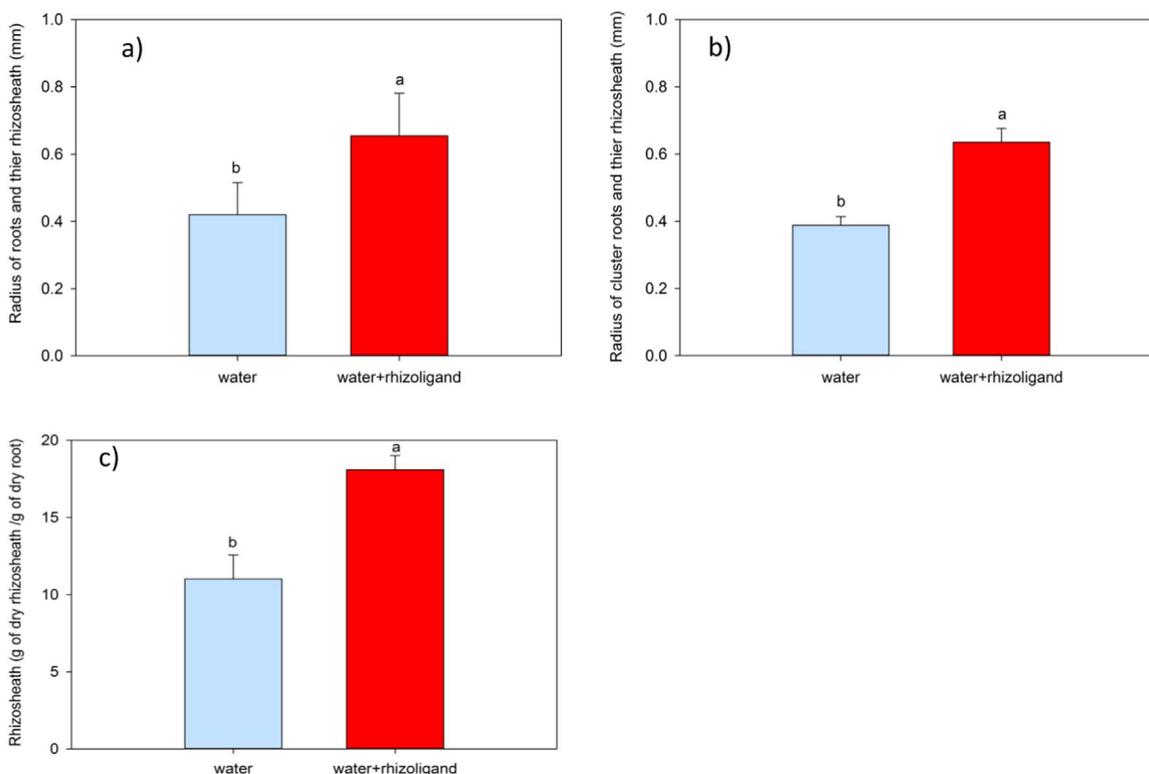


Fig. 6. Radius of roots and their rhizosheath calculated for bare roots (a) and cluster roots (b) of plants irrigated with water and rhizoligand solution. c) Dry mass of rhizosheath adhering to the entire root system of plants irrigated with water and rhizoligand (weights are normalized by dry mass of roots). The values are averages of four plants and lower case letters indicate a significant difference at $P < 0.05$.

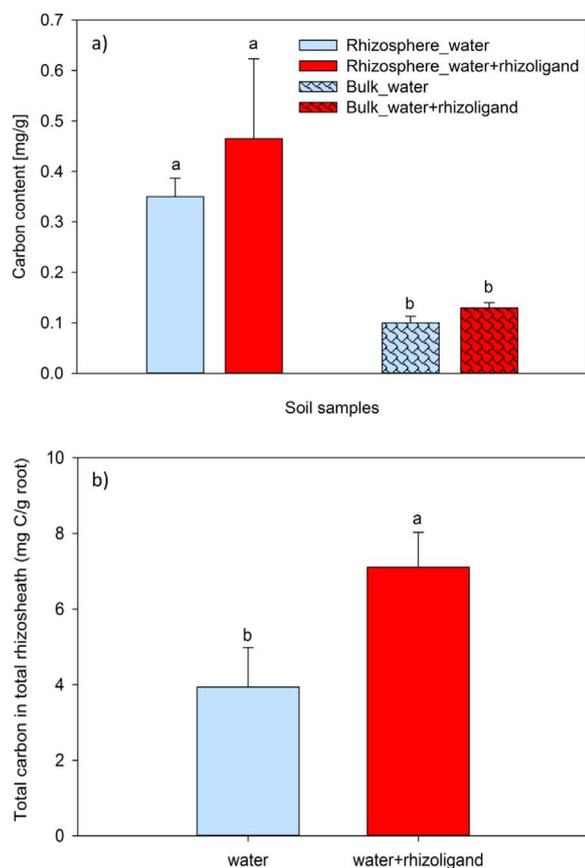


Fig. 7. a) Carbon content (mg) per gram of rhizosheath (two simple patterns in the left-hand side) and bulk soil (two hatch patterns in the right-hand side). b) total carbon in the rhizosheath of plants irrigated with water and rhizoligand solution. The values are averages of four plants and lower case letters indicate a significant difference at $P < 0.05$.

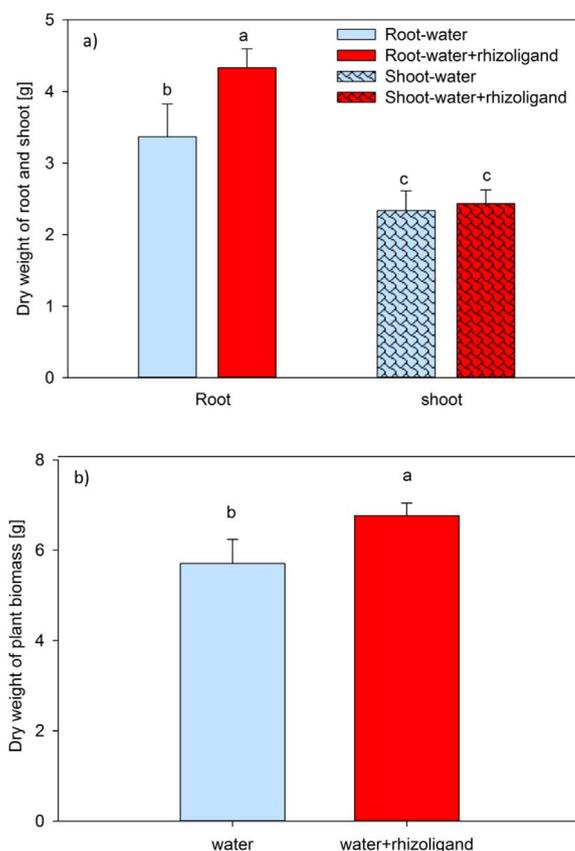


Fig. 9. a) Dry weight of roots (two simple patterns in the left-hand side) and shoots (two hatch patterns in the right-hand side) of lupins irrigated with water and rhizoligand solution. b) Total biomass of plants irrigated with water (left-hand side) and rhizoligand solution (right-hand side). The data are average of four samples. Lower case letters indicate a significant difference at $P < 0.05$.

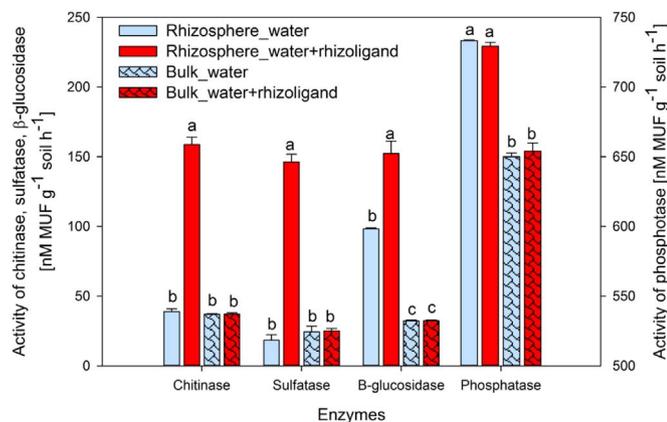


Fig. 8. Activity of four enzymes in the rhizosheath and bulk soil of plants irrigated with water and rhizoligand solution. The values are averaged of four samples. Lower case letters indicate a significant difference at $P < 0.05$ done for each enzyme individually.

(2015) and Chaichi et al. (2015), who showed that surfactants increase water use efficiency in alfalfa and corn under water limitation. Further studies are needed to understand why surfactant reduce transpiration.

Rhizoligand addition significantly increased rhizosheath formation in plants subjected to several drying and rewetting cycles (Fig. 6). This effect was attributed to the reduction in maximum swelling of mucilage after treatment with rhizoligand (Fig. 2). We expected that a lower swelling results in a higher viscosity and therefore, in a stronger capacity of mucilage treated with rhizoligand to bind soil particles together, as proposed in the model of Albalasmeh and Ghezzehei (2014). The observation that the tested surfactant reduced mucilage

swelling is in line with the observations of Simovic et al. (1999), who showed that interactions between non-ionic surfactants and a hydrophobically modified polymer increased the stability of this complex. This observation was attributed to the presence of an extra attractive force binding non-ionic surfactants to the hydrophobic groups of the polymer. These interactions increase viscosity of the complex (Simovic et al., 1999). We believed that a similar mode of action -one of biopolymer cross linking- could have occurred in our experiment, however further studies are needed to prove that such cross-linking of root mucilage is occurring.

We expected to observe a greater carbon content in the rhizosheath of plants irrigated with rhizoligand solution. We hypothesized that lower mucilage swelling and its higher viscosity would reduce the diffusion of mucilage and other root exudates far from the roots, resulting in greater carbon content in the rhizosheath of the plants treated with rhizoligand. However, the carbon content of rhizosheath on a unit weight basis was not significantly different between two treatments. While carbon content on a unit weight basis was not influenced, rhizoligand treatment resulted in larger rhizosheath volume. The carbon content in the rhizosheath of plants treated with rhizoligand was therefore averaged over a larger distance from the root surface. Since the carbon content typically decreases with increasing distance from the root surface (Kuzyakov et al., 2003; Sauer et al., 2006) the fact that in both treatments the carbon content was similar possibly indicates a higher carbon content in the rhizoligand treated sand. This speculation remains to be confirmed by measurements of carbon content at higher spatial resolution, e.g. by ¹⁴C imaging (Pausch and Kuzyakov, 2011; Pausch et al., 2013).

The total amount of carbon contained in the rhizoligands was much smaller compared to the increase in total carbon content in the

rhizosheath of the plants treated with rhizoligand (Fig. 7b). Plants were irrigated six times with the rhizoligand solution at concentration of 0.05 g per liter. Based on the carbon content of the tested surfactant and the total volume of the rhizoligand solution added during six irrigations, we estimated that a total of 3.5 mg carbon was added to each sample. This value is smaller compared to the changes in the total carbon in the rhizosheath of plants irrigated with rhizoligand solution (Fig. 7b).

The activities of three enzymes (chitinase, sulfatase and β -glucosidase) significantly increased in the rhizosheath of the plants treated with rhizoligand (Fig. 8). The greater activity of these three enzymes in the rhizosphere of plants irrigated with rhizoligand solution can be explained by 1) improved wettability of the rhizosphere and 2) by stimulated microbial activity in the rhizosphere. The greater wettability of the rhizosphere maintains microbial activity during severe drought. We hypothesize that rhizoligand treatment may have resulted in stabilized rhizodeposits that remained close to the root. These rhizodeposits composed of mucilage, inorganic and organic substances, and dead root cells would be an energy source for microorganisms and in turn be the driver of the increased enzyme activities in the rhizosheath, although our data are not sufficient to prove it (Kuzyakov and Domanski, 2000; Hinsinger et al., 2009; Jones et al., 2009; Brzostek et al., 2013).

The effect of rhizoligand on enzyme activity was limited to the rhizosheath and the rhizoligand had no effect on enzyme activity in the bulk soil. Phosphatase activity was not affected by the rhizoligand. Generally, plants and microorganisms employ many mechanisms to increase availability of phosphorus e.g. releasing extracellular phosphatases (Richardson et al., 2011). Lupin, in particular, releases large quantities of phosphatase as well as carboxylates (mostly citrate and malate) through cluster roots to increase mobility and availability of phosphorus in soil (Weisskopf et al., 2006). We suspect that in our experiment, large amounts of phosphatase released by lupin suppressed activity of microorganisms to release phosphatase, as suggested in Landi et al. (2006) and Weisskopf et al. (2006), and it was not affected by the rhizoligand.

The biomass of plants irrigated with rhizoligand solution was 1.18 fold greater than that of plants irrigated with water (Fig. 9b). This effect might arise from multiple processes, such as the greater water content in the rhizosphere, thicker rhizosheath and elevated rhizosphere enzyme activity. All such effects might imply a greater accessibility of water and nutrient resources, particularly when plants experience severe drying. Further studies, with rhizoligands tested with varying plant species and agriculturally important soils, are needed to generalize our results and demonstrate the rhizoligands increase nutrient uptake and plant tolerance to diverse abiotic stresses.

5. Conclusions

We introduce a new concept to engineer the biophysical properties of the rhizosphere. A commercial surfactant was employed as a rhizoligand, which was defined as an additive that facilitates the rewetting of the rhizosphere and reduces mucilage swelling. We present experimental evidence that addition of a rhizoligand results in ongoing wettability and better mechanically connection of the rhizosphere to the root surface and increases rhizosheath formation and plant biomass. Rhizoligand addition also resulted in increased rhizosphere activity which are strongly associated with increasing plant nutrient availability. These rhizosphere modifications would have the potential to improve plant tolerance to abiotic stresses and to improve agricultural sustainability in drought-prone areas.

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