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Lignin-based hydrogel alleviates drought stress in maize

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ABSTRACT

Global water shortage is a crisis for all living systems, including agricultural cropping systems. Water absorbent hydrogels from synthetic polymers can retain moisture from irrigation or rainfall and release it in response to crop water demands, but have a limited functional lifespan and may release byproducts that are harmful to plants or pollute the soil environment. Hydrogels fabricated from plant-based polymers are more suitable for agricultural cropping systems because they could improve the soil water availability to the crop and gradually biodegrade to harmless carbon dioxide and water. The objective of this work was to determine how biodegradable lignin hydrogel affected the soil water availability to maize grown under drought conditions. The lignin hydrogel was applied at 0.3 % and 0.6 % (by weight) in pots subjected to water deficit or sufficiency, and compared to sodium polyacrylate hydrogel that was applied at the same rates. Maize plants were taller, had greater phosphorus content and more biomass when grown in soil with lignin hydrogel and sodium polyacrylate hydrogel than without hydrogel. Furthermore, soil receiving 0.6 % lignin hydrogel produced significantly ($P < 0.05$) greater maize biomass and relative leaf water content, with an 86 % reduction in leaf proline content and 10 % less electrolyte leakage under severe drought conditions than without hydrogel. There was more soil soluble Na and Na uptake by maize in the sodium polyacrylate-amended soil due to solubilization of the Na^+ ions from the sodium polyacrylate. We recommend lignin hydrogel as a soil additive to increase water availability to crops experiencing drought stress that will not release undesirable byproducts into the cropping system.

1. Introduction

Plants need water for normal physiological activities and development. Global water shortages from 1980 to 2015 were responsible for yield losses that reduced wheat (*Triticum aestivum* L.) production by up to 21 % and maize (*Zea mays* L.) yield potential by as much as 40 % (Daryanto et al., 2016). Rising temperature associated with global climate change is increasing the drought stress in many vulnerable regions such as southern Asia, the Mediterranean, and the southwestern United States (IPCC, 2014). Innovative approaches are needed to improve the soil water balance for crops in these regions. Application of superabsorbent hydrogels to agricultural soils can increase water holding capacity and improve plant growth (Chen et al., 2014; Yang et al., 2013). These hydrogels are created through chemical stabilization of hydrophilic polymers in a 3-dimensional network (Montesano et al., 2015), which can absorb and retain more than $100\,g^{-1}$water of hydrogel (Jin et al., 2011). For example, sodium polyacrylate hydrogel (hereafter referred to as synthetic hydrogel) absorbs water during irrigation and rainfall events, and swells to as much as 200 times its original mass. As the soil dries, water is released gradually from the hydrogel by diffusion. This diffusion process is responsive to crop water demands, since the crop roots will deplete soil water reserves through evapotranspiration and create a potential gradient that causes water desorption from the hydrogel, leading to greater water uptake and better growth performance (Galeş et al., 2016; Liu and Chan, 2015). Most superabsorbent hydrogels are made from petroleum-based monomers like acrylic acid and acrylamide, so they are not easily biodegradable and may remain in the soil as micro- and nano-plastic residues. Furthermore, the functional lifespan of superabsorbent hydrogels is limited by their low salt tolerance and poor mechanical properties. (Franssen et al., 2013; Ma et al., 2011; Passauer, 2012)

Hydrogels could also be fabricated from natural biodegradable

Abbreviations: EC, electrical conductivity; FC, field capacity; FW, fresh weight; PEGDGE, poly ethylene glycol diglycidyl ether

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polymers such as polysaccharides (Guilherme et al., 2015; Parvathy and Jyothi, 2012), Alginate (Feng et al., 2017) and cellulose (Liu and Huang, 2016; Moon et al., 2011), both of which have potential to absorb water. The disadvantage of these materials is that they begin to biodegrade within weeks to months of soil application because degradative enzymes that hydrolyze these substances are produced by most prokaryotic and eukaryotic soil organisms. In contrast, lignin is a slowly biodegradable material with a residence time of around 20 years in soil (Marschner et al., 2008). Lignin is a constituent in almost all vascular plants and the second most plentiful natural polymer in the world (Heitner et al., 2016). The aromatic ring structure is bound through aliphatic groups containing methoxyl, carbonyl, carboxyl, and hydroxyl functional groups (Kai et al., 2016), which provide reactive surfaces for adsorption-desorption of soluble ions and contribute to soil buffering. Lignin-based hydrogels (hereafter referred to as lignin hydrogel) were already used successfully for drug delivery, seed coating, and absorption of trace metal ions (Li and Pan, 2010). In soil-free conditions, lignin hydrogel absorbed 34 g water g^{-1} and did not cause phytotoxicity of wheat seeds (Mazloom et al., 2019). This suggests that lignin hydrogel has potential to reduce drought stress of crops. In addition, the slow biodegradation of lignin may extend its functional lifespan beyond that of synthetic hydrogels, and thereafter a lignin hydrogel would contribute to the soil organic matter pool (Passauer et al., 2011). However, very little is known about the water-absorbent properties of lignin hydrogels in soil, compared to superabsorbent hydrogels with high swelling capacities.

The objective of this study was to determine if drought stress in maize could be alleviated with soil-applied lignin hydrogel. This was tested by applying lignin hydrogel and synthetic hydrogel to soil at the same rates (0.3 % and 0.6 %, by weight), and comparing maize growth of the hydrogel-treated soil to a no-hydrogel control soil under severe and moderate water stress. We hypothesized that lignin hydrogel will have less phytotoxic effect on maize than synthetic hydrogel, particularly as drought stress increases. Furthermore, we predicted that lignin hydrogel would increase soil extractable P, result in higher total P content in maize especially in soils with P deficiency, due to its greater reactive surface area than synthetic hydrogel.

2. Materials and methods

2.1. Lignin hydrogel synthesis

Lignin alkali (low sulfonate content) was selected because it is a common and inexpensive polymer backbone, and mixed with poly ethylene glycol diglycidyl ether (PEGDGE), a non-toxic cross-linker in the adjusted alkali solution of NaOH to prepare water absorbent soil conditioner. Synthesis of the lignin hydrogel was described in our previous work (Mazloom et al., 2019), and generated a lignin hydrogel with an estimated biodegradability of 59 % mass loss y^{-1}. Sodium polyacrylate hydrogel and lignin alkali for this study was purchased from Sigma-Aldrich (Ontario, Canada).

2.2. Soil physico-chemical properties

Soil was collected from the 0–30 cm layer of a field in Mashhad, Iran (59°26′ E, 36° 20′ N), air-dried and sieved (< 0.02 mm) and stored at 25 °C until use. Soil was classified as an Aridisol with a sandy loam texture, pH 7.2 and electrical conductivity of 1.7 dS m^{-1}. The soil contained 6.5 g organic C kg^{-1} according to the Walkley-Black method (Nelson and Sommers, 1982) with 0.65 g Kjeldahl-N kg^{-1}. Soil fertility was low to medium, with 11.7 mg P kg^{-1} (sodium bicarbonate extract; Olsen 1954) and 70.4 mg K kg^{-1} (ammonium acetate extract). Soil moisture content at field capacity was 300 g water kg^{-1}.

2.3. Greenhouse experiment and treatments

The experiment was done in a greenhouse (25 − 35 °C) under natural daylight conditions. It was an unbalanced factorial design that tested the combined effect of hydrogel sources (5 treatments) × drought stress (3 levels). There were two types of hydrogel (lignin hydrogel, synthetic hydrogel) applied at two rates (0.3 % and 0.6 % by weight) plus a no-hydrogel control. These five treatments were applied to pots under three drought conditions: extreme water shortage (45 % of field capacity), moderate water shortage (65 % of field capacity) and sufficient soil moisture (90 % of field capacity). The 15 factorial treatments were replicated four times, for a total of 60 pots.

Each pot (21 cm tall, 23 cm diam.) was filled with 7 kg of soil (air-dry basis) that was mixed with hydrogel treatment. Then, N, P and K fertilizers were added to each pot as follows: 80 mg kg^{-1} N as $Ca(NO_3)_2.4H_2O$, 25 mg kg^{-1} P as $CaH_2PO_4.H_2O$, and 90 mg kg^{-1} K as K_2SO_4. Soil moisture in the pot was adjusted by adding tap water to reach the target moisture content (45, 65 or 90 % of field capacity). Maize (*Zea mays* L. var. saccharata) seeds were pre-germinated on moist paper and then three seedlings were transplanted into the pot. Every day, the pot was reweighed and soil moisture was adjusted according to the water loss from the pot.

Maize were harvested after 45 d (vegetative growth stage: V12-V14) by cutting plants at the soil surface. Before harvesting, we measured the average plant height (in cm) per pot. Fresh samples of the three young, fully-developed leaves from one plant per pot were used to assess the proline concentration, relative water content and electrolyte leakage in leaf tissues. The stem and non-sampled leaves of this plant, plus shoots of the other two plants per pot were placed in a paper bag and dried (65 °C for 16 h) and dry biomass was recorded. Dry plant tissue was ground (< 2 mm) and digested with concentrated HNO_3 before measuring the P concentration by ascorbic acid colorimetry (Chapman and Pratt, 1962) and Na concentration by flame photometry (Watson and Isaac, 1990). Soil was removed from each pot, dried and sieved (< 2 mm) to remove roots and other plant residues, then analyzed for pH and Na concentration in soil slurries (1:2 soil: deionized water) and the P concentration in sodium bicarbonate extracts.

2.4. Leaf measurements: proline content, water content, electrolyte leakage

Fresh leave samples were removed from the second fully-formed leaf from the apical meristem of one maize plant per pot for the leaf measurements. A subsample (0.5 − 1 *g*) of each pot was frozen in liquid nitrogen placed in a refrigerator before the determination of proline concentration. Afterward, 0.5 *g* of frozen tissue was homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged for 5 min at 20 °C using a benchtop centrifuge according to (Bates et al., 1973). Ninhydrin buffer (100 μL of 3% sulfosalicylic acid, 200 μL glacial acetic acid, 200 μL acidic ninhydrin) and 100 % acetic acid were added to 2 mL of supernatant (1:1:1 v/v/v). The tube was incubated at 96 °C for 60 min. After cooling, the sample was extracted by toluene and the optical density of the upper organic phase was read at 520 nm. The proline concentration was determined using a standard L-proline concentration curve and expressed as μmol g^{-1} fresh weight (FW) leaf.

Relative water content was determined on the leaf samples of the selected plant. The fresh weight (w_f) of this leaf was recorded, then it was placed into a beaker of deionized water for 1 d, then the turgid weight (w_t) was measured. Finally, this leaf was dried at 70 °C for 48 h and weighed again (w_d). Relative water content was calculated as ((w_f-w_d/w_t-w_d) × 100) (González and González-Vilar, 2001).

Electrolyte leakage, an indicator of the membrane stability of plant cells under stress conditions, is the relative difference in ions released from a chopped leaf before and after boiling (Zhao et al., 2004). About 0.15 g of the leaf sample was shaken in 100 mL of deionized water and the initial electrical conductivity (EC_i) was measured. Then, the leaf-water mixture was boiled (100 °C for 15 min) and the final electrical conductivity (EC_{max}) was recorded. Electrolyte leakage was (EC_i/EC_{max}) ×100.

2.5. Statistical analysis

The effect of hydrogel source × drought stress on maize height, biomass and nutrient accumulation, leaf physiology and soil properties were determined by analysis of variance (ANOVA), following the procedure of Piepho et al. (Piepho et al., 2006) to accommodate the no-hydrogel controls in the unbalanced factorial structure. Normal distribution of the residuals was verified using the UNIVARIATE procedure. When the hydrogel source × drought stress effect was significant ($p < 0.05$), means were compared with a post-hoc Tukey's test using SAS statistical software (version 9.1, SAS Institute Inc., Cary, NC). Differences in leaf physiology between hydrogel treatments as a function of the drought stress were determined by regression analysis.

3. Results

3.1. Effects of lignin hydrogel and synthetic hydrogel on maize growth under water deficits

3.1.1. Maize growth parameters

As expected, maize height and biomass declined significantly ($P < 0.05$) with drought stress (Figs. 1 and 2). Maize was taller in pots amended with lignin hydrogel and synthetic hydrogel than the no-hydrogel control when soil was well-watered (90 % FC) and under moderate water shortage (65 % FC), and the lignin hydrogel contributed to taller maize in pots under extreme water shortage (45 % FC; Fig. 1). Maize biomass was greater in the lignin hydrogel and no-hydrogel treatments than with synthetic hydrogel in soil at 90 % FC ($P < 0.05$; Fig. 2). There was more maize biomass with the lignin hydrogel than with synthetic hydrogel and the no-hydrogel control in soil at 65 % FC (Fig. 2). Application of 0.6 %. lignin hydrogel supported significantly ($P < 0.05$) more maize biomass than the other hydrogel treatments in soil at 45 % FC (Fig. 2).

3.1.2. Plant P and Na contents

Generally, the P content and concentration in maize shoots declined with water shortage and the no-hydrogel control had the lowest P content and concentration (0.22 to 0.53 g P pot^{-1} or 0.34 to 0.44 %, Table 1). Shoot P content and concentration with lignin hydrogel (0.3 % and 0.6 %) and the 0.3 % synthetic hydrogel treatments were higher under both sufficient water condition or moderate water shortage, and the lignin hydrogel always produced a higher shoot P content and concentration than the 0.6 % synthetic hydrogel treatment (Table 1). Maize grown with severe water shortage had greater shoot P content and concentration with lignin hydrogel than synthetic hydrogel (Table 1).

Shoot Na content and concentration was lowest in the no-hydrogel control (0.04 to 0.11 g Na pot^{-1} or 0.06 to 0.12 %), which was similar to the 0.3 % lignin hydrogel treatment, but significantly ($P < 0.05$) less than with 0.6 % lignin hydrogel (0.21 to 0.28 g Na pot^{-1} or 0.30 to 0.34 %; Table 1). Shoot Na content was always higher in pots amended with synthetic hydrogel, and there was significantly ($P < 0.05$) more Na in maize shoots in the 0.6 % than 0.3 % synthetic hydrogel treatment (Table 1). Overall, shoot Na content was greatest with 0.6 % synthetic hydrogel > 0.3 % synthetic hydrogel > 0.6 % lignin hydrogel ≥ 0.3 % lignin hydrogel = no-hydrogel control, regardless of the drought stress.

3.1.3. Leaf proline content, relative water content, electrolyte leakage

Proline content was highest in maize leaves under extreme water shortage, and declined exponentially as soil water content increased (Fig. 3a). Maize grown with extreme water shortage had the highest proline content in leaves from the no-hydrogel control, followed by the 0.3 % synthetic hydrogel treatment > 0.6 % synthetic hydrogel ≥ 0.3 % lignin hydrogel > 0.6 % lignin hydrogel (Fig. 3a). The lowest proline content of 0.64 μmol g^{-1} FW in the 0.6 % lignin hydrogel treatment was about 8-fold lower than the no-hydrogel control (Fig. 3a). The proline content remained elevated in the no-hydrogel control compared to the other treatments under moderate water shortage, but there was no difference in proline content between treatments in the well-watered pots (Fig. 3a).

The second youngest fully-formed leaf of maize had a lower relative water content when grown under drought stress than in well-watered conditions. In the no-hydrogel control, the relative water content increased from 63 % under extreme water shortage to more than 80 % in the moderate water shortage and well-watered conditions, following a quadratic relationship (Fig. 3b). Maize leaves from the hydrogel-treated

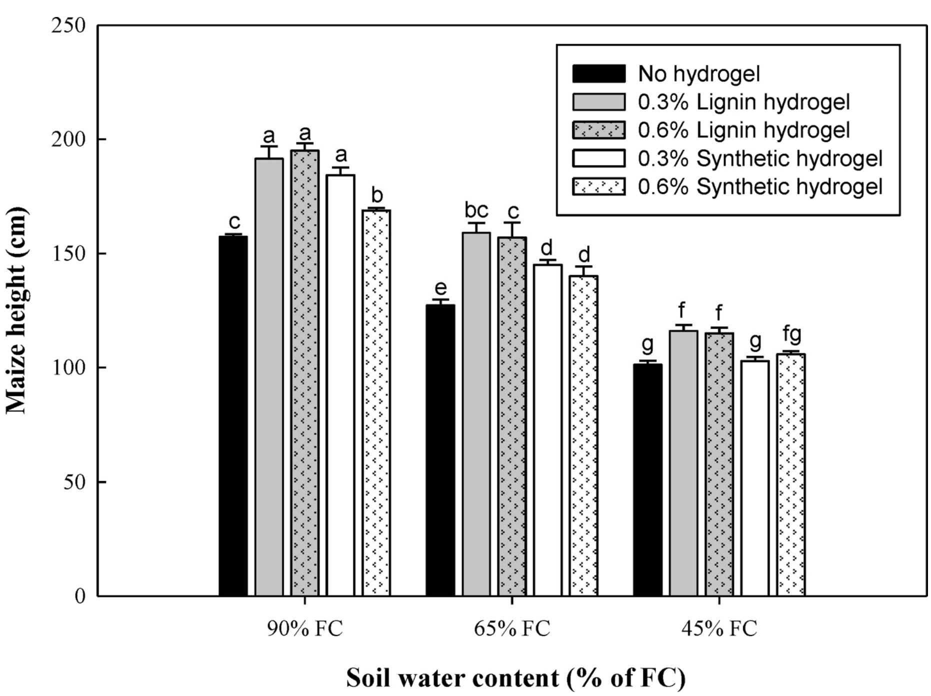

Fig. 1. Maize height (average of three plants per pot) after 45 d of growth in soil amended with lignin-based hydrogel and synthetic hydrogel, compared to the no-hydrogel control. Soil water content was adjusted daily to maintain the well-watered (90 % field capacity, FC), moderate water shortage (65 % FC) and extreme water shortage (45 % FC) conditions. Data are the mean values (n = 4). Bars with different letters were significantly different at $p < 0.05$.

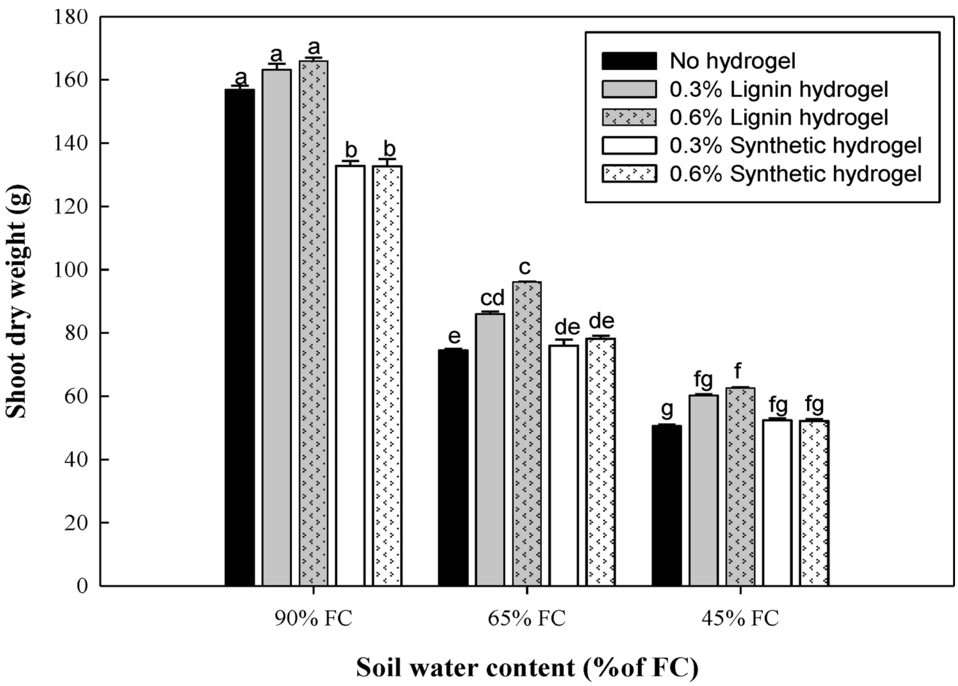

Fig. 2. Maize biomass (average of three plants per pot) after 45 d of growth in soil amended with lignin-based hydrogel and synthetic hydrogel, compared to the no-hydrogel control. Soil water content was adjusted daily to maintain the well-watered (90 % field capacity, FC), moderate water shortage (65 % FC) and extreme water shortage (45 % FC) conditions. Data are the mean values (n = 4). Bars with different letters were significantly different at $p < 0.05$.

Table 1
Accumulation of P and Na in maize shoots after 45 d growth in soil amended with lignin-based hydrogel and synthetic hydrogel, compared to the no-hydrogel control. Soil water content was adjusted daily to maintain the well-watered (90 % field capacity, FC), moderate water shortage (65 % FC) and extreme water shortage (45 % FC) conditions. Data are the mean values (n = 4).

Treatment	P (g P pot^{-1})	P (%)	Na (g Na pot^{-1})	Na (%)
		Well-watered (90 % FC)		
No-hydrogel control	0.53 ^{h}	0.34 ^{h}	0.11 ^{hi}	0.07 ^{j}
Lignin hydrogel (0.3 %)	1.53 ^{a}	0.94 ^{e}	0.18 ^{fgh}	0.11 ^{hij}
Lignin hydrogel (0.6 %)	1.38 ^{b}	0.83 ^{f}	0.23 ^{ef}	0.14 ^{h}
Synthetic hydrogel (0.3 %)	1.30 ^{b}	0.99 ^{e}	0.47 ^{d}	0.47 ^{e}
Synthetic hydrogel (0.6 %)	0.85 ^{efg}	0.64 ^{g}	0.89 ^{b}	0.67 ^{d}
		Moderate water shortage (65 % FC)		
No-hydrogel control	0.27 ^{i}	0.37 ^{hi}	0.04 ^{i}	0.06 ^{ij}
Lignin hydrogel (0.3 %)	1.05 ^{cd}	1.23 ^{cd}	0.13 ^{ghi}	0.15 ^{h}
Lignin hydrogel (0.6 %)	1.10 ^{c}	1.14 ^{cd}	0.28 ^{e}	0.30 ^{g}
Synthetic hydrogel (0.3 %)	0.94 ^{de}	1.24 ^{c}	0.80 ^{c}	0.80 ^{c}
Synthetic hydrogel (0.6 %)	0.83 ^{ef}	1.14 ^{d}	0.99 ^{a}	1.27 ^{b}
		Extreme water shortage (45 % FC)		
No-hydrogel control	0.22 ^{i}	0.44 ^{h}	0.06 ^{i}	0.12 ^{hi}
Lignin hydrogel (0.3 %)	0.93 ^{de}	1.56 ^{a}	0.10 ^{hi}	0.16 ^{h}
Lignin hydrogel (0.6 %)	0.92 ^{e}	1.48 ^{ab}	0.21 ^{efg}	0.34 ^{fg}
Synthetic hydrogel (0.3 %)	0.78 ^{fg}	1.50 ^{ab}	0.50 ^{d}	0.50 ^{d}
Synthetic hydrogel (0.6 %)	0.75 ^{g}	1.45 ^{b}	0.91 ^{ab}	1.76 ^{a}

Within each column, data with different letters were significantly different at $p < 0.05$.

pots were better hydrated under extreme water shortage (on average, 67–73% relative water content), and showed a linear increase in relative water content as soil water content increased (Fig. 3b). There was no difference ($P > 0.05$) in the relative water content of maize leaves from hydrogel-treated pots, regardless of the hydrogel type and application rate, at each level of drought stress level.

Drought stress was associated with more electrolyte leakage from maize leaves, which declined linearly with increasing soil water content (Fig. 3c). Maximum electrolyte leakage (71 %) occurred in the no-hydrogel control under extreme water shortage, and overall there was significantly ($P > 0.05$) greater electrolyte leakage from leaves in the no-hydrogel control than hydrogel-treated soils when maize was grown under extreme and moderate water stress (Fig. 3c). The lowest electrolyte leakage (44–45 %) was from maize leaves in the lignin hydrogel-treated pots that were well-watered (Fig. 3c).

3.1.4. Soil parameters

After maize harvest, the effect of hydrogel treatments and drought stress on soil properties were evaluated. Regardless of the watering regime, soil pH was significantly ($P > 0.05$) greater in pots amended with synthetic hydrogel (pH 7.9–8.3) than the no-hydrogel control (pH 7.56–7.58), which had similar pH values as the lignin hydrogel (pH 7.52–7.7; Table 2). Soil P concentration varied between 7 and 9.2 mg extractable P kg^{-1} and was significantly ($P < 0.05$) lower in the no-hydrogel control than the hydrogel-amended soils that were well-watered or maintained under extreme water shortage (Table 2). Soluble Na concentration in soil after maize harvest depending on the level of drought stress. In well-watered pots, there was significantly ($P < 0.05$) more soluble Na with 0.6 % synthetic hydrogel than the no-hydrogel control, which had a similar Na concentration as the other hydrogel treatments (Table 2). Under moderate and extreme water shortage, there was generally more soluble Na in hydrogel-treated soils than the no-hydrogel control, and the soluble Na concentration was always significantly ($P < 0.05$) greater with synthetic hydrogel than lignin hydrogel (Table 2).

4. Discussion

The greenhouse pot study provided insight into the hydrogel × drought stress interactions in a controlled environment where soil water content was maintained at a constant level by daily monitoring and adjustment. Drought stress levels imposed in this study (i.e., 65 % and 45 % of field capacity) were based on climate change predictions for drought-prone regions of the world (Cammalleri et al., 2016). This approach allowed us to determine the efficacy of lignin-based hydrogel

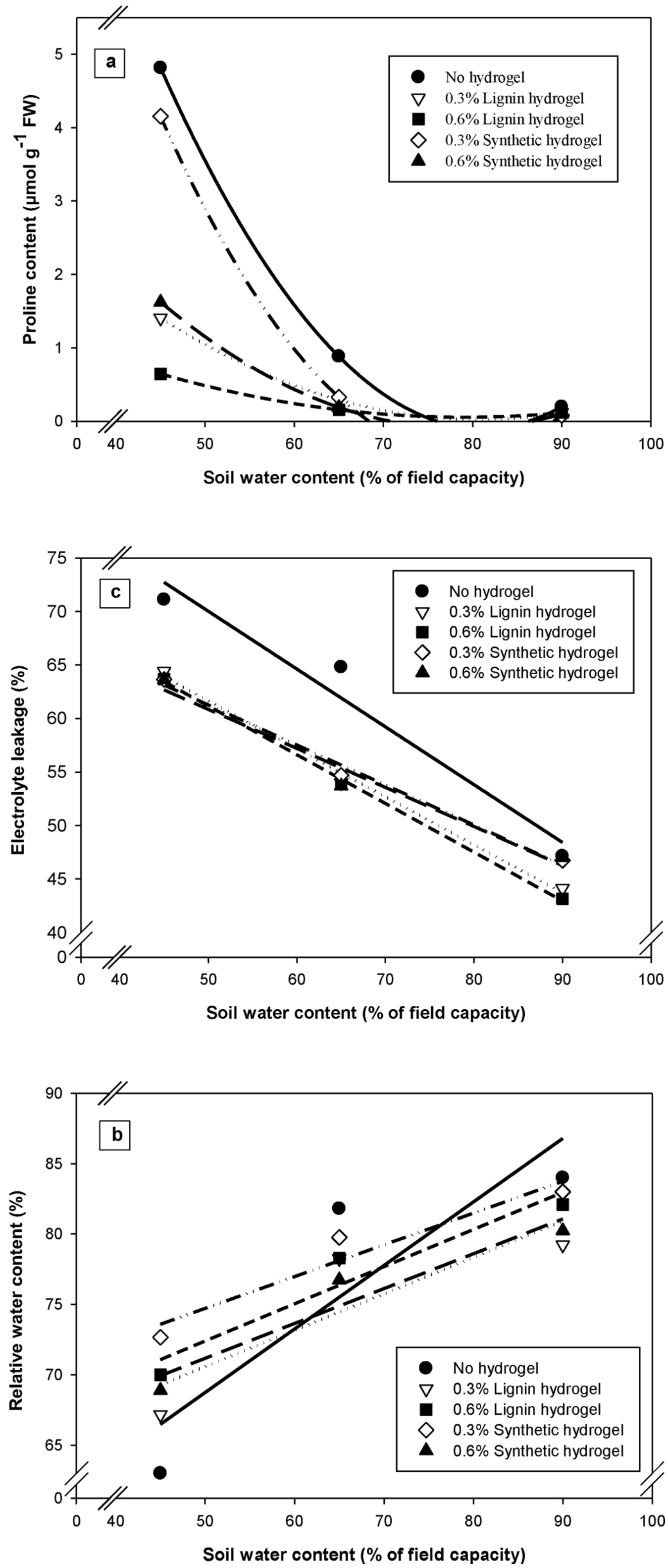

Fig. 3. Relationships between maize leaf physiology and drought stress in soil amended with lignin-based hydrogel (0.3 % and 0.6 % by weight), synthetic hydrogel (0.3 % and 0.6 % by weight) and a no-hydrogel control. Physiological parameters were (a) proline concentration, (b) relative water content and (c) electrolyte leakage. The best-fit line for each treatment were fitted with linear and non-linear regression analysis.

Table 2
Soil pH, extractable P and soluble Na concentrations following maize harvest from soil amended with lignin-based hydrogel and synthetic hydrogel, compared to the no-hydrogel control. Soil water content was adjusted daily to maintain the well-watered (90 % field capacity, FC), moderate water shortage (65 % FC) and extreme water shortage (45 % FC) conditions. Data are the mean values (n = 4).

Treatment	pH	Extractable P (mg P kg^{-1})	Soluble Na (mg Na kg^{-1})
	Well-watered (90 % FC)		
No-hydrogel control	7.56 [ef]	6.96 [g]	354.3 [f]
Lignin hydrogel (0.3 %)	7.59 [ef]	8.42 [bcde]	347.2 [f]
Lignin hydrogel (0.6 %)	7.52 [f]	9.09 [abcd]	370.5 [ef]
Synthetic hydrogel (0.3 %)	7.87 [cd]	8.29 [cde]	407.4 [def]
Synthetic hydrogel (0.6 %)	8.08 [b]	9.23 [a]	440.8 [d]
	Moderate water shortage (65 % FC)		
No-hydrogel control	7.57 [ef]	7.35 [fg]	384.8 [def]
Lignin hydrogel (0.3 %)	7.58 [ef]	8.53 [bcde]	440.6 [d]
Lignin hydrogel (0.6 %)	7.59 [ef]	9.11 [ab]	553.4 [c]
Synthetic hydrogel (0.3 %)	7.90 [c]	7.83 [f]	731.9 [a]
Synthetic hydrogel (0.6 %)	8.30 [a]	8.13 [def]	688.1 [a]
	Extreme water shortage (45 % FC)		
No-hydrogel control	7.58 [ef]	7.87 [ef]	350.9 [f]
Lignin hydrogel (0.3 %)	7.58 [ef]	8.30 [cde]	452.2 [d]
Lignin hydrogel (0.6 %)	7.70 [de]	8.80 [bcd]	547.9 [c]
Synthetic hydrogel (0.3 %)	7.98 [bc]	8.32 [cde]	658.6 [ab]
Synthetic hydrogel (0.6 %)	8.14 [ab]	8.96 [bcd]	611.6 [bc]

Within each column, data with different letters were significantly different at $p < 0.05$.

and sodium polyacrylate hydrogels to alleviate water deficits in maize that experienced drought stress during its vegetative growth (until the V12-V14 stage), a critical period for biomass accumulation and development of reproductive components necessary for grain formation (Ritchie et al., 1982). Logistic constraints resulted in tests with a single soil type and one maize cultivar. Consequently, our findings cannot predict hydrogel responses under realistic field conditions with non-uniform soils, heterogeneous plant growth and climate fluctuations, but they do contribute to a mechanistic understanding of hydrogel functions in a model soil-plant system.

4.1. Superior effects of lignin hydrogel

4.1.1. More maize growth and biomass accumulation

Maize growth and biomass accumulation was limited by drought stress, but some of the hydrogel treatments alleviated this effect. Maize grew taller in pots amended lignin hydrogel than the no-hydrogel control, regardless of the soil water content, suggesting that lignin hydrogel may elicit stem elongation. In contrast, synthetic hydrogel stimulated stem growth of maize that was well-watered or had moderate water shortage, but not under extreme water shortage, compared to the no-hydrogel control (Fig. 1). Biomass accumulation was less affected by hydrogel treatments, although the 0.6 % lignin hydrogel treatment produced more maize biomass than the no-hydrogel control in the moderate and extreme water shortage conditions (Fig. 2).

The fact that maize plants were consistently taller in pots amended with lignin hydrogel than other treatments is interesting and similar to the behavior of other natural hydrogels. For instance, Montesano et al. (Montesano et al., 2015) observed growth enhancement of water-limited cucumber grown in soil treated with a biodegradable cellulose-based hydrogel. In a related study, Parvathy et al. (2014) (Parvathy et al., 2014) observed superior plant growth in pots amended with a cassava starch-based superabsorbent hydrogel and irrigated every 3 days, compared to the no-hydrogel control. Moreover, Islam et al., (Islam et al., 2011) reported that application of super absorbent hydrogel increased maize height by 11.3 % in comparison with control. One reason for longer maize stems could be that lignin hydrogel released water in synchrony with biological activities that support cell division and elongation, whereas the synthetic hydrogel was less efficient in this regard. This seems unlikely because synthetic hydrogels can absorb up to 200 g water g^{-1} and the lignin hydrogel retained 34 g water g^{-1} (Mazloom et al., 2019; Passauer et al., 2011), meaning that the synthetic hydrogel would theoretically have more water to release than the lignin hydrogel. A second possibility is that lignin hydrogel stimulated root growth and the root-derived phytohormones (e.g., auxins) were transferred to shoots, where they promoted stem elongation. There is one report in the literature suggesting that alkaline lignin addition to soil promotes root activity (Lv et al., 2017), but the authors did not explain why. It will be interesting to determine whether lignin hydrogel can stimulate plant growth hormone production, either in plants or by rhizobacteria associated with plant roots. Auxin production by rhizobacteria is beneficial for the growth and yield of water-limited maize and many other crops (Barnawal et al., 2019), however it is not known whether lignin hydrogel can stimulate auxin production by rhizobacteria and this needs to be determined.

4.1.2. More leaf water content, less proline concentration and electrolyte leakage

Cell membranes are sensitive to environmental stressors, including drought stress, and this can be assessed by measuring cell membrane integrity (Bajji et al., 2002) or biomarkers such as proline, an amino acid that maintains turgor, buffers against reactive oxygen species and maintains redox homeostasis to prevent dehydration of plant cells (Ashraf and Foolad, 2007). It has been reported that under water stress condition, proline accumulation in plants may reach a level more than 70-fold higher than unstressed condition (Sharma and Verslues, 2010). Our results confirm that drought stress damaged cell membranes, releasing more proline and electrolytes, and reducing the relative water content of the youngest fully-formed maize leaves (Fig. 3). In general, maize grown in hydrogel-treated soil had less cell membrane injury (based on proline concentration and electrolyte leakage) than those in the no-hydrogel control. The relative water content, which is the balance between water absorption and transpiration from leaves (González and González-Vilar, 2001), was 23 % lower in the no-hydrogel control but only 8–14% lower in the hydrogel-treated soils under extreme water shortage. This confirms that lignin and synthetic hydrogels were effective in supplying water to maize grown in water deficit conditions as reported by (Abobatta, 2018). Yang et al., (Yang et al., 2019) reported that soil super absorbent significantly increased the grain number, biomass and yield of maize as compared to control by 8.2 %, 11.5 % and 12.0 %, respectively.

Our results show that lignin hydrogel was superior to synthetic hydrogel in extreme water shortages because it reduced proline production, a water stress biomarker, in maize leaves. Pots amended with lignin hydrogel had lower proline concentration under moderate and extreme water shortage conditions than the synthetic hydrogel and no-hydrogel control (Fig. 3a). This suggests that there was less water limitation for maize in the lignin hydrogel-amended pots than in the other treatments, and is consistent with the maize growth (height) in the presence of lignin hydrogel. Demitry et al., (Demitri et al., 2013) proposed that the controlled release of water from a cellulose-based hydrogel cross-linked by carbodiimide during drought could maintain soil moisture for plant uptake for a long period of time. We agree that water release from hydrogel is a dynamic process, but in the same soil type planted with the same maize cultivar, the diffusion gradient responsible for physically transferring of water molecules from the hydrogel to the plant should be the same for lignin and synthetic hydrogels. We assert that lignin hydrogel has an additional biological function, possibly on the root system or with the root-associated rhizobacteria, that alleviated water stress in maize, but this remains to be confirmed.

4.1.3. More maize P content

Drought interferes with nutrient uptake, notably P acquisition by crops, because lack of water inhibits the P biogeochemical cycle (i.e., mineralization and solubilization reactions) (Sanaullah et al., 2012; Schimel et al., 2007) and interferes with the diffusion of phosphate ions from soil solution to the roots (Chapin, 1991; Lambers et al., 2008). In addition, plants growing with water limitation may synthesize fewer P transporter proteins or the transporter protein activity may be diminished due to water shortage in cells (Fahad et al., 2017; Voothuluru et al., 2016). Our work showed the benefit of hydrogel treatments for P uptake, which resulted in a 2-fold increase in the shoot P content in well-watered maize and 3–4 times greater shoot P content in maize grown under moderate and extreme water shortage (Table 1).

At each level of drought stress, the maize P content was always greater in pots with lignin hydrogel than synthetic hydrogel. This was not related to the soil extractable P concentration, which was similar in all hydrogel treatments and drought stress levels, so there must be another explanation. The first possibility is that the diffusion rate of water and P ions was greater in the presence of lignin hydrogel than synthetic hydrogel. We reject this explanation because the small amount of lignin hydrogel added in this study (0.6 % by weight, or less) is unlikely to change the soil physical matrix in a way that alters the diffusion path (tortuosity) or diffusion rate (controlled by maize evapotranspiration as well as cohesive forces that are a function of the soil texture and soil organic matter content). A second possibility is that the lignin hydrogel maintained more soluble P ions in the soil water than synthetic hydrogel through chemical reactions. Lignin hydrogel and synthetic hydrogel have reactive surfaces with hydroxyl and carboxyl functional groups (Duval and Lawoko, 2014; Peng et al., 2014), which can covalently bond phosphate ions (primarily $H_2PO_4^-$ in this slightly alkaline soil with an initial soil pH 7.2 that increased to pH 7.6 after maize growth). Phosphate ions often precipitate with soluble Ca, Fe and Al to form secondary minerals, or bind to positively charged clay and organic matter surfaces, which makes them unavailable for plant uptake (Shen et al., 2011). Temporary adsorption of phosphate ions onto charged hydrogel surfaces would prevent P fixation, and those P ions could be released along with water moving from the hydrogel to the maize roots. We posit that lignin hydrogel has more anion exchange capacity than synthetic hydrogel. This is based on the fact that lignin hydrogel buffered soil pH (pH 7.5–7.7) by absorbing OH^- from the soil solution, whereas synthetic hydrogel had less capacity for anion exchange and higher soil pH values (pH 7.9–8.3; Table 1).

In natural soils, lignin and its byproducts (humic-like substances) can increase soil extractable P by occupying anion exchange sites, which increases P desorption rates, and forming complexes with Ca, Fe and Al compounds to prevent P precipitation (Perassi and Borgnino, 2014; Urrutia et al., 2014). The addition of 0.6 % (by weight) of lignin hydrogel seems insufficient to cause such changes in the soil physicochemical environment. However, Savy et al. (Savy et al., 2016) reported that soluble fractions of commercial lignin may function as a plant growth promoter, similar to other humic-like substances that increase plant biomass. Humic-like materials can stimulate the H^+-ATPase activity, which is the driving force for active uptake of many nutrients (Canellas et al., 2002). In addition, humic-like substances are bioactive molecules that can elicit the growth of rhizobacteria, which have multiple functions in plant growth promotion (Barnawal et al., 2019). It was beyond the scope of this study to determine if lignin hydrogel had humic-like characteristics and consequences for soil biological functions, but it should be investigated in the future.

4.2. Synthetic hydrogel improves soil water availability, but releases Na in pots with maize

Addition of 0.6 % synthetic hydrogel was beneficial for maize growth under extreme water shortage, resulting in more relative water content in the second youngest fully-formed maize leaf, 66 % lower proline concentration, 11 % less electrolyte leakage and greater shoot P content than the no-hydrogel control (Fig. 3; Table 1). The main issue with synthetic hydrogel is that it contained appreciable Na concentration, which resulted in substantial accumulation of Na in maize shoots (Table 1) and in soil (Table 2). Uptake of Na by plants can decrease the K^+/Na^+ ratio in shoots and may reduce or inhibit the activity of enzymes that require K^+ as a cofactor, including kinase (Maathuis et al., 2014).

In addition to the detrimental effect on plants, the synthetic hydrogel has undesirable effects for soil chemistry due to the dissolution of Na from the sodium polyacetate ($[C_3H_3NaO_2]c_n$) in soil solution. Mobile Na ions leach through the soil profile with water moving through mass flow, but this does not occur under drought conditions or in semi-arid regions where evaporation exceeds precipitation. Soils treated with synthetic hydrogel had a higher soil pH and higher soluble Na concentration than the no-hydrogel control, and these factors could contribute to soil sodicity as follows:

$$(C_3H_3\boldsymbol{Na}O_2)_n + H_2O \Leftrightarrow (C_3H_3\boldsymbol{H}O_2)_n + Na^+ + OH^- \quad (1)$$

In this reaction H^+ is inactivated by exchange with Na^+ in synthetic hydrogel. The displaced Na does not bind to OH^-, which increased the OH- concentration and increased soil pH. If all Na in the synthetic hydrogel is dissolved according to Eq. (1), then the application of 0.6 % synthetic hydrogel would consume 0.02 mol H^+ from the soil solution and increase the soil pH by 1.6-units. This would be undesirable from an agronomic perspective, since high soil pH interferes with the nutrient uptake by plants and many micro-nutrients like zinc, copper, manganese and iron become deficient in alkaline soils (Tinus, 1980). We recommend caution when using sodium polyacetate hydrogel as a soil amendment due to the possibility that it will increase soil pH and reduce the fertility of alkaline soils.

5. Conclusion

Lignin hydrogel, a superabsorbent polymer, has potential as a green solution to alleviate water scarcity in agricultural soils. Lignin hydrogel increased water availability, reducing the water stress in maize leaves and increasing P uptake in maize shoots. Synthetic hydrogel also supplied water to maize, but released Na ions that accumulated in maize shoots and soil solution, increased soil pH and contributed to soil sodicity. Lignin hydrogel buffered the soil pH and slightly increased the Na concentration in maize shoots and in soil solution, but at lower levels than synthetic hydrogel. The beneficial effects of lignin hydrogel for crop water availability need to be confirmed for other soil types and with more crop varieties, under realistic field conditions with fluctuations in soil water content. It is also necessary to evaluate the effects of lignin hydrogel on soil moisture characteristic curve in the laboratory. Moreover, field testing is required to evaluate the long-term fate of lignin hydrogel in soil. Although lignin is an abundant polymer and byproduct of paper-making and biorefinery industries that could be transformed into hydrogel, more works are needed to develop inexpensive and efficient industrial processes to separate suitable lignin precursors from biomass feedstocks.

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CRediT authorship contribution statement

Najmeh Mazloom: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Visualization, Investigation. **Reza Khorassani:** Supervision, Methodology, Funding acquisition. **Gholam Hossein Zohury:** Supervision, Methodology. **Hojat Emami:** Validation, Writing - review & editing. **Joann Whalen:** Validation, Writing - review & editing.

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