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Research article

Cellulose Hydrogel and Its Impact on Mung Bean Growth

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Keywords

cellulose hydrogel;
seed germination;
plant growth;
water retention of soil

Abstract

Cellulose hydrogels were prepared from commercial cellulose using NaOH/urea and LiCl/DMAc methods. For NaOH/urea method, alkali hydrates ($OH^-(H_2O)_x$), urea hydrates (urea$(H_2O)_y$), and water were adsorbed into cellulose fiber to break hydrogen bonds between cellulose molecules yielding HG_Urea. For LiCl/DMAc method, the Lithium-DMAc complexes, $[Li(DMAc)_n]^+Cl^-$, were seeped into cellulose fibers and the hydrogen bonds between the fibers were substituted by O-H…$Cl^-[Li(DMAc)_n]^+$ bonds to produce transparent cellulose gel, HGL. Both freeze-dried hydrogels show the characteristics of cellulose vibrational modes and nano-sized fibrous SEM images. For water retention study, there was no significant difference when different amounts of HG_Urea were being used. Covering mung bean seeds directly helps improve the growth of roots, leaves and stems by 410, 28 and 27%, respectively. HGL and HGL in water were also tested on the germination of the mung bean seeds. As compared with HGL, the average lengths of roots, stems and leaves of mung bean on HGL-water were increased by 82, 32 and 14%, respectively.

1. Introduction

Cellulose is the most abundant polysaccharide, and is considered as a source of raw material for the increasing demand for environmentally friendly and biocompatible products [1]. It can be used to prepare hydrogel, a three-dimensional network of hydrophilic polymer absorbing and retaining a significant amount of water. Cellulose hydrogel has been used in various fields like drug delivery [2], medicine [3], cosmetics and agricultures [4] due to its excellent hydrophilicity, permeability, compatibility and low coefficient of friction. In agricultural field, the water holding capacity of hydrogel helped accelerate the growth of corn [5], increase the survival time of cherry tomatoes in

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low humidity soil [6] and the seedling growth of sesame seeds [7]. Apart from enhancing the water absorptivity of soil, hydrogel can be a good candidate for soilless culture in agricultural application [8]. To prepare cellulose hydrogel, the hydrogen bond interactions in cellulosic chains must be overcome by stronger cellulose-solvent interactions. Various solvent systems [9] such as NH_4F/DMSO, N-methyl-morpholine-N-oxide (NMMO), ionic liquid, LiCl/DMAc, and NaOH/urea have been successfully attempted. Among these methods, NaOH/urea [10] and LiCl/DMAc [11] are quite suitable in preparing cellulose hydrogel due to their cost effectiveness and easy handling. Both cases produce active species which can bind effectively with hydroxyl groups of cellulosic chains. In NaOH/urea method, alkali hydrates and urea hydrates penetrate through the three-dimensional networks of cellulose creating new hydrogen bond interactions. In a similar manner, the lithium-DMAc complex, $[Li(DMAc)_n]^+Cl^-$, is generated in LiCl/DMAc method and effectively bound with cellulose *via* hydrogen bonds. The hydrogels obtained from these two methods are quite different. LiCl/DMAc method yields a strong and transparent gel while NaOH/urea method gives a wax-like opaque gel.

In this research, cellulose hydrogels, HG_Urea and HGL were prepared from two solvent methods, NaOH/urea and LiCl/DMAc, respectively. Due to the difference in gelling property of these two hydrogels, they were attempted for plant culture in two different ways, with and without soil. HG_Urea was applied to increase the water retention of soil and to enhance the growth of mung beans. However, having a potential as a growth support for soilless culture, HGL was attempted in the absence of soil for the germination of mung bean seeds.

2. Materials and Methods

2.1 Materials and instruments

Cellulose powder, LiCl, and N, N-dimethylacetamide (DMAc) were purchased from Fluka while urea, NaOH and acetic acid were obtained from Sigma-Aldrich. All chemicals and solvents were of analytical grade and used as received. The functional group analyses were performed in Attenuated Total Reflectance mode using FT-IR spectrophotometer (Perkin Elmer). The surface morphology was studied by scanning electron microscopy (SEM) using a TESCAN MIRA 3 electron microscope.

2.2 Preparation of cellulose hydrogel

2.2.1 HG_Urea

Commercial cellulose (4 g) was soaked in 300 mL of deionized water at room temperature for 24 h and filtered. It was then added to 96 g of freshly prepared NaOH/urea solution (weight ratio of NaOH: urea: water = 8:15:77) at -5.0 to -10.0ºC. The low temperature helps decrease the side reaction between NaOH and urea. Under this temperature, the mixture was continuously stirred until clear solution was obtained. It was poured into a plastic plate and left at room temperature for 4 days and subsequently neutralized with 1 M acetic acid to obtain the wax-like opaque gel, HG_Urea. After that, HG_Urea was freeze-dried and analyzed using FTIR spectroscopy and SEM.

2.2.2 HGL

Cellulose powder (1.5 g) was soaked with deionized water for 24 h and filtered. The fiber was then swelled in 80 mL of DMAc for 1 h and filtered. In a separate flask, 150 mL of DMAc was heated

under nitrogen atmosphere to 110ºC for 15 min. DMAc was further heated to 165ºC and the treated fiber was subsequently added. After 1 h, temperature was decreased to 100ºC and 15.0 g of LiCl was added with stirring until dissolved. The solution was cooled to 40ºC and the clear colorless solution was obtained. The solution was then filled and kept in a vial for a week to obtain HGL-DMAc. HGL-DMAc was then left in deionized water to obtain HGL. It was freeze-dried prior to analysis by FTIR spectroscopy and SEM.

2.3 Water retention of soil using HG_Urea

Soil was dried under sunlight and sieved using 2.0 mm sieve. Dried soil (50 g) was filled into each of five 100 mL beakers. HG_Urea was mixed with soil in each beaker in varied amounts: 1.0, 2.0, 4.0 and 8.0 wt% (see Figure 1). Water (20 mL) was then added into each beaker. The weight of each beaker was measured every 24 h for 20 days. The water retention was calculated using the following equation:

$$\%\text{Water retention} = \frac{\text{Weight of water in the dried mixture}}{\text{Weight of water in the initial mixture}} \times 100$$

Figure 1. Five beakers containing soil and different amounts of HG_Urea
(◆ = HG_Urea, ⬭ = soil)

2.4 Study on the growth of mung bean seeds using hydrogel

2.4.1 Growth of mung bean in soil mixed with HG_Urea

Three transparent plastic containers were labeled as S1, S2 and S3. Each was then filled with 46 g of dried soil (see the preparation in 2.3). In S2, 4 g of HG_Urea was well mixed with dried soil. Three mung bean seeds were planted in S1-S3. For S3, the seeds were covered with 4 g of HG_Urea. Each seed was buried at 1.0 cm depth. Each container was watered (5.0 mL) every 2 days. The set-up of S1-S3 are presented in Figure 2. The stem lengths and leaf sizes were measured on day 3, 5 and 7 while the lengths of roots were measured on day 7.

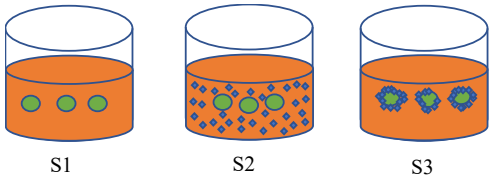


Figure 2. Three plastic containers labeled S1 (dried soil, a control), S2 (dried soil mixed well with HG_Urea) and S3 (dried soil and HG_Urea covering mung bean seeds)
(◆ = HG_Urea, ● = mung bean seed, ⬭ = soil)

2.4.2 Germination of mung bean on HGL

Two HGLs were placed in two petri dishes: with 20 mL water and without water. A mung bean seed was placed on top of each HGL (see Figure 3). The stem lengths and leaf sizes were measured on day 3, 5 and 7 while the lengths of roots were measured on day 7.

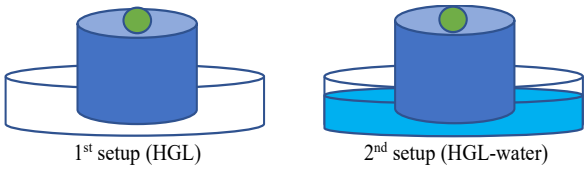


Figure 3. Set-ups of mung beans on HGLs in petri dishes. (= mung bean seed, = HGL)

3. Results and Discussion

3.1 Preparation of cellulose hydrogel

3.1.1 HG_Urea

HG_Urea, a wax-like opaque gel, was successfully prepared in NaOH/urea solution as shown in Figure 4. When the cellulose was treated with NaOH/urea solution, alkali hydrates ($OH^-(H_2O)_x$), urea hydrates ($Urea(H_2O)_y$), and water were adsorbed into cellulose fiber to break hydrogen bonds in cellulose molecules until it was completely dissolved. The detailed mechanism is shown in Figure 5 [10].

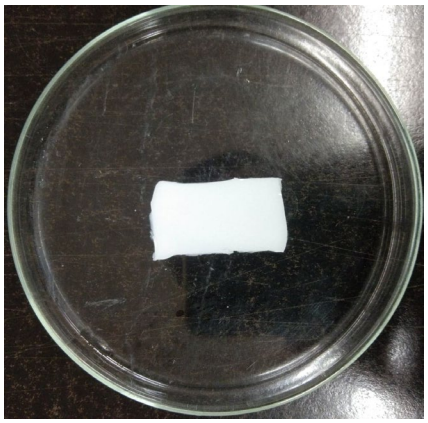


Figure 4. HG_Urea

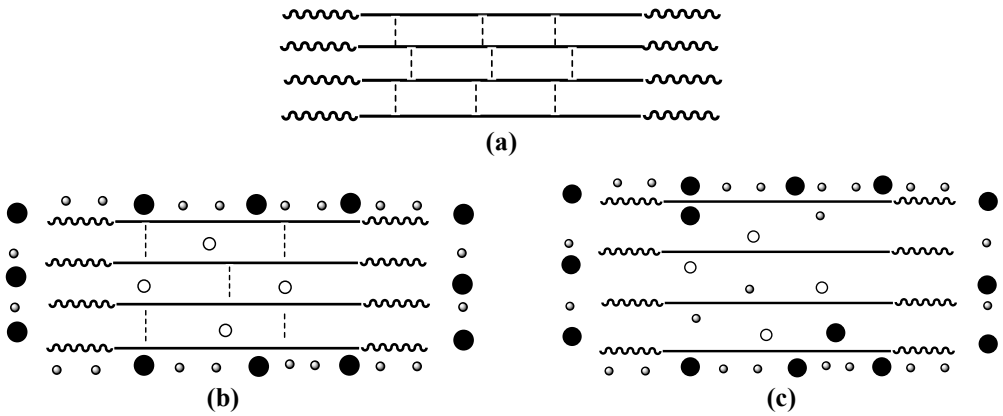


Figure 5. Mechanism of the cellulose dissolution in NaOH/urea (a) cellulose (b) separated cellulose fibers (c) cellulose solution [10]
(○= water, ○= alkali hydrates ($OH^-(H_2O)_x$), and ● = urea hydrates ($Urea(H_2O)_y$)

The FT-IR spectra of commercial cellulose and HG_Urea were recorded in the range of 4000-450 cm^{-1} as shown in Figure 6 and Table 1. HG_Urea shows similar characteristics to commercial cellulose. However, the absorption band for the O-H vibrations (3334 and 1633 cm^{-1}) of HG_Urea are broader as compared with those of commercial cellulose. This is due to the hydrogen bond interactions between hydroxyl groups of the loosely bound cellulose chain of HG_Urea and water.

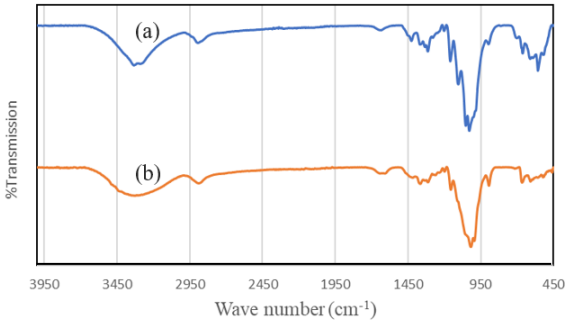


Figure 6. FT-IR spectra of (a) Commercial cellulose and (b) HG_Urea

Table 1. Bond vibrations of commercial cellulose and HG_Urea

Bond vibration	Wave number (cm^{-1}) of the sample	
	Commercial cellulose	HG_Urea
ν (O-H)	3341	3334
ν (C-H)	2896	2891
δ (O-H)	1636	1633
δ (C-H)	1428	1419
ν (C-O-C)	1163	1157
ν (C-O)	1030	1026

The SEM images of HG_Urea were shown in Figure 7. Most cellulosic fibers in nanometer size (estimated from the SEM image) were still bundled together. However, in certain area, the chains were separated to produce voids for water molecule. This makes the gel more opaque and poorer water capacity as compared with HGL.

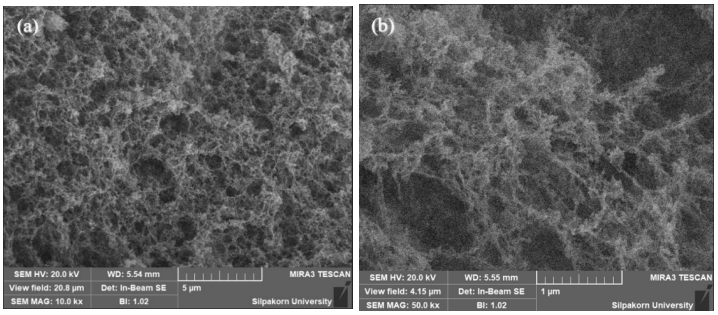


Figure 7. SEM images of HG_Urea at (a) 10,000x and (b) 50,000x magnification

3.1.2 HGL

HGL was successfully prepared by LiCl/DMAc method to obtain HGL-DMAc. The detailed mechanism is presented in Figure 8. Carbonyl oxygens in DMAc molecules were bonded with Li^+ to form $[Li(DMAc)_n]^+Cl^-$. The Lithium-DMAc complexes then seeped into cellulose fibers and the hydrogen bond in the fibers were substituted by $O\text{-}H\ldots Cl^-[Li(DMAc)_n]^+$ bonds [11].

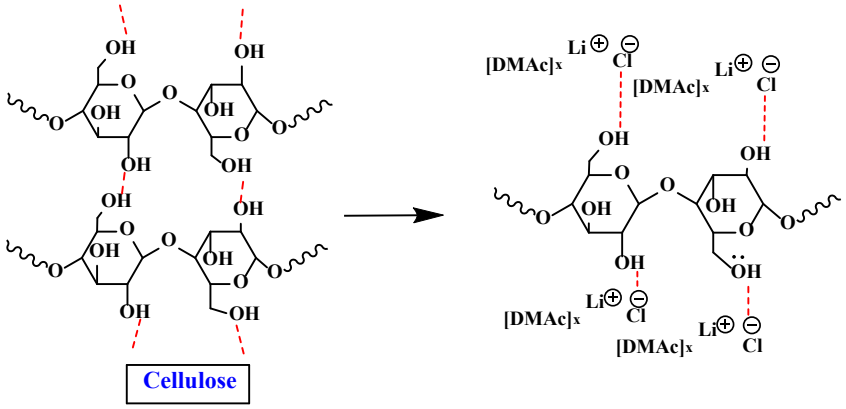


Figure 8. Mechanism of cellulose dissolved in LiCl/DMAc [11]

DMAc molecules in HGL-DMAc were replaced by water to obtain a hydrogel lump, HGL (see Figure 9). Both HGL-DMAc and HGL showed a transparent cylinder shape with strong gel matrix. The sizes and weights of HGL-DMAc and HGL are illustrated in Table 2. The size and weight of HGL was decreased due to the smaller molecular size of water as compared with DMAc. The shrinkage of HGL slightly increased the opacity of the hydrogel matrix.

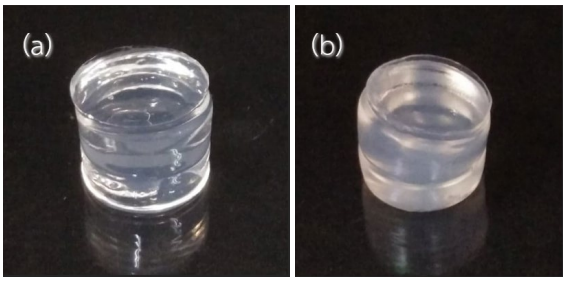


Figure 9. The shape and gel characteristics of (a) HGL-DMAc, (b) HGL

Table 2. The sizes and weights of HGL-DMAc and HGL

Sample	x̄±SD, n=10		
	Height (mm)	Diameter (mm)	Weight (g)
HGL-DMAc	15.34±0.9	17.72±1.8	3.8149±1.38
HGL	13.04±0.73	14.96±1.84	2.7811±1.36

The FT-IR spectra of freeze-dried cellulose samples were recorded in the range of 4000-450 cm^{-1}. HGL shows the characteristic vibrations of cellulose similar to commercial cellulose as shown in Figure 10 and Table 3. In the case of HGL_DMAc, the spectrum shows only the signal of DMAc in the pore of cellulose gel. The DMAc was not removed by the freeze-dried process due to its high boiling point (165°C). It is of interest that the HGL has a very broad signal of OH stretching due to the hydrogen bond interaction between hydroxyl groups of cellulose and residual water left in the pore of the HGL. This broad signal hindered the appearance of the CH stretching of the cellulose in the HGL.

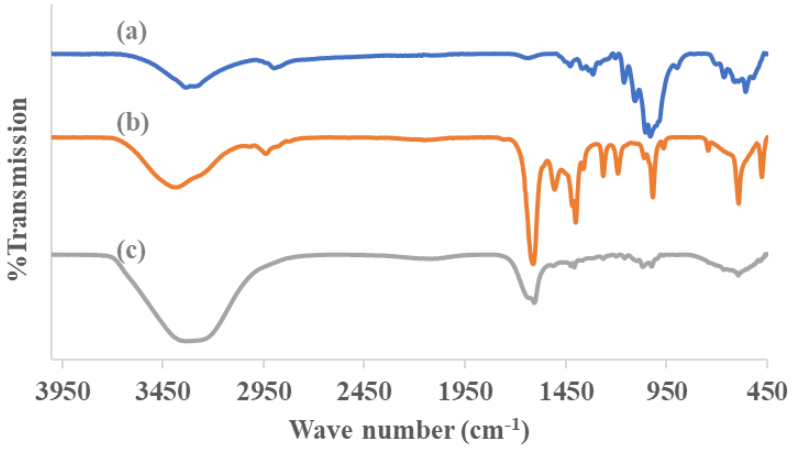


Figure 10. FT-IR spectra of (a) Commercial cellulose, (b) HGL_DMAc and (c) HGL

Table 3. Bond vibrations of commercial cellulose, HGL_DMAc and HGL

Bond vibration	Wave number of sample (cm^{-1})	
	Commercial cellulose	HGL
ν (O-H)	3341	3340
ν (C-H)	2896	-
δ (O-H)	1636	1607
δ (C-H)	1428	1408
ν (C-O-C)	1163	1162
ν (C-O)	1030	1023

The SEM images of HGL are shown in Figure 11. The cellulosic fibers in nanometer size were well separated to produce voids for water molecules. They bound to the cellulosic hydroxyl groups *via* hydrogen bonds. These interactions are strong enough to hold water molecules in the hydrogel matrices.

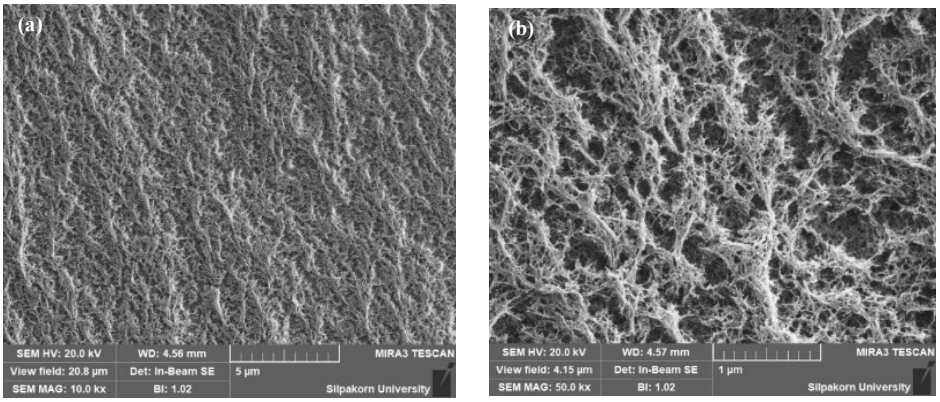


Figure 11. SEM image of HGL with (a) 10,000x and (b) 50,000x magnification

3.2 Water retention of soil using HG_Urea

After 20 days of the study, the trends of water retention are shown in Figure 12. HG_Urea mixed in the soil slightly increased water retention in the samples. The addition of 8% HG_Urea showed the highest water retention. However, there was still no significant difference when different amounts of HG_Urea were used. This may indicate the difficulty in changing the water retention property of the whole soil matrix using HG_Urea.

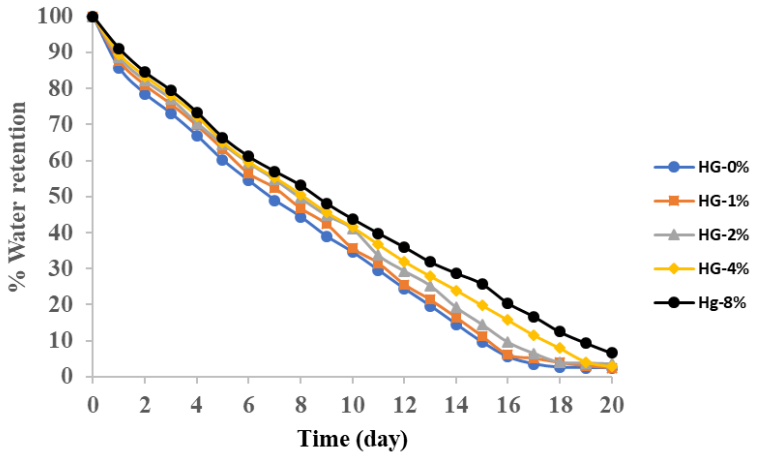


Figure 12. Plot of water retention percentage in the soil and HG_Urea mixture

3.3 The impact of hydrogel on the growth of mung bean seeds

3.3.1 Growth of mung bean in soil mixed with HG_Urea

In this study, HG_Urea was mixed with soil using two methods, mixing with soil (S2) and covering the mung bean seeds (S3). The dried soil (S1) was set up as a control. The idea of seed covering comes from the finding in section 3.2 in which direct mixing of hydrogel (up to 8%) with soil leads to a small change in the water retention of soil. The mung beans in S3 had a greater growth rate than those in S2 and S1. The length data of stems, leaves and roots are shown in Table 4. The photos of the mung bean trees taken on day 3, 5 and 7 are presented in Table 5 and Figure 13. From these results, S3 showed the highest stem and leave growth rates. On day 7, all mung bean trees were carefully pulled out from soil in order to measure the length of the roots. The longest root length from S3 confirmed the highest growth rate result. As comapred with dried soil (S1) the lengths of roots, leaves and stems were increased by 410, 28 and 27%, respectively. The high water capacity of HG_Urea helped provide water for the mung bean seeds effectively. Moreover, hydrogel covering the plant seeds reduced the water evaporation rate. This method can be applied for planting in arid area.

Table 4. The physical data of the mung bean trees in S1, S2 and S3

Sample	Average length (cm) ($\bar{x}$±SD, n=3)						
	Day 3		Day 5		Day 7		
	stems	leaves	stems	leaves	stems	leaves	roots
S1	6.5±1.5	1.4±0.2	19.9±1.8	1.6±0.2	24.6±2.1	1.8±0.4	2.0±2.7
S2	11.8±1.2	1.0±0.8	21.3±2.2	1.8±0.4	27.4±1.8	1.9±0.3	1.9±2.2
S3	14.4±0.8	1.8±0.4	22.8±0.6	2.1±0.2	31.2±0.7	2.3±0.5	10.2±1.3

Table 5. The mung bean trees in S1, S2 and S3 on day 3 and 5

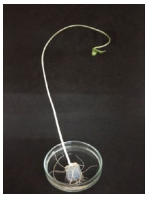
Day 3		
S1	**S2**	**S3**
		
Day 5		
S1	**S2**	**S3**
		

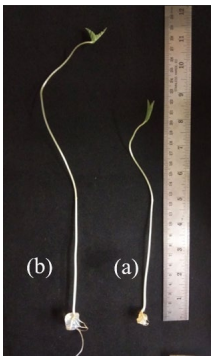
Figure 13. The mung beans trees on day 7 from (a) S1, (b) S2 and (c) S3

3.3.2 Germination of mung bean on HGL

HGL, the transparent lump of hydrogel prepared from LiCl/DMAc method, was attempted for the germination of mung bean. The growths of mung bean trees on both HGL and HGL in water were monitored and recorded, as shown in Table 6 and Figure 14. The mung bean on HGL-water has a higher growth as compared with that on HGL. On day 7, the average length of roots in HGL-water was increased by 82% as compared with that in HGL while the average lengths of stems and leaves were improved by 32 and 14%, respectively. It is of interest that the average lengths on day 7 of the stems, leaves and roots using HGL-water and HG_urea-soil (S3) are very similar. This promising result showed a high potential of HGL as a growth support in soilless culture.

Table 6. The mung bean trees in HGL set-ups at day 3, 5 and day 7

Sample	Day 3	Day 5	Day7
HGL			
HGL in water			



Sample	Average length (cm) ($\bar{x}$ ± SD, n=3) on day 7		
	stems	leaves	roots
HGL	1.5 ± 22.7	0.5 ± 2.2	2.3 ± 5.5
HGL in water	0.9 ± 30.0	0.2 ± 2.5	1.8 ± 10.0

Figure 14. The mung bean trees planted on (a) HGL (b) HGL in water, and the tabulated average length data of stems, leaves and roots on day 7

4. Conclusions

Cellulose hydrogels were successfully prepared from commercial cellulose using NaOH/urea and LiCl/DMAc methods. The growth of mung beans in soil was enhanced by covering seeds with HG_Urea (8% by soil weight). The average lengths of roots, leaves and stems were increased by 410, 28 and 27%, respectively. HGL in water also helped increase the germination of the mung bean seeds. The average lengths of roots, stems and leaves were increased by 82, 32 and 14% respectively.

5. Acknowledgements

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