Hydrogels improve plant growth in Mars analog conditions

Frédéric Peyrusson

Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Belgium

Address for correspondence:

Frédéric Peyrusson

Avenue E. Mounier 73 B1.73.05

1200 Bruxelles, Belgium

Phone: +32.2.764.73.76

frederic.peyrusson@uclouvain.be

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Abstract

Plants will be fundamental components of future human missions to Mars, and cultivation will probably suffer from the lack of optimal soils as well as of available liquid water. Additionally, the question as to whether Mars soils are suitable for plant cultivation is still open. Hydrogels are polymers able to absorb large quantity of water and have been shown to increase water retention in the soil, thus increasing plant establishment and growth. This work reports on the short-term assessment of plant growth in Mars soil analogs supplemented with hydrogels, in the constrained environment of a simulated life-on-Mars mission. Soil analogs consisted in sand and clay-rich material, sampled in the vicinity of the Mars Desert Research Station, a Mars analog facility surrounded by soils sharing similarities with mineralogical and chemical composition of Martian soils. Heights and dry biomass of *Mentha spicata* and seed germination of *Raphanus sativus* were monitored. Results revealed that clay-containing soil was the most unfavorable for the growth of control plants. The deleterious effects of Mars soil analogs on plant growth were potentiated under limited irrigation. By contrast, hydrogel amendment allowed for an overall improvement of plant endurance in Mars soil analogs, more pronounced under low watering conditions, leading to almost constant masses across conditions. Additionally, results point to an increase of seed germination rate in sand, which creates a dense medium with low water retention, that hydrogels might help loosening. The results suggest that Mars soil analogs can support the growth of plants in the tested conditions, and raise the hypothesis that *in situ* soils might be suitable for future exploration of Mars.

1. Introduction

In the next long-duration exploration of Mars, human settlement will require the use of plant-based bioregenerative advanced life support (ALS) systems, as a source of food and air and water regeneration systems [1].

Due to the necessity of working with *in situ* resources, the question as to whether plants can grow on Martian soils is still uncertain [2, 3]. In view of fleets of orbital and landed spacecraft, our understanding of Martian soils has improved considerably over the last few decades [4]. Indeed, although Martian soils contain elements which are necessary nutrients for the growth of plants [5], some other chemical elements, as well as their low water retention capacity could be barriers for the growth of plants [2, 3, 6]. Additionally, the availability of liquid water will be very limited on Mars [7, 8]. The study of Terrestrial analogs of Martian regolith on plant growth in the context of limited resources are thus critically needed.

The Mars Desert Research Station (MDRS), operated by the Mars Society, is a Mars analog facility in Utah that allows Earth-based research to address the issues raised by space exploration, in the constrained environment of simulated life-on-Mars missions. MDRS is surrounded by a landscape that is an actual geologic Mars analog, with a mineralogy comparable to Mars, consisting of deposits of sands, clay minerals, iron oxides and traces of carbonates [9, 10].

Hydrogels are polymers able to absorb large quantities of water and fixing on seedling roots. By improving water availability, they have been shown to reduce water stress and improve plant growth and survival [11]. However, some studies report negative effects on different soil types [12], and no study has been conducted so far with Mars soil analogs. This article reports on the effect of hydrogels on plant growth in two Mars soil analogs. All experiments were conducted during the UCL to Mars 2018 campaign within the constraints of *in situ* operations in the MDRS station [13, 14]. Results demonstrate that hydrogels improve the growth of plants, despite soils unfavorable for control plants, when monitoring height and dry biomass of *Mentha spicata* and seed germination of *Raphanus sativus*. These effects were more pronounced in Mars soil analogs under limited irrigation. The present work suggests that hydrogels could represent an interesting strategy for the use of *in situ* Mars soils.

2. Material and Methods

2.1 Soils sampling

Soil samples were collected in the vicinity of MDRS station, at an average altitude of 1391m, and consisted in a white sand layer and a brown-reddish clay-rich material (hereafter referred as "sand" and "clay", respectively) (Table 1). These soils were previously analyzed for their composition during the EuroGeoMars 2009 campaign [5], including elemental composition of nitrate, potassium, phosphorous, organic matter and carbonates. Soils were selected for their similarities with Mars soils, based on their (i) mineralogy (i.e., sand and phylosilicates [clay minerals]) and (ii) composition (i.e., low amount of organic matter, iron oxides, and traces of carbonates) [15-17]. Additionally, soils were analyzed *in situ* for their pH in water, indicating pH of 9.2 and 9.06 for sand and clay soils, respectively.

2.2 Water holding and release properties of hydrogels

Hydrogels are polymers that are able to increase water retention, and enhance plant growth [11, 18, 19]. The superabsorbent polymer used in the present study was the commercial hydrogel STOCKOSORB® 660 Medium (Evonik Industries; hereafter referred as "hydrogel"), a crosslinked potassium polyacrylic acid designed to remain active in the soil for 1 to 3 years. Soils were supplemented with 0.1% (w/w) hydrogel (according to the manufacturer's instructions) prior to plant transplantation. Hydrogels were first analyzed for their ability to regenerate after drying, to mimic absorption by plant roots. A sample of 2 g of dry hydrogel was saturated with water, incubated approximately 10 min prior weighing. This sample was dried on absorbing paper at room temperature for 24 h, and subjected to three additional hydration/dehydration cycles.

2.3 Experimental design for plant growth

All experiments were conducted in the greenhouse facility of MDRS station during the UCL to Mars 2018 campaign, from Mars 12, 2018 (Sol 0) to Mars 25, 2018 (Sol 13), in a controlled atmosphere with an average temperature of 24 °C during the experimental period. During this period, mean length of visible light was 14 hours.

2.4 Effect of hydrogels on plant growth

For a short-term assessment of plant growth, *Mentha spicata* plants were selected for their rapid growth and their robustness under nutrient-poor conditions. Experiments aims at addressing the effect of hydrogel amendment in Mars soil analogs, in normal or reduced irrigation conditions. To that end, experiments were divided into three groups of soils: potting medium ("pot. medium"), sand supplemented with 10% (w/w) potting medium ("mix sand"), and clay supplemented with 10% (w/w) potting medium ("mix clay"). Each group was tested under normal or reduced irrigation regimes, performed in three independent experimental units. This experimental set-up resulted in three soils x two irrigation conditions x three replicates.

For each condition, seedlings of *Mentha spicata* were transplanted at Sol 0 in single pots of 9 cm length and 10 cm depth with or without 0.1 % (w/w) hydrogel supplementation. Plants were manually overhead irrigated with water, daily for normal irrigation conditions, and each 4 days for reduced irrigation conditions. Shoots heights were followed daily and dry shoots masses were recorded for all plants 13 days after transplantation (Sol 13). For dry shoots masses, plants were dried in a forced air oven at 60 °C until constant mass [20].

2.5 Effect of hydrogels on seed germination

To monitor the first growth stages, seeds of *Raphanus sativus* were selected for their fast germination. Experiments imply to address the effect of hydrogel supplementation in Mars soil analogs on seed germination. To that end, experiments were divided into five groups of soils: potting medium ("pot. medium"), sand supplemented with 10% (w/w) potting medium ("mix sand"), clay supplemented with 10% (w/w) potting medium ("mix clay"), sand and clay. This resulted in five soils x three replicates. For each condition, five seeds were positioned per pot at the same burial depth. The percentage of seed germination were recorded at Sol 13.

2.6 Statistical Analysis

Statistical analyses were performed with GraphPad Prism versions 4.03, GraphPad InStat

v3.10 (GraphPad Software) and SPSS v25.0 (IBM Statistics). For comparison between hydrogel and control conditions, statistical differences were determined using unpaired Student's t-tests, with a threshold of statistical significance set to 0.05. *P* values strictly inferior to 0.05, 0.01 and 0.001 were used to show statistically significant differences and are represented with *, ** or *** respectively. For multiple comparison between soils, statistical differences were determined using one-way ANOVA with Tukey's post hoc tests, with a threshold of statistical significance set to 0.05. Different letters indicate statistically significant differences.

3. Results

3.1. Water holding and release properties of hydrogels

Hydrogels are polymers that can absorb large quantity of water during expansion (Fig. 1a). When submitted to consecutives cycles of hydration and dehydration in order to mimic absorption by plant roots and watering, respectively, data indicated that they efficiently regenerate for sustained periods of time (Fig. 1b). Additionally, roots of *Mentha spicata* transplanted in potting medium amended with 0.1% (w/w) hydrogel were able to fix on hydrogels (Fig. 1c). These data indicate that hydrogels present interesting water holding and release properties that could constitute a method for improvement of plant growth.

3.2. Effect of hydrogels on plant growth

For a short-term assessment of plant growth, *Mentha spicata* plants were selected for their rapid growth and their robustness under nutrient-poor conditions. All experiments were conducted in the greenhouse facility of MDRS station from Mars 12, 2018 (Sol 0) to Mars 25, 2018 (Sol 13), in a controlled atmosphere and a 14-h photoperiod. Importantly, the mixtures of soils and potting medium (groups mix sand and mix clay) resulted in final pH of 8.5 and 8.4, respectively, in the range of pH of Martian soils [21].

Data indicated that either soils or limited irrigation could affect the growth of plants (Fig. 2a). Indeed, when compared with potting medium, soils containing clay induced a stress leading to an arrest of growth during the course of experiments. Under reduced irrigation, this effect was potentialized by water stress in clay and sand-containing

soils, leading to plant heights of 97% and 101% their initial values, respectively. Moreover, a wilting of main branches of plants was observed in clay-containing soil, which appeared to be cohesive and unfavorable for plant growth. On the other hand, the growths recorded with hydrogel supplementation were not significantly affected across conditions, except for clay-containing soils during limited watering. These observations are consistent with studies with *Argania spinosa* on arid region [22], and on cucumber and basil plants in sandy soils [11]. Under limited irrigation, hydrogel supplementation led to significantly improved growth in potting medium, sand- and clay-containing soils with heights of 108%, 101% and 107% their initial heights, respectively.

Similar trends were observed when monitoring biomasses, for which constant masses in plants amended with hydrogels were observed (Fig. 2b). In control plants, the water stress resulted in decreased masses, particularly pronounced in clay and sandy soils, resulting in significant differences with hydrogel amendment.

3.3. Effect of hydrogels on seed germination

The effects of hydrogel supplementation in Mars soil analogs on seed germination was also assessed (Fig. 3). To that end, seeds of *Raphanus sativus* were used due to their fast germination. All the seeds germinated in pots filled with potting medium, indicating the absence of toxicity of hydrogels on germination, in these conditions. A reduced germination was observed in soils containing clay, and sand to a lesser extent. In control conditions, no germination was observed in pots only filled with clay and sand, which create dense media unfavorable for germination.

Although sand is considered unfavorable to germination due to low water retention [23], hydrogel supplementation allows for the germination of 27% of seed in average in sand. Results suggest here that hydrogel could be useful for the germination in soils as the cohesive sand-like soils encountered in Mars.

4. Discussion

Human missions to Mars will raise considerable number of challenges, among which the questions related to food self-sufficiency. Additionally, plant will be fundamental components of advanced life support systems, as a potential source of air and water regeneration systems.

The question of habitability remains open, and has been mainly studied on bacterial species [24-27], but there are relatively few reports for plants. As a precursor to human visitations, research regarding plant response to simulated Martian environment is of chief importance.

Our current knowledge on Mars surface highly suggest that some mineral and chemical properties encountered might be a barrier for plant growth. Firstly, Mars is covered with vast expanses of dust [28] and coarse, sand-sized or finer-grained particles. Sampling by Phoenix, and Spirit and Opportunity rovers revealed high soil cohesion [29, 30], and additionally, traces of clay minerals have been confirmed by Curiosity [31]. Low amounts of organic matter [32] and carbon stored in carbonates [15] have been found. Secondly, the measurements so far indicate that soils are alkaline, with pH ranging from 8 to 9 [21], which may be problematic for many plant species [33]. Finally, although liquid water has been evidenced, its hypersalinity may be a major limiting factor for its *in situ* use [3].

The proposed study tends to integrate these different constraints, and to assess the effect of hydrogel on plant growth in Mars soil analogs. Although experiments were limited to growth indices and germination parameters, the results indicate that Mars soil analogs appeared less suitable for control plants as a comparison to potting medium, by creating a nutrient-poor and alkaline environment - well beyond Mentha species requirement – and cohesive soils, especially clay-containing ones. This led to the wilting of main branches in soils containing clay. For the two soil analogs tested, these effects were even more pronounced under limited irrigation. By contrast, hydrogel supplementation allowed for an overall improvement of growth in Mars soil analogs, particularly significative under low watering conditions. Additionally, results point to an increase of seed germination in sand, and suggest that hydrogels could help loosening very dense soils.

5. Conclusions

This work is a short-term assessment conducted within the constraints of *in situ* operations and warrants further studies on other plants, hydrogels and soil analogs, but could be considered as a proof of concept for the use, at least in part, of *in situ* soils. In these conditions, *in situ* Mars soils would not necessarily preclude the growth of plants. The results suggest that hydrogels could be suitable for a potential use in human missions to Mars.

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Declaration of competing interest

F.P. certify that no parties have a direct financial interest in the results of this article.

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Tables

Table 1. Characteristics of soils sampled at the Mars Desert Research Station location. Adapted from Ehrenfreund *et al.* [5].

Soil nature	Location	pH	Organic matter (%)	N-element (ppm)	P (ppm)	K (ppm)	Carbonates (%)
Sand	N38.40746° W110.79280°	9.2	1	>75	12	175	<1
Clay	N38.42638° W110.78342°	9.06	2	>75	100	200	2

Figures

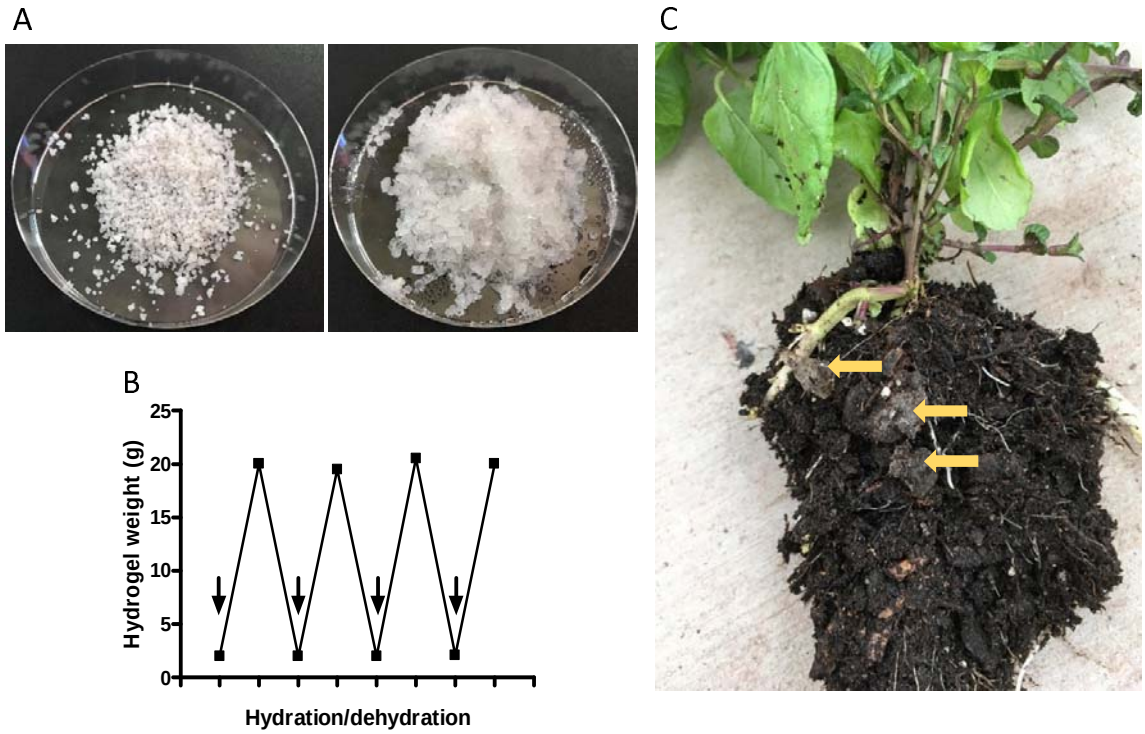

Fig. 1. Water holding and release properties of hydrogels. (**a**) Sample of 1g of dry hydrogel (left). The same sample was saturated with water and incubated 10 min (right). (**b**) Hydrogels weight during hydration/dehydration cycles. A sample of 2 g of dry hydrogel was saturated with water (arrows), incubated 10 min prior weighing, and dried on absorbing paper at room temperature for 24 h. (**c**) Hydrogels (arrows) fixed on *Mentha spicata* roots.

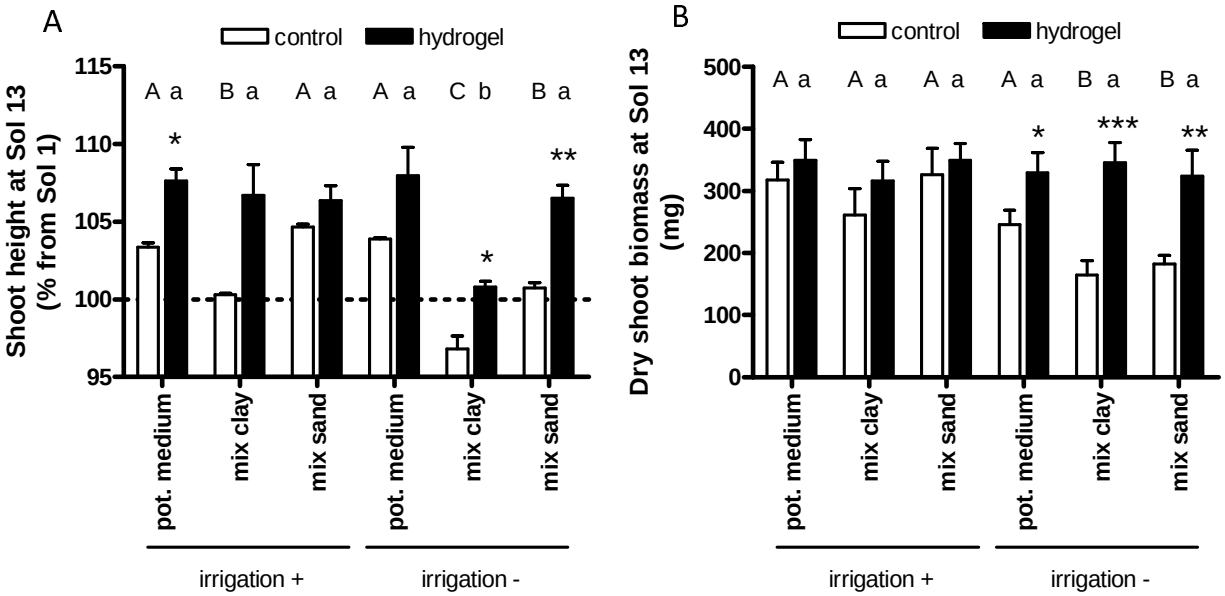

Fig. 2. Shoots heights (**a**) and dry shoots biomasses (**b**) of *Mentha spicata* 13 days after transplantation (Sol 13), with (hydrogel) or without (control) 0.1 % (w/w) hydrogel supplementation, under varying irrigation conditions. Pots were filled with soils from MDRS location (see Table 1) supplemented with 10% (w/w) potting medium or with potting medium alone (mix clay, mix sand and pot. medium, respectively). Plants were either daily watered (irrigation +) or each four days (irrigation –). All data are means of three replications. Statistical analysis for comparison between control and hydrogel conditions: unpaired Student's t-test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. For comparison between soils: one-way ANOVA with Tukey's post hoc test. Different uppercase (control) or lowercase (hydrogel) letters indicate values significantly different from each other.

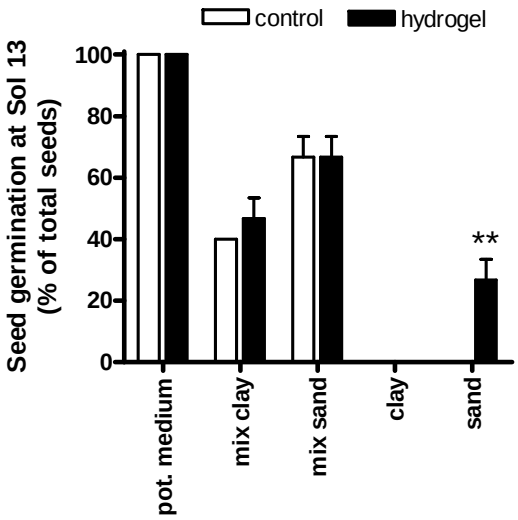

Fig. 3. Percentage of seed germination of *Raphanus sativus* at Sol 13. For each condition, five seeds were positioned per pot, filled with potting medium, or soils from MDRS location (see Table 1) supplemented with 10% (w/w) potting medium, or soils alone, respectively, with (hydrogel) or without (control) 0.1 % (w/w) hydrogel supplementation. Data are means of three replications. Statistical analysis for comparison between control and hydrogel conditions: unpaired Student's t-test. $^{**}P < 0.01$.