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Comparative of Shoot and Root Growth of Water Spinach (*Ipomoea aquatica*) Cultivated in Mars Global Simulant MGS-1 With or Without Addition Earth Soil Illuminated by LED Light in Indoor Environment

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ABSTRACT

Mars has been a target for human colonization for centuries. When the time comes for humans to settle Mars, they will need food for survival. It is implausible to send a lot of fresh food to Mars. Therefore, we could instead cultivate plants at the site itself, using Martian soil in an indoor environment. In this study, we evaluated the shoot and root growth of water spinach (Ipomoea aquatica) in Mars Simulant or combination of Mars Simulant with earth soil at indoor environment using LED lighting. The result showed that water spinach can grow on earth soil and Mars Global Simulant MGS-1 for 28 days (about 4 weeks) without any addition of nutrients aside from water. Growth in earth soil is much better than on Mars Global Simulant MGS-1, and even 75 % Mars Global Simulant MGS-1: 25 % earth soil is slightly better than 100 % Mars Global Simulant MGS-1. The growth of water spinach shoots in Mars Simulant was compared to poor water spinach root growth in Mars Simulant. Our results provided the insight that it is possible to grow water spinach in Mars Simulant indoors and to boost its growth, physical properties and chemical composition of Mars Simulant improvement was needed.

Introduction

The prospect of achieving a self-sustaining human presence on Mars has captivated scientists and space enthusiasts alike. However, Mars's inhospitable conditions and scarcity of resources pose significant obstacles to sustaining life, including food provision. Traditional agricultural practices relying on Earth's fertile soil and sunlight may not directly applicable to the Martian surface due to differences in soil composition, lack of atmospheric protection, and limited access to water and nutrients (Cockell, 2014).

To address these challenges, researchers have turned their attention to regolith simulants—artificial materials that closely resemble Martian soil in composition and physical properties. These simulants, composed of volcanic rocks and minerals like those found on Mars, serve as valuable tools for studying plant growth potential in Simulant Martian environment (Cannon et al., 2019; Kasiviswaneathan et al., 2022; Wamelink et al., 2014). Moreover, current advance in artificial light technology makes it possible to growth plant in indoor environment 5 to protect the plant from harsh martial environment.

Water spinach (Ipomoea aquatica), a leafy vegetable rich in essential nutrients, has been identified as a potential candidate for cultivation on Mars due to its easy to propagate 6, rapid growth, and high nutritional value 7,8. However, the suitability of water spinach for growth in a Martian in indoor environment using Mars Global Simulant MGS-1 remains unexplored.

Root is plant organ that directly contact with growth media, such as Mars Global Simulant MGS-1. Root absorbs nutrient and water from the media and distribute to all plant organ to support plant growth(Bengough et al., 2006). Leave, plant organ responsible for photosynthesis, depend on the nutrient form the root for its growth 10. Low nutrient supply from the root can be observed from leave size or color. Any condition that affected leaf growth finally will reduce supply of energy and carbon source form plant growth and development11. The aim of this study was to evaluate the shoot and root growth of water spinach in Mars Global Simulant MGS-1 with or without additional of earth soil to get some insight of opportunity to growing water spinach in Mars Global Simulant MGS-1.

Methods

Density and pH of Growth Media

Medium pH was measured by suspended 50 g media with 100 ml distillate water. After 15 minutes, the liquid was filter and the pH was measured using a portable pH meter (https://www.adwainstruments.com/support/knowledge-base/how-to-test-soil-ph). Soil densities were done by measuring the weight of 50 ml media using digital balance. Soal density was calculate using following formula:

Plant Growth Experiment

Ipomoea aquatica used in this experiment was bought from an online shop while Mars Global Simulant MGS-1 was provided by Exolith Lab as part of Plant Mars challenge competition. Ipomoea aquatica seeds were soaked in water for 12 hours and set to drain and pre-germinate for another 24 hours. Pregerminated seeds were divide into 4 groups (three seed per group) and transferred into 50 falcon tubes containing 35 ml of 100% earth soil (E), mixture of 75% (v/v) of Mars Global Simulant MGS-1 and 25% (v/v) of earth soil (3M1E), 50% (v/v) of Mars Global Simulant MGS-1 and 50% (v/v) of earth soil (1M1E) or 100% simulant (M). The bottom of the falcon tubes contains two holes to avoid water lodging. After germinating, seeds were grown in a box (Figure 1) illuminated with 7-watt cold white LED (4000K, Toshiba) lamps with photoperiod 12 h-1d-1. The LED lights were adjustable, and their position was set at 15 cm above the plant. The plants were irrigated manually with tap water (pH 9.02 and electrical conductivity about 481 µS/m) using a 3 ml syringe to provide 1 ml of water in the first two weeks, 2 ml in the third week and 3 ml in fourth week, two times a day. Humidity and temperature were not controlled, but the temperature recorded ranged from 26.9 to 31.2 0C and relative humidity from 48 to 56.

Growth and development of the plants was observed at 14 days (14D) and 28 days (24) after germination by measuring the shoot high and number of leaves developed. Shoot length was measured from the base of the shoot to the tip of the longest leaf.

At 28 days (24D) after germination, plants were harvested. Media was removed carefully by dripping water to the media and the depth of root penetration was measured. Root lengths were measured from the boundary between root and shoot until the tip of longest root. Shoot and root fresh weight was measured using a digital balance (Sartorius). Leaf area was measured using ImageJ software. Only green leaves were included in this measurement. The color of the leaf was compared based on the R G B method using image J software.

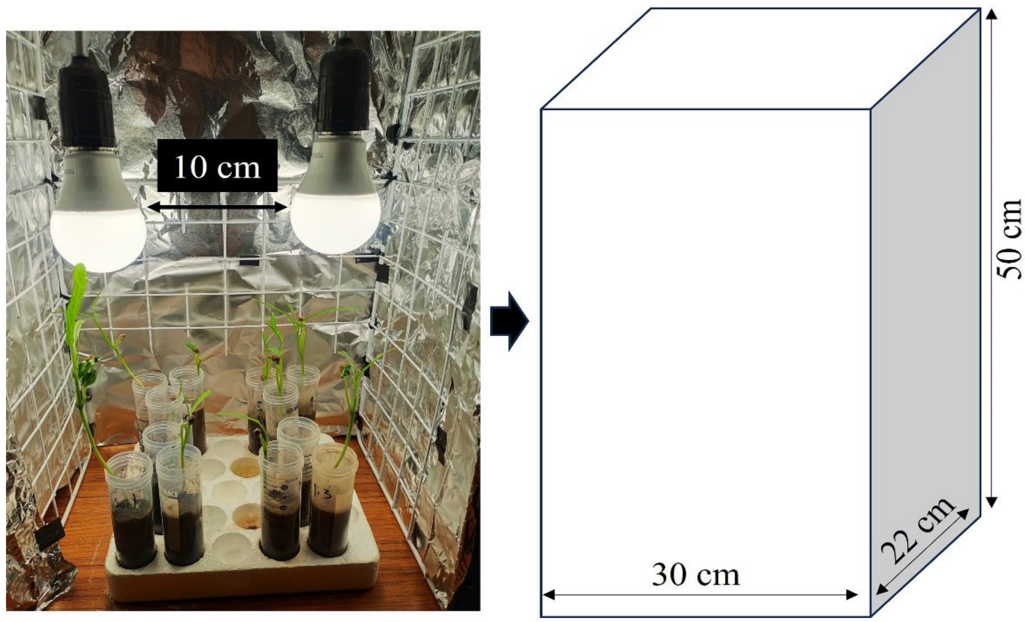

Figure 1. Diagram of grow box. The box was cover with aluminum foil in 4 sites (upper, left, right and back side)

Results and Discussion

Result

Plant Growth and Development

Mars Global Simulant MGS-1 has high pH and density (Table 1). Compared to earth soil, pH of MGS-1 is about 2 unit higher and 2 time denser. Addition of earth soil to MGS-1 reduces both its pH and density.

Table 1. pH and Density of four different media. Each value in the table was average from two replications.

Media properties	M	3M1E	1M1E	E
pH	9.86	9.05	8.49	7.215
Density (g/L)	1634.6	1376.6	1203.25	917.4

All plants exhibited growth within the 28 days (about 4 weeks) after germination (28D). However, one plant from 3M1E and E group showed abnormal growth and therefore was not involved in growth measurement (Figure 2A).

All plants showed similar development at 14 days (about 2 weeks) after germination (Figure 2B). The average shoot length of water spinach at M, 3M1E, 1M1E and E media were 10.5, 11, 12 and 13.5 cm, respectively. Most of shoot growth in this period occurred due to hypocotyl elongation. Except for the M group, all plants significantly showed growth from 14D to 28D. The shoot length of water spinach in M, 3M1E, 1M1E and E increased by about 39.7, 73.2, 86.1 and 142.6%, respectively, from 14D to 24D (Figures 2C and D). Shoot length at 24D showed that adding earth soil to Mars Global Simulant MGS-1 can improve the growth of water spinach. Water spinach grown in the media 50% of earth soil (1M1E) exhibited significantly longer shoots compared to water spinach grown in Mars Global Simulant MGS-1 alone. Leaf development showed a similar pattern with shoot growth (Figure 2 C and E). All water spinach only had one expanded leaf at 14D (Figures 2 B and E). Rapid leaf development occurred from 14D to 28D. At 24D, water spinach growth in M, 3M1E, 1M1E and E media had averages of 4, 4.5, 5.7 and 6.5 fully expanded leaf sizes, respectively.

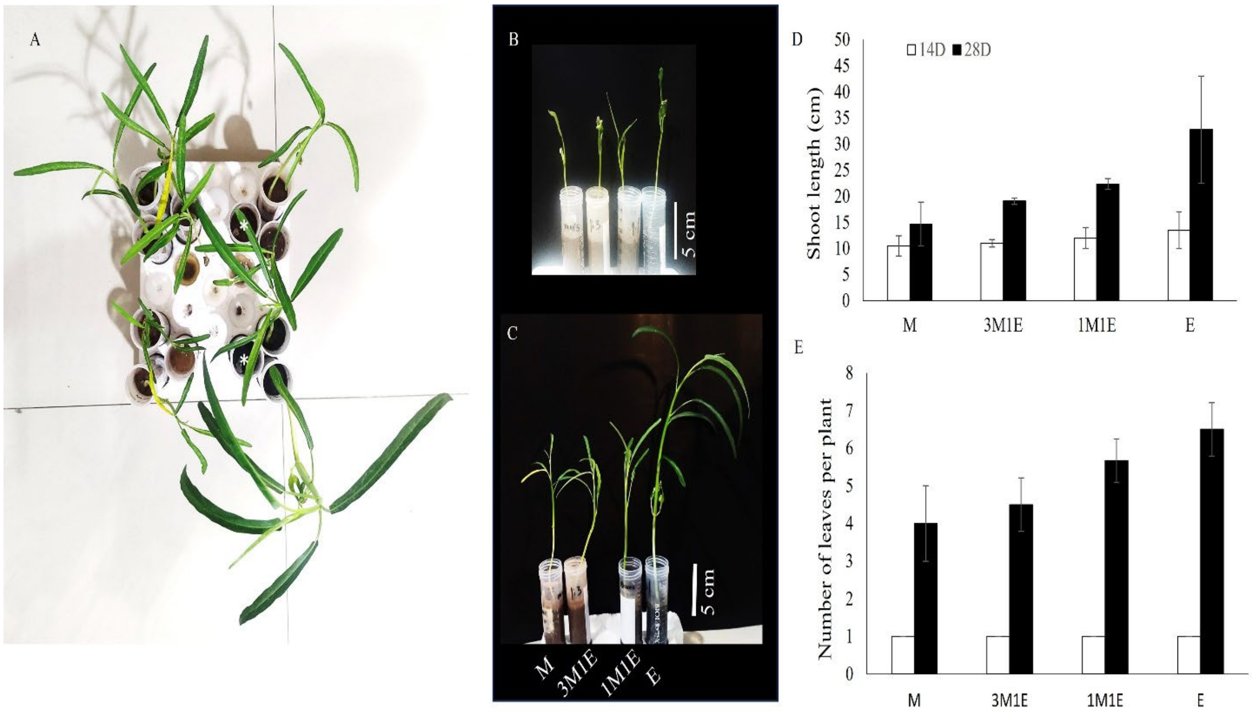

Figure 2. Growth and development of spinach at 14 and 28 Days after culture in Mars Global Simulant MGS-1, earth soil, or combination of Mars Global Simulant MGS-1 and earth soil. A. shoot growth at 14 days (about 2 weeks). B. Root growth at 28 days (about 4 weeks). C. Shoot length at 14 and 28 days (about 4 weeks). D. Number of leaves on 14 and 18 days (about 2 and a half weeks). Bar indicates SD of three or two replications. * Seed that develops abnormally.

Observation on the leaf organs showed that media composition influenced the leaf length, width, and area (Figure 3). Compared to water spinach growth in earth soil, water spinach in mars simulant showed lower leaf length (Figure 3A), width (Figure 3B) and area (Figure 3C) starting from the first leaf. The difference in those three parameters was the increase from first leaf to fourth leaf. Addition of earth soil to mars simulant can improve leaf development starting from the first leaf. In addition, yellow leaves were observed in water spinach grown in M, 3 M1E and 1M1E, but not in E media.

Figure 3: Leaf development of water spinach in fourth different media. A. leaf length. B leaf diameter. C. Leaf area. D the comparison of leaf size. Bar indicates SD of three or two replications.

Plant Morphology at 24 days After Culture

Water spinach grown in Mars Simulant and earth soil showed contrasting morphology in both shoot and root parts at 28D of culture (Figure 4 and Table 1, 2 and 3). Water spinach grown in earth soil has more leaves, taller shoots, higher shoot fresh weight, wider stems and higher total leaf area compared with water spinach grown in mars simulant (Table 1). In contrast, observation of second leaf from shoot apex (functional leaf) only showed a slight difference in red, green, blue, and blue green color (Table 2). Roots of water spinach in earth soil showed more branching and were longer compared to water spinach grown in mars simulant (Figure 4B). Additionally, root fresh weight of water spinach in earth soil was five times higher than water spinach grown in mars simulant (Table 3). Roots of water spinach in earth soil also could penetrate 3 times deeper than water spinach in mars simulant. Moreover, shoot to shoot ratio of water spinach in earth soil two times higher than water spinach in Mars Simulant (Figure 4).

The addition of earth soil to mars simulant also improves the morphology of water spinach. Most of the morphological parameters of water spinach growth in 3M1E dan 1M1E showed higher values than water spinach in M but lower than E (Figure 4 and Table 1, 2 and 3). Addition of 25% earth soil increased shoot length, shoot fresh weight and leaf area by about 1.29, 1.67 and 2.94 times, respectively, compared to water spinach in mars simulant (Table 1). For plants grown in 50% of earth soil, shoot length, shoot fresh weight, and leaf area by about 1.52, 2.72 and 3.70 times, respectively, compared to water spinach in mars simulant (Table 1). The presence of earth soil provides an obvious change in root development. Water spinach grown in the media containing 25 (3M1E) to 50% (1M1E) earth soil shower longer roots and more root branching (Table 3). The roots of water spinach grown in 25% soil for 26 days (about 3 and a half weeks) were 2.45 times heavier in fresh weight, 1.5 times longer and had 2.3 times deeper root penetration compared to water spinach grown in mars simulant. Fresh root weight, root length and root penetration were improved by about 3.15, 1.87 and 2.74 times more respectively, when 50% earth soil were mixed in the growth media. In contrast to other parameters, addition of earth soil up to 50% had a slight effect on stem diameter leaf color (Table 2) and shoot to root ratio (Figure 5).

Figure 4: Root and shoot morphology of water spinach at 28 Days after culture in Mars Global Simulant MGS-1, earth soil and combination of Mars Global Simulant MGS-1 and earth soil. A. the morphology of whole plant. B the root morphology.

Table 2: Shoot fresh weight, shoot diameters, and total leave area of water spinach culture in different media for 28 days. Data show in the table was average from three or two replication ± SD

Media	Shoot fresh weight (g)	Shoot diameter (mm)	Total leave area (m2)
M	0.38 ± 0.11	2.17 ± 0.06	3.23 ± 1.04
3M1E	0.63 ± 0.03	2.30 ± 0.14	9.50 ± 0.91
1M1E	1.02 ± 0.15	2.80 ± 0.79	11.97 ± 1.05
E	2.43 ± 1.43	4.35 ± 0.78	26.81 ± 11.08

Table 3: Leaf color of second leaf from the shoot apex of water spinach in four different media for 28 days. Data show in the table was average from three or two replication ± SD

Media	R	G	B	G+B
M	28.89 ± 2.83	48.37 ± 2.55	22.74 ± 4.71	71.11 ± 2.83
3M1E	28.72 ± 0.97	53.75 ± 0.34	17.53 ± 0.63	71.28 ± 0.97
1M1E	32.02 ± 0.86	55.68 ± 3.36	12.30 ± 3.27	67.98 ± 0.86
E	29.62 ± 2.23	49.27 ± 0.98	21.11 ± 3.21	70.38 ± 2.23

Table 4: Root fresh weight, Root length and root penetration of water spinach culture in different media for 28 days. Data show in the table was average from three or two replication ± SD

Media	Root fresh weight (g)	Root length (cm)	Root penetration (cm)
M	0.11 ± 0.02	6.17 ± 1.44	2.92 ± 1.04
3M1E	0.26 ± 0.04	9.25 ± 2.47	6.75 ± 1.77
1M1E	0.34 ± 0.03	11.33 ± 1.26	8.00 ± 0.00
E	0.31 ± 0.11	10.50 ± 2.83	8.00 ± 0.00

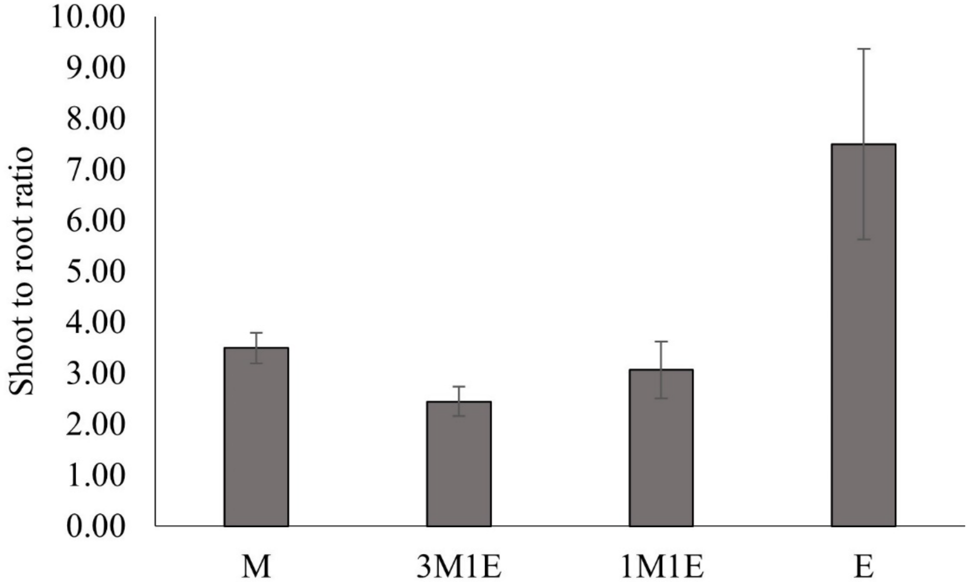

Figure 5. Shoot and root ration of water spinach at 28 Days after culture in Mars simulant, earth soil or combination of Mars simulant and earth soil. Bar indicates SD of three or two replication.

Discussion

All water spinach was still at seedling stage with single true leave after 14 DAG (Figure 2B). At early seedling stage, plant growth depends on food reserve inside seed endosperm and less dependent of nutrient from soil 11,12. Our current finding showed only slightly different at 14DAG growth between water spinach grown in M,3M1E, 1M1E and E media. The effect of the growth media on water spinach growth and development obviously showed from 14 DAG to 28DAG (Figures 2 D and E). During this period, all groups showed an increase in leaf numbers (Figure 2E). Water spinach grown in Mars Simulant produces the least number of leaves (average 4 leaves per plant), followed by water spinach in 2M1E, 1M1E and E media. Not only number of leaves, the size of 1st, 2nd, 3rd and 4th leaf of water spinach in M media was smaller than water spinach in in 2M1E, 1M1E and E media (Figure 3). Leaf is a plant organ responsible for photosynthesis which provides source of energy and carbon backbone for plant growth and development 13. Cumulative plant photosynthesis is influenced by chlorophyll content (green pigment) in the leaf and total leaves area14. Analysis of leaves color indicates only slightly different in leaves color between group (Table 3). Based on that data, small size of leaves of water spinach in comparison with other group might predominantly limit their ability to provide energy and Carbone backbone for plant growth. As result, shoot and root parameter of water spinach at 28DAG were the lowest among all group.

Beside the energy and Carbone backbone from leaf photosynthesis, the nutrient deliveries from root to shoot also play key role in plant growth, especially at transition from food reverse dependent to fully photoautotrophic condition. Many reports showed the connection between root and shoot development 15–17. Current research also shows the connection between shoot and shoot growth of water spinach (Table 2 and 4). Water spinach in M showed root growth inhibition as shown by low root fresh weight, length, and less deep root penetration (Table 4). One factor that might cause this root growth inhibition is related to soil physical property. Mars is more compact than other media (Table 1). Previous research showed compact media inhibit root growth that indicate by short root and less deep root penetration 18,19.

The addition of earth soil to mars simulant improves media properties indicated by increase root growth and penetration (Table 4). Comparison of root and shoot fresh weight of water spinach in M, 3M1E and 1M1E showed that increase root biomass was followed by increase in shoot biomass with similar shoot to root ratio (around 3). An interesting result was found in water spinach growth in E media. Plants in this media have shoot to root ratio 2 times higher than other group which means that with the same root biomass, the plant in E media can support 2 times higher in shoot growth compared to another group (Figure 5). Many reported showed shoot to root ratio effected by nutrient in the media. In general, high shoot to root ration occur in the media with rich nutrient 17,20. This result might indicate that Mars Simulant not only has physical properties that inhibit root growth but also contains less nutrient needed for water spinach to grow.

Conclusion

Mars Simulant can support the growth of water spinach. However, improving physical and nutrient properties, such as by adding substance that has character like earth soil is needed to improve its growth.

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