Green on red: phenotypic and molecular study of Italian ryegrass grown on Martian regolith simulant

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Introduction

If human settlements will ever be established on Mars or the moon, it will be crucial to produce edible plant biomass *in situ*.

Preliminary data were gathered on the response of *Lolium multiflorum* Lam. to the growth on Martian regolith simulant (MMS1, Mojave Mars Simulant) without supplying any fertilizers**.** This monocot was chosen as a representative Gramineae and because the biomass from previous harvests could be used as organic amendment [1].

Previous studies reported that plants can grow on Martian soil simulants [1]; however, **molecular studies**, such as gene expression analysis, **are still missing**. This work aims at **filling this gap**, by **providing gene expression data** coupled with **optical microscopy**.

Experimental design

Figure 1: Image showing the experimental set-up.

- Two germinated seeds were placed in 50 mL tubes filled either with soil or MMS1 (3 biological replicates each composed of a pool of 6 plants; Fig. 1).
- The plants were grown under controlled conditions (16h light at 25° C/8h dark at 20° C) and were watered every 3 days with deionized water.
- The plants were harvested after 7 and 14 days, then they were cut and the molecular response of newly formed leaves was studied after 7 days.

Results: Microscopy

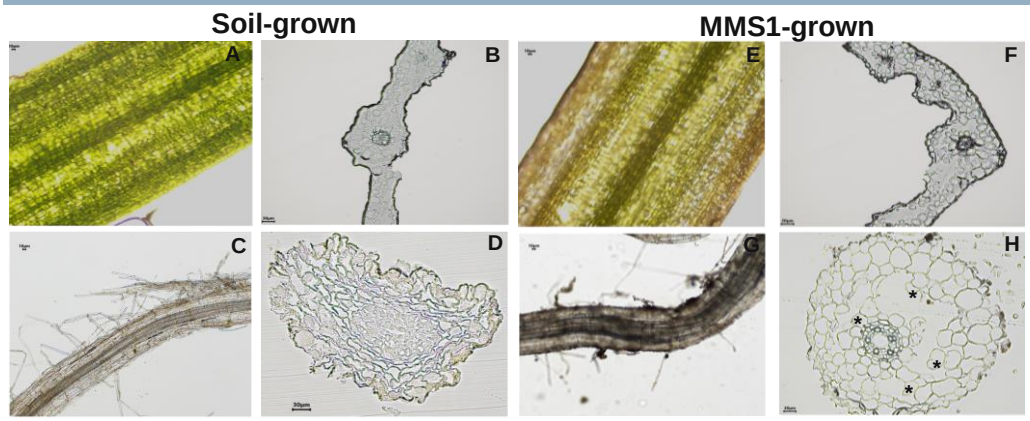

Figure 2: Light microscope images of leaves and roots grown on soil (A-D) or MMS1 (E-H).

The leaves of MMS1-grown plants were **chlorotic**, **curled** and showed a **less developed xylem** tissue (Fig. 2E-F) compared to control ones (Fig. 2A-B).

Fewer root hairs formed on MMS1 (Fig. 2G) compared to regular soil (Fig. 2C) and **cortical lacunae** were well visible in the roots (Fig. 2H, asterisks).

The results confirmed that MMS1 caused nutrient deficiency, as evidenced by the presence of chlorotic leaves and lacunae in the roots [2].

Gene expression analysis

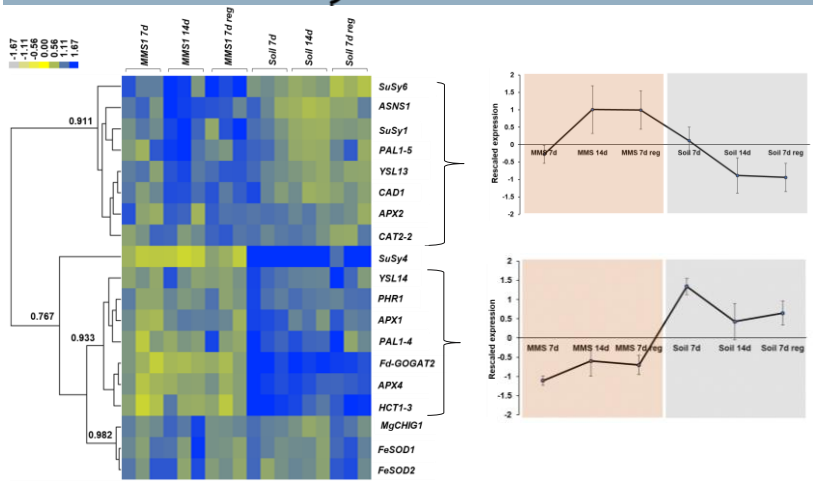

Figure 3: Heatmap hierarchical clustering of the gene expression data obtained from leaves after 7-14 days and 7 days regrowth after the cut. The rescaled expression values of genes in the 2 main clusters are shown on the right. The numbers refer to the Pearson's correlation coefficients.

A gene expression analysis was carried out on the leaves. Transcripts involved in C and N metabolism, stress response and lignification were targeted.

Two main clusters of genes can be observed (Fig. 3) showing **induction** or **repression on MMS1** (upper and lower cluster, respectively).

Isoforms of genes involved in C metabolism behaved differently depending on the growth conditions: sucrose synthase (*SuSy*) 1 and 6 were induced when plants grew on MMS1, while *SuSy4* was down-regulated.

The expression profile of a key gene involved in N metabolism, asparagine synthetase (*ASNS1*) [3], suggests a response involving **N remobilization** in plants grown on MMS1.

References:
[1] Wamelink GW, et al. (2014) *PLoS One* 9:e103138.
[2] Hu B, et al. (2014) *Ann Bot.* 113:181-189.
[3] Avila-Ospina L, et al. (2015) *J. Exp. Bot.* 66:2013-2026.

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