Effect of lunar soil mixtures on physiological parameters of *Pea sativa* cv. P. Freezer and *Triticum aestivum* cv. Chagual

by Space Growers

Introduction

Plant as sessile organisms are subjected to environmental changes and pressures, their capacity to survive are key in terms of evolution of ecosystems. Food and Agriculture Organization (FAO) estimates that the population for 2050 will be reach to 10,000 million of persons around the world and water and crops will be an important limit (Hunter et al., 2017). For this reason, the astrobiological exploration of new soils to increase the crop efficiency thinking in the future colonization of outer bodies is an important goal for plant scientists (Wamelink et al., 2014). The aim of the present work is to determine the effect of mixtures of lunar and organic soil in the growth and physiology of two important crops: wheat and peas.

Cereals are important model of study, represent an important fraction of cultured crops and provides the most important font of food (basically carbohydrates) for human nutrition (McKevith, 2004), on the other hand, legumes are important font of nitrogen (the principal in plant-based fooding) and represents an important crop in improve nitrogen availability in soils for their capacity to generate symbiotic interaction with nitrogen-fixing bacteria (Jacob et al., 2016).

Extraterrestrial soils are previously studied, at gross mode, are composed by oxygen (42%), silicon (21%), iron (13%), calcium (8%), aluminum (7%), magnesium (6%) and other substances (3%) (Lucey, 2004), this soil represent a poor font for plant requirements growth and the aim of this work is understand the effect of mixtures of lunar soil with organic soil in wheat and pea physiology and morphology.

Theory.

According to the presented antecedents, we analyze the hypothesis that toxic substances present in lunar soils alters physiology and inhibit growth in wheat and pea.

Measurement methods.

Plant material and experimental design. Seeds of commercial crops of wheat and pea was obtained from local market, selected cultivars were wheat cv. Chagual and pea cv. P. Freeze. Before to sowing, seeds were sterilized using bleach 5% 3 min at room temperature, washed three times and activated during one hour in water. Five seeds were sowed in pots of each species, and fully watered. Selected soils were 100% of organic soil (control) and 3:2 proportion of organic:lunar soils (OS:LS) with n=6 per condition. Seeds and plantlets were cultured at 26±4 ºC with 18/6 h light/darkness for five weeks (2.782 lux).

Physiological parameters. After five weeks, samples were collected and analyzed according to the following procedures:

a. **Germination efficiency:** Total seeds germinated were analyzed and expressed as percentage.
b. **Total growth and rates:** Plantlets were collected, and soil was removed with water. Total aerial and radicular elongation were registered.
c. **Total leaves and brown/green rate:** Total leave count was registered according both phenotypes, old or new leaves and brown or green.
d. **Photochemical parameters:** We analyze using PEA (Hansatech) the photosynthetical efficiency (Fv/Fm) and the electron transport rate (ETR @1000 µmol m^{-2} s^{-1}) of leaves (Contreras et al., 2019).
e. ~~**Photosynthetic pigments:** 100 mg of leaves were grinded in mortair and pestle using liquid nitrogen and were extracted using 80% acetone, the extracted pigments were analyzed using spectrophotometer (Agilent 8454) at 470, 649 and 665 nm and pigment concentrations were obtained according previously described in literature.~~

Soil parameters. We analyze pH and conductivity using soil pH/conductivi-meter (Hanna) and the content of soluble nitrate and sodium were determined using 1:10 dilution of soil in MilliQ water, extracted during 2 h at 25 ºC by orbital mixer with selective electrodes (Laquatwin Horiba).

Statistical analysis. All analysis were realized with n=5 and analyzed with ANOVA.

Analysis and results.

1. **Plant morphology and physiology.**

In order to determine the effect of LS, we register the anatomy and morphology of plantlets collected in the experiment with pictures (Fig. 1). The results reveal some anatomical effects, first, when we compare wheat in OS vs OS:LS 2:3, we determine differences in the branching and root growth, second, the wheat in OS:LS mixture shows the presence of spikes. Second, in the case of pea, we determine an effect in the elongation of aerial tissue and a strong effect on branch angle, in both cases (OS and OS:LS) peas shows development of flowers and tendrils. In both species, wheat and pea, we observe chlorosis and necrosis of older leaves, determined by the browning and loss of water content. Finally, in the case of pea, we determine succulency in green leaves.

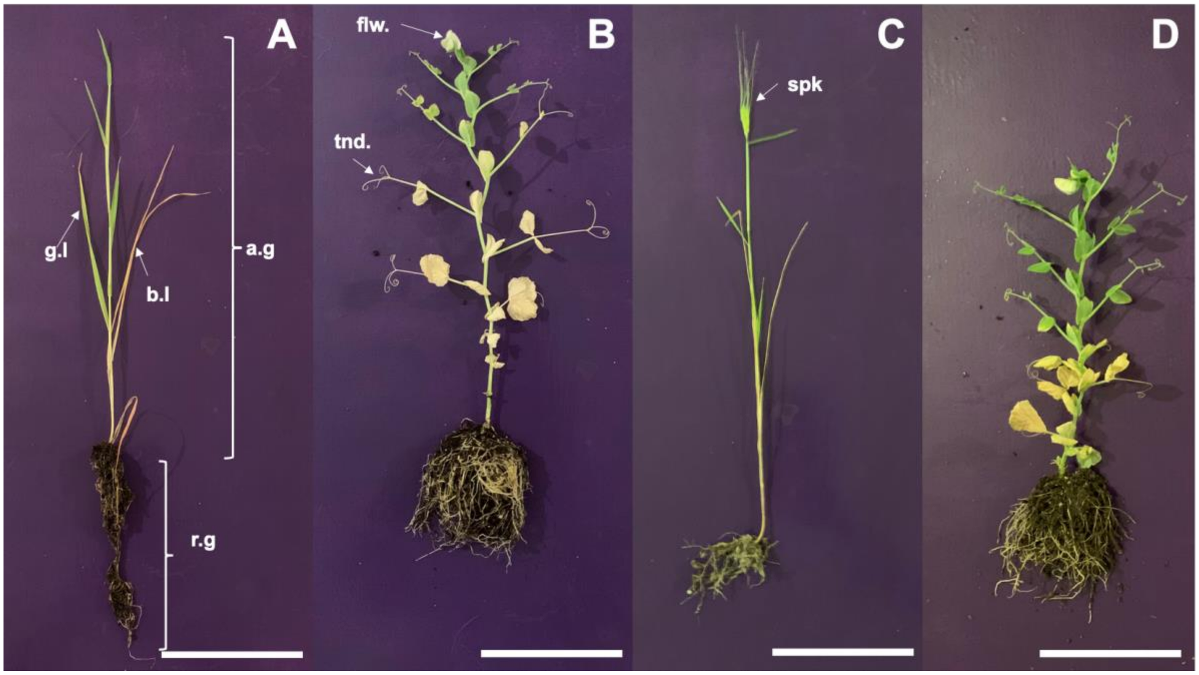

Figure 1. Picture of 5 weeks-old plantlets. (A) wheat in 100% of OS, (B) pea in 100% of OS, (C) wheat in 3:2 OS:LS and (D) pea in 3:2 OS:LS. g.l: green leaves, b.l: brown leaves, r.g: root growth, a.g: aerial growth, flw: flower, tnd: tendrils and spk: spike. Bars represent 10 cm.

To complement with quantitative data, we analyze the anatomical parameters. First, we determine the germination efficiency, the results shown a negative effect of OS:LS in the wheat germination, in pea, results do not show a significant effect on germination.
On the other hand, when we analyze quantitatively the growth (as elongation), as germination, wheat shows a partial inhibition in the aerial elongation, and peas does not show significant differences, in the case of root elongation, the mixture of OS:LS shows a strong and significant inhibition of root elongation in wheat, when we analyze the rate between aerial and root elongation, the results reveals the effect on root inhibition on wheat (Fig. 2).

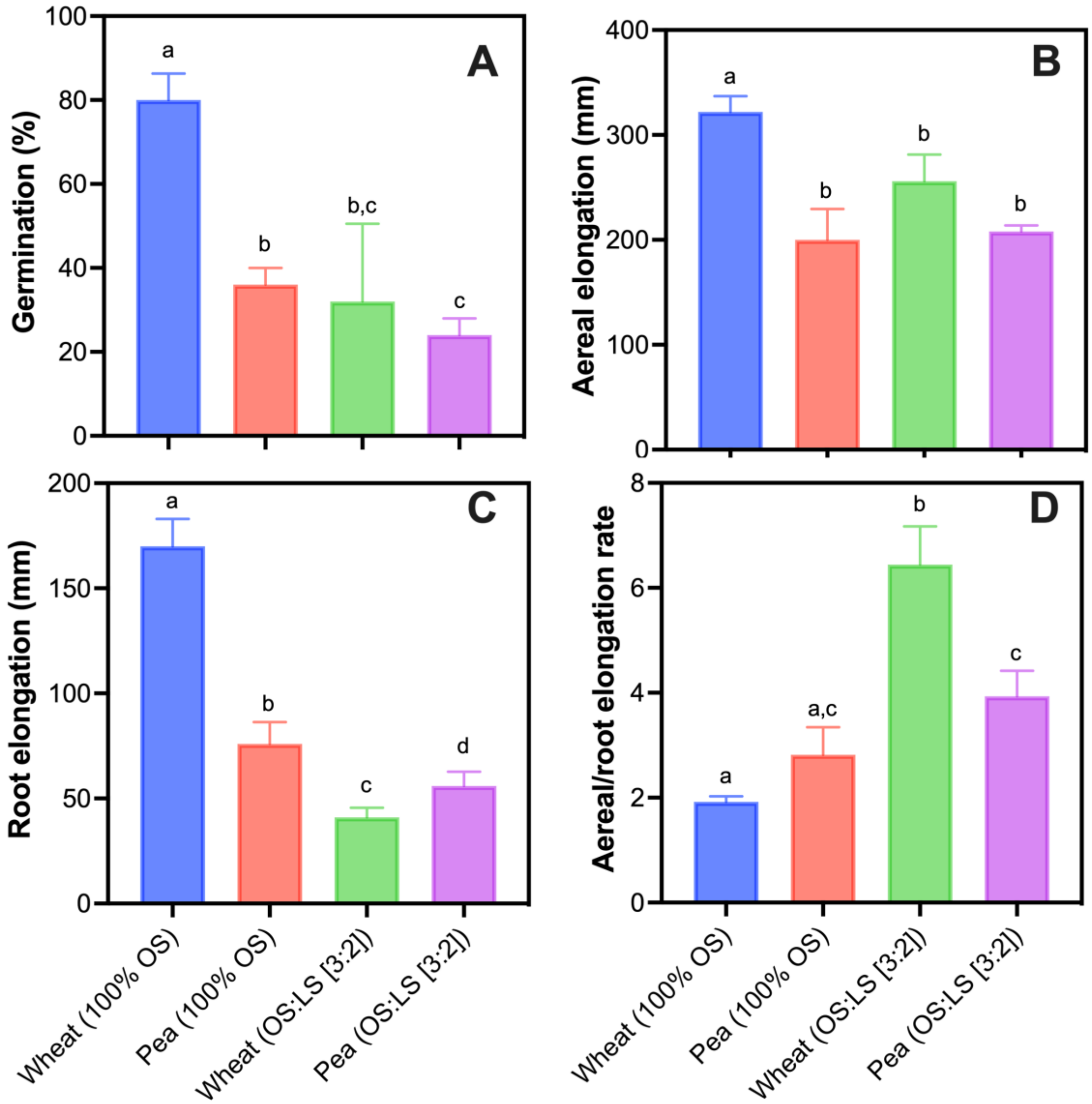

Figure 2. Physiological parameters of plantlets grown under control and 3:2 OS:LS soils. (A) germination efficiency expressed as percentage, (B) aerial and (C) root elongation, and (D) aerial/root elongation rate. Bars correspond to a mean (n=5) plus standard deviation. Significant differences were determined using one-way ANOVA with Tukey's post-test (p<0.01).

In the same way, we analyze the effect in the development of leaves. The first observation is that wheat does not shows effect in total leaves, however, the plantlets that grown in OS:LS shows a major proportion of brown leaves, respect to plantlets that grown in OS. In the case of pea, we observe a similar pattern, with an increase of brown leaves in plantlets that grown in OS:LS (Fig. 3).

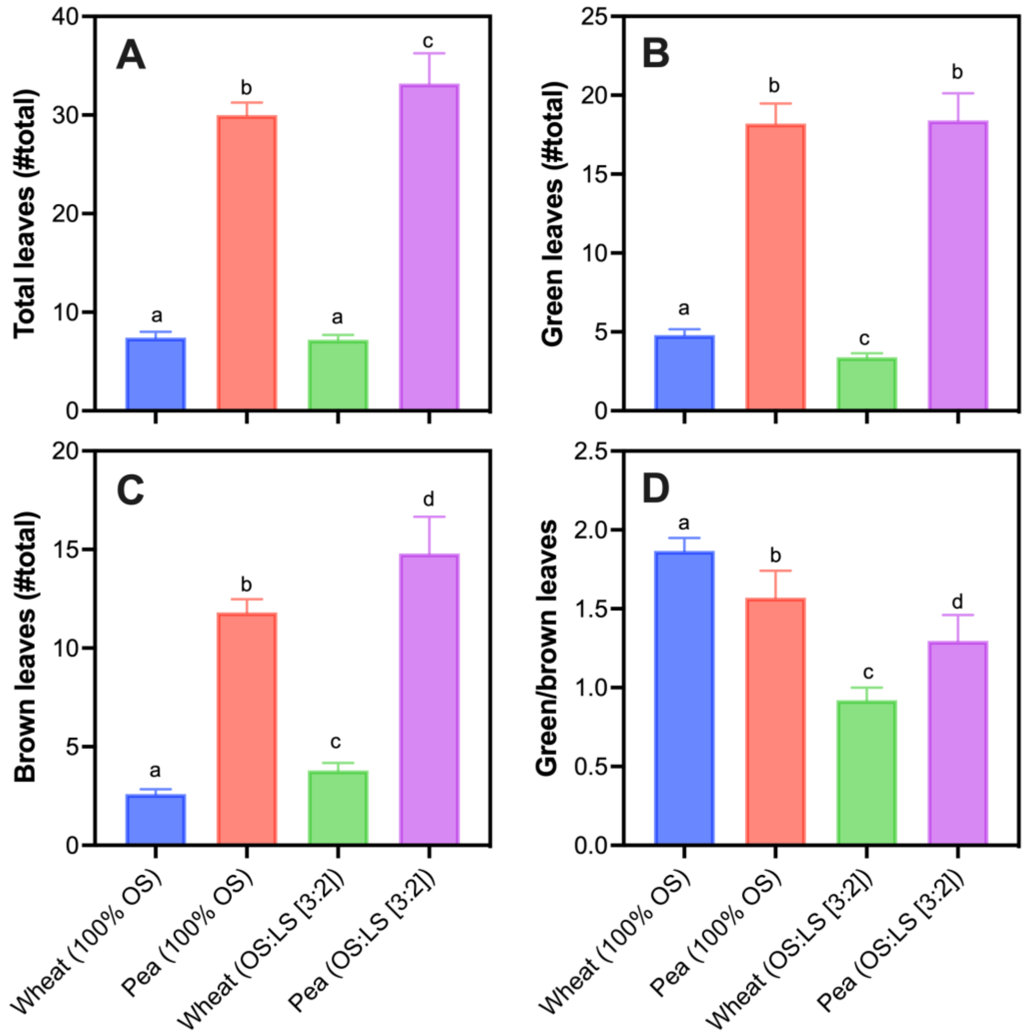

Figure 3. Total leaves (green and brown) of plantlets grown under control and 3:2 OS:LS soils. (A) total leaves per plant, (B) green and (C) brown leaves, and (D) green/brown leaves number rate. Bars correspond to a mean (n=5) plus standard deviation. Significant differences were determined using one-way ANOVA with Tukey's post-test ($p<0.01$).

To understand at the physiological level, we determine photochemical parameters (Fig. 4). First the maximum photochemical efficiency of photosystem II (PS II), the results show first as the brown leaves does not have photochemical activity, second, both species have less PS II photochemical activity when are compared with OS grown plantlets (Fig. 4A). On the other hand, when we analyze the electron transport rate (ETR) with constant light source, we observe a significant negative effect of OS:LS in both species, especially in wheat (Fig. 4B), ETR were not registered in brown leaves.

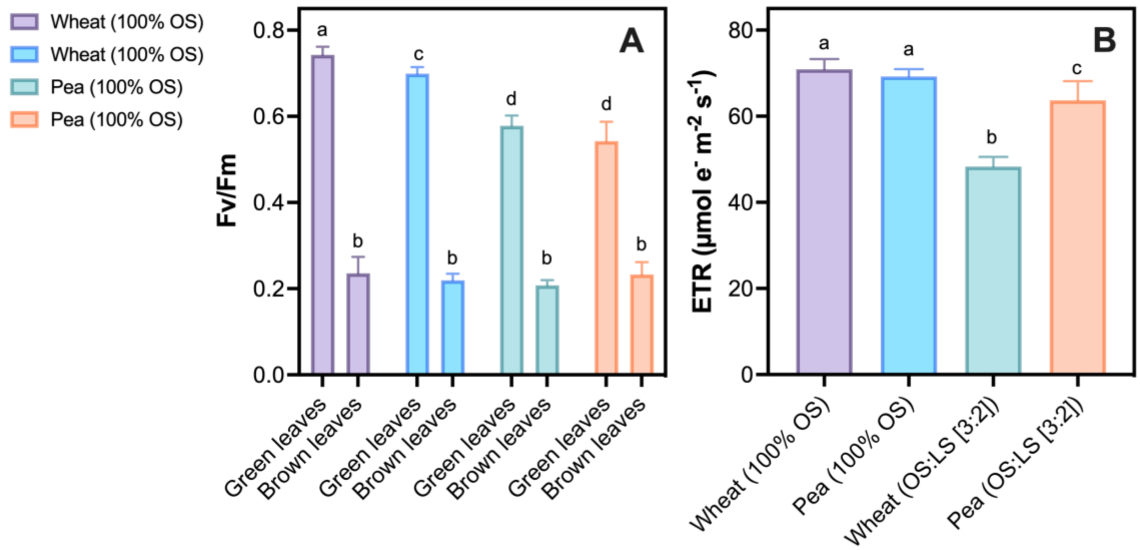

Figure 4. Photochemical parameters. (A) PSII photochemical efficiency (Fv/Fm) and (B) electron transport rate at 1000 µmol m^{-2} s^{-1}. Bars correspond to a mean (n=5) plus standard deviation. Significant differences were determined using two-way ANOVA (Fig. 5A) and one-way ANOVA (Fig. 5B) with Tukey's post-test ($p<0.01$).

2. Soil parameters.

In order to determine the relationships of soil composition and the anatomical and physiological parameters, we analyze pH, conductivity, nitrate and sodium content (Fig. 5). First, the results reveal that plantlets germination and development does not have significant influence in soil pH, the LS have a basic pH and generate a decrease in soil acidity when is mixed with OS (Fig. 5A). In terms of conductivity, pea grown in OS generate an increase in conductivy (Fig. 5B). Nitrate content is higher in OS when is compared with OS:LS mixture, in the case of plantlets grown in OS, results show a significant decrease in nitrate content in wheat grown soil, in case of pea, the results does not show significant differences (Fig. 5C). Finally, the sodium content in OS:LS mixture is higher than OS, but in both soils after harvesting the plantlets, have same level than OS control, a lower than OS:LS (Fig. 5D).

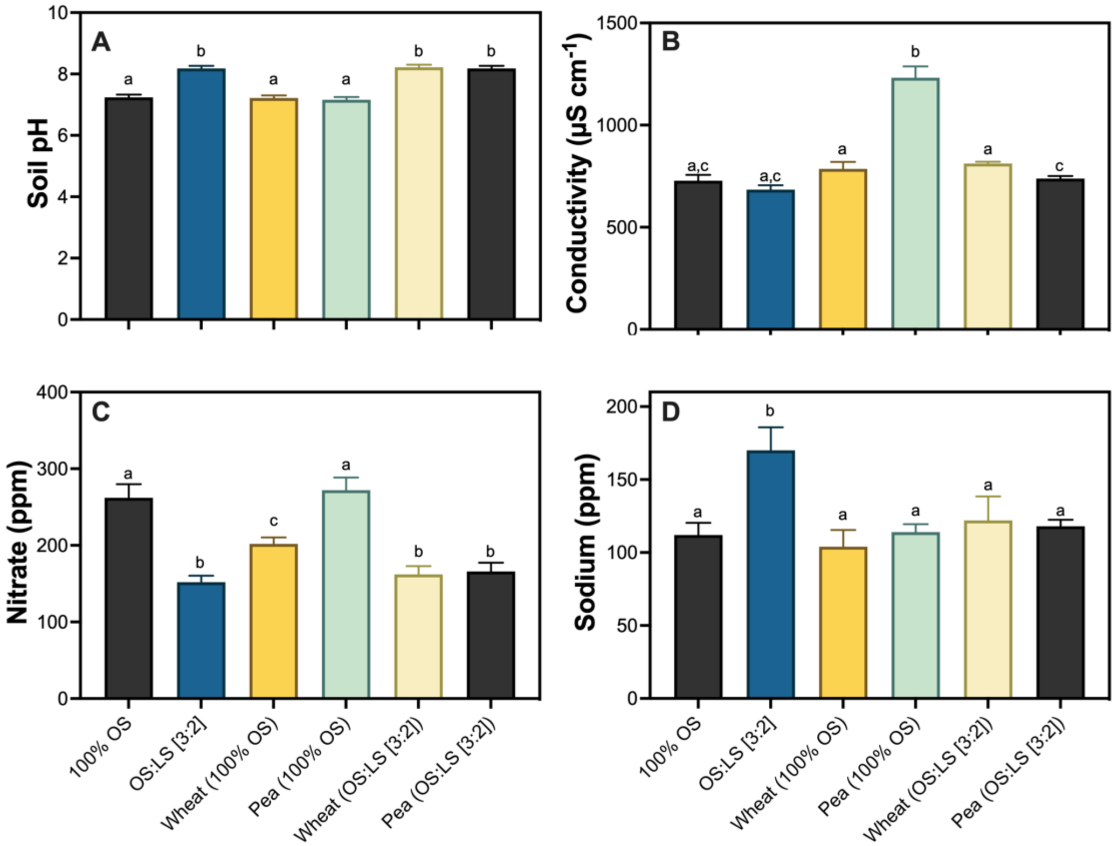

Figure 5. Physicochemical parameters of soil. (A) pH, (B) conductivity, (C) nitrate, and (D) sodium content. Bars correspond to a mean (n=5) plus standard deviation. Significant differences were determined using one-way ANOVA with Tukey's post-test ($p<0.01$).

Discussion.

Plants are good biomarkers for environmental studies, the incapacity to migrates position them as an ideal model to understand the extreme conditions. As mentioned before, FAO estimates important growth of population for 2050 and the demand for safe and quality food is a fact, for this reason scientist will be research in new technologies and conditions to understand forms to project the fooding technology, as part of solution, the astrobiology explores the possibility to start sustainable cultures outer the Earth.

This work explores the effect of lunar soil mixed with organic soil in the physiology of two important crops: wheat as font of carbohydrates and pea as font of plant-based proteins. The results shows in first that the lunar soil decrease acidity of soil and is poor in nitrate (important font of nitrogen for plants, especially for production of proteins in legumes) in general, the physiology is negatively impacted by lunar soil, but does not induce the plant death, both type of soils show chlorosis and leaf death in old leaves in wheat and pea, this result suggest a stress for absence (o low concentration) of mobile nutrients in organic soil used (nitrogen, potassium, phosphorous, magnesium, molybdenum or zinc), considering the observation of partially yellow nerves and necrotic areas in young leaves, probably is low concentration of potassium, we need to analyze soil to determine the macronutrient composition. On the other hand, succulence in peas and the low elongation of roots (with high ramification), suggest stress by excess of iron in soil, considering the discrete differences in conductivity and sodium content in soil, plus the root inhibition, the results discard an osmotic or salt stress effect. The presence of toxic substances such as iron in toxic sub-lethal levels explain, almost in part, the symptoms observed when we compare both species in the two culture conditions (Wang et al., 2019)(Sharma et al., 2020)(Spiller et al., 1987)(Nenova, 2008).

Analyzing the effect of lunar soil in the anatomy, the results suggest an effect in hormone synthesis and/or signaling. Gibberellins and brassinosteriods are widely studied in terms of promotion of tillering in cereals and branching in dicots (Best et al., 2017)(Tong et al., 2014), our results show an inhibition of both in plantlets developed in lunar soil, but we need to analyze the hormone profile to determine the complete effect of both hormones, apical dominance was not significantly different, but peas in lunar soil mixture shows dwarfism, indicating complex hormone regulation, plus the effect of pressures of ethylene that explains the leaves abscission, and dwarfism, on the other hand the presence of sub-lethal toxic concentration of iron explains in part the phenomenon of root inhibition in wheat grown in lunar soil mixture (Ward et al., 2008). As a complement some authors describe the effect of metallic substances as inhibitors of gibberellins, auxin and brassinosteroid metabolism(Ahmad et al., 2018)(von Wirén & Bennett, 2016)(Matsuoka et al., 2014)(Wamelink et al., 2014)(Li et al., 2015), supporting the hypothesis of iron substances in sublethal conditions in lunar soil.

Conclusion

Despite the negative effects of lunar soil in the studied species, both, peaa and wheat growth and develop chlorophylls, activates their photochemical systems and complete their life cycle including flowering, for this reason lunar soil generates stress in plantlets but in sub-lethal condition. We need to understand in deep the possible toxic substances that alters the physiology of plants and if our theoretical hypothesis of toxic levels of iron alters their physiology, alternatives as incorporate chelating agents probably improve the performance of plantlets in mixtures with lunar soils.

For the future is important determine the capacity to generate symbiotic interaction of legumes and nitrogen-fixing bacteria in presence of lunar soil, the result of this study does not show the presence of nodules.

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