

Tolerance of high mountain quinoa to simulated extraplanetary conditions. Changes in surface mineral concentration, seed viability and early growth

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ABSTRACT

We studied the tolerance of one species of quinoa achenes from ecotype RQ252 to simulated extraplanetary conditions in a vacuum chamber (high low-pressure 10^{-2} to 10^{-7} Torr, UV laser simulated plasma radiation, and cryogenic temperature). The selection of this ecotype of quinoa achenes was a condition to previous studies, where RQ252 shows evidence of high efficacy in grow adaptation in the South America Puna between 3800–4500 m asl subjected to low oxygen and increased UV radiation exposition. After extraplanetary experiment exposure, we evaluated quinoa tolerance to experimental conditions through germination and early growth responses under a controlled laboratory standard atmosphere. Rate and final germination subjected to high low-pressure treatments during 4 h, 8 h, and 16 h were not different to control. Laser plasma application accelerated the germination rates. Final germination always reaches values up to 90%. SEM-EDS analysis showed structural changes on the pericarp surface, especially in high low-pressure and high low pressure + plasma treatments. EDS revealed that the quinoa pericarp subjected to different treatments showed changes in mineral content. Potassium ions decreased under high low-pressure and high low pressure + laser plasma irradiation (between 32 and 42%) but increased in a prolonged vacuum (35%) and more when plasma was added (96%). Early growth was affected by the different treatments, being the radicle length the most affected parameter. Our results suggest that quinoa achene ecotype RQ252 viability has excellent tolerance to extraplanetary conditions.

1. Introduction

Long-term space exploration missions require life support systems to ensure astronaut survival. Future missions to Mars and further planetary exploration will involve long-term stays in space, which will depend on the development of life support systems for food production and resource regeneration. NASA has made some crucial contributions to this issue with the programs for the development of Closed Ecological Life Support Systems (CELSS) and experiments performed at the International Space Station (ISS) [1]; the BIOMEX group with the project “the habitability of Mars and limits of life” [2], and others [3]. These projects

performed tests on biological samples and plants through ground-based simulation and space experiments, laying the foundations for establishing Bioregenerative Life Support Systems (BLSSs). As such, these researches will contribute to future short-duration missions like low earth orbit, *cis-lunar* or lunar surface missions, and long-duration and distance exploration missions, like missions to Mars.

Growing plants in space require the knowledge of their growth responses not only to all environmental factors acting on Earth but also to specific space constraints such as altered gravity, ionizing radiations and confined volume; cultivation techniques need to be adjusted considering such limitations and the type and intensity of environmental factors to

Abbreviations: CELSS, Ecological Life Support Systems; ISS, International Space Station; BLSS, Bioregenerative Life Support System; pl, plasma irradiation.

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analyzed depend on the mission scenarios [3]. We can use plants to provide fresh food and revitalize the atmosphere by photosynthesis continuously. When designing a BLSS for food production/resource regeneration, the selection criteria for choosing the crop, species or cultivars candidates to grow is an essential subject for future human missions [1,3].

Various species such as cereals, fruits, tubers and leafy vegetables have been tested as potential candidates for food production in space [4]. The main selection criteria for these species were their adaptability to environmental constraints, short growing cycles; compact plant sizes; high productivity; tolerance to high plant densities; high light and water use efficiency; their tolerance to osmotic stress as well as their nutritional value and the presence of biologically active compounds related to health promotion, disease prevention and psychological health, with a particular preference for vegetables requiring little or no preparation to be eaten [1,3].

Also, the scientific community suggested that microgreens or sprouts are optimal candidates because they are richer in phytonutrient and biologically active metabolites content, particularly pigments such as carotenoids, phenols, anthocyanins and betalains considered as an indicator of the antioxidant properties of edible plants [1,5].

Quinoa (*Chenopodium quinoa* Wild) originated in the Andes Mountain (Titicaca Lake, between Bolivia and Peru border), is a traditional native crop used by the Andean inhabitants as food and beverage [6]. From this origin centre, quinoa was spread up to the north to Colombia and the south to Chile and Northwest of Argentina. Quinoa can be cultivated from sea level to high mountain (near 4000 m asl) and tolerates contrasting temperatures between day and night, drought, high salinity and poor or marginal soil with low nutrient concentrations.

For its high nutritional value and adaptability to extreme conditions, quinoa was recognized as an excellent complementary crop not only for marginal soils in many countries all over the world. Schlick and Bubenhien [30] evaluated quinoa as crop for Controlled Ecological Life Support System (CELSS) with results that showed a rapid growth and a good seed production. However, knowledge about quinoa survival rates, growth and development when exposed to extraterrestrial environments are necessary.

We hypothesized that achenes from a native Altiplano ecotype [36] named RQ252, from a high mountains region (3800–4500 m asl) with high photosynthetic assimilation [31] (despite being a C₃ species), shorth growth cycle (110–115 days against other ecotypes with 150–160 days) and tolerance mechanisms to extreme conditions on Earth such as high radiation, low pressure and low temperatures, would be an excellent candidate for BLSSs exploration.

The quinoa tolerance to the constraints mentioned above is attributed to its genetic plasticity, which allowed this species to develop tolerance/resistance mechanisms to low pressures [7], low temperatures [8], different intensities of UVB radiation [9–11] and salinity [10,12,13]. Molecular research has demonstrated that quinoa has a significant genetic diversity [14] that allows its adaptation to extreme environments [15].

The erroneously called grain, seed or cereal, quinoa fruit is an achene formed by the pericarp (two-layered fruit) and the seed itself. Moreover, the seed is formed by the seed coat (bitegmic, constituted by a testa and a tegmen), a peripheral curved embryo, a large central perisperm with starch as reserve substance and a micropylar endosperm rich in proteins and lipid bodies in the form of a cone surrounding the radicle tip [16–18].

The more conspicuous feature in quinoa fruit is the presence of different organic and inorganic compounds with high nutritional values. Quinoa has all the essential amino acids, more than 40 major, trace and ultra-trace minerals, free sugars, vitamins, antioxidants and essential fatty acids [19–24]. Probably the essential features in quinoa are its high biological value (82.8) compared to 59.0 for the wheat [25] and the energetic value of 1820 kJ/100 gr [20,26]. Moreover, quinoa has the advantage that it cannot only be consumed as a seed but also as fresh

sprouts or as green leaves in salads [27]. Quinoa sprouts are rich sources of magnesium, leucine, vitamin C, β-carotene, anthocyanins, flavonoids and phenols [28,29].

Thus, the aim of this study was to determine structural modifications, viability, germination rates and early growth responses of quinoa achenes ecotype RQ252 exposed to simulated extraplanetary conditions (high low-pressure, cryogenic temperature and plasma application in combination) during different periods of time. This study offers a new exploration of quinoa ecotypes that exist in the high mountains of South America and specially those growing around 4000 m asl or more.

2. Materials and methods

2.1. Experimental set up

Quinoa samples of achenes belonging to an Argentinian native ecotype called RQ252 were collected in Abra Pampa (Jujuy, Argentina –22°48'07.9"S; –65°49'27.6"W, 3800–4500 m asl) by collaborators from *Estación Experimental Agropecuaria Abra Pampa- Instituto Nacional de Tecnología Agropecuaria* (EEA-INTA, Agricultural Experiment Station - National Institute of Agricultural Technology), where Voucher specimens are maintaining at the National Institute of Agriculture and Technology (INTA) germplasms bank. Achenes and seedlings were studied in the *Instituto de Ecología, Comportamiento y Conservación* and *Instituto de Morfología Vegetal* at the *Fundación Miguel Lillo* (Institute of Ecology, Behavior and Conservation; and Institute of Plant Morphology at the Miguel Lillo Foundation), Tucumán, Argentina, in order to determine changes caused by space simulated conditions in achene morphology, surface mineral concentration, germination and early growth development. Space simulated experimental conditions, scanning electron microscopy with energy dispersive X-ray microanalysis (SEM- EDS) were performed at Planetary Instrumentation Exploration Laboratory, Lassonde School of Engineering, York University (Toronto, Canada).

2.2. Plant material. Quinoa achenes

For each experiment, quinoa achenes from ecotype RQ252 without any kind of previous preparation were used. Forty eight samples of 1 g of achenes (approximately 400 achenes) each, were taken and distributed in eight containers of six borosilicate glass petri dishes with a diameter of 50 mm. The achenes were ordered taking care that they were not stacked on top of each other (Fig. 1). Of each of the 48 samples, 16 remained as controls while the other 32 were subjected to the different experimental conditions. Each experimental condition (described in 2.5. section, Achene's exposure) was performed in duplicate and repeated twice independently.

2.3. Vacuum chamber

For lunar surface and space conditions simulation, quinoa achenes samples were studied in vacuum chamber (Fig. 1 B) in the Canadian Planetary simulator Centre for Research in Earth and Space Science (CRESS laboratory) at York University (Canada). This planetary simulator is a pressure vessel capable of reaching high vacuum using cryogenic pumps in conjunction with a mechanical roughing pump. For this study, high low-pressures of 10⁻⁷ Torr were achieved. The temperature control system consists of liquid helium cryo-coolers that heat and cool a platen and shroud. The temperature range of the platen depends on the method of heating and cooling, with ranges from LN₂ cooled –60 °C with heating up to 150 °C. During this experiment, temperatures were gradually changed from 20 °C to –80 °C in the shroud and platen. In addition, we introduced liquid nitrogen and obtained –200 °C controlled temperature during the experiment at the sample surface. The vacuum chamber experiment was monitored by LABView in-souse software during study. This software achieves advanced data logging

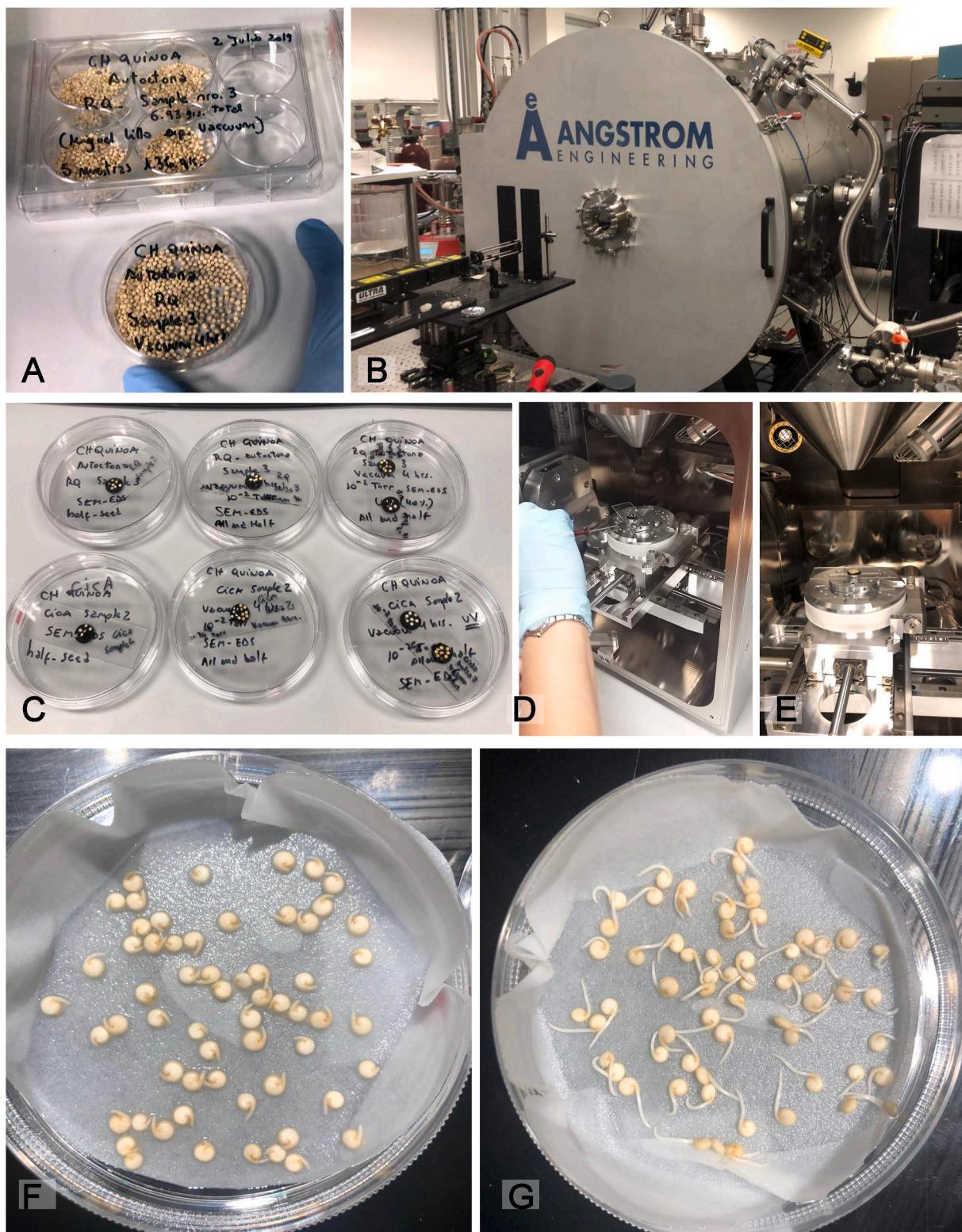


Fig. 1. A. Samples of 1 g of achenes of RQ252 quinoa (approximately 400 achenes) distributed in 8 borosilicate glass petri dishes. B. Vacuum chamber and 1064 nm 16 Quantel ULTRA Nd:YAG laser in the Canadian Planetary simulator Centre for Research in Earth and Space Science (CRESS laboratory) at York University (Canada). C-E. Preparation and mounting of samples for SEM-EDS analysis. F-G. Germination assays.

and process tracking ensuring consistent and repeatable processes and high-resolution control of the experiment.

2.4. Laser irradiation

Some of the most essential parameters to simulate in space are extreme radiation, electromagnetic radiation and high-velocity dust impact. In this regard, several groups succeeded in reproducing the

optical property change expected as space weathering, using nanosecond pulse laser irradiation simulating high-velocity dust impacts and electromagnetic radiation. The most common practice to simulate space weathering on minerals is using a solid-state Nd-YAG pulse laser beam with a pulse duration of 6–8 ns [32,33], which is comparable with real dust impacts [34]. However, in the case of the effect of radiation in organics, the laser has to be focused and hit a metallic surface like thin aluminum foil and subsequently electromagnetic radiation is created and directed to the material under study. To achieve solar plasma irradiation (pl) simulation a laser system was mounted externally and, through a window, was focused inside the vacuum chamber described above. The laser is a 1064 nm Quantel ULTRA Nd:YAG laser (Fig. 1 B) equipped with a variable attenuator to control the laser power from 1.2 mJ to 102 mJ, and 10 ns pulse width. The light from the laser was collimated and expanded, using a Keplerian beam-expander, from 2 mm to a 2 cm beam diameter. Subsequently, the beam passed through a Sapphire window into the vacuum chamber. The beam inside the chamber was focused on the samples using a 2-inch off-axis parabolic mirror with a focal length of 15.4 cm and a spot size of 33 μm (FWHM). The samples were set in an aluminum plate covered with thick aluminum foil under an off-axis parabolic at ~15 cm inside the chamber. The laser power for creating the laser ablation and space weathering on the aluminum foil was 10% corresponding 12.1 mJ. The peak power density at the target was on the order of 100 GW/cm² allowing the simulation and acceleration of particle photon-bombardment in space conditions.

2.5. Achene's exposure

In order to reproduce extraplanetary conditions two samples per treatment were exposed to high low-pressure simulation (10^{-2} to 10^{-7} Torr), at -200°C during 4 h (V4h), 8 h (V8h) and 16 h (V16h). A second set of experiments was designed to determine the effect of the combined high low-pressure simulation plus simulated solar plasma (pl) with laser. For this experiment the samples were placed in petri dishes covered with thick aluminum foil and subject to -200°C , for 4 h (V4h + pl), 8 h (V8h + pl) and 16 h (V16h + pl). After being subjected to the different treatments, Petri dishes were removed from the chamber and exposed to controlled normal atmosphere (near 757.5 Torr). Immediately after, a number of individual achenes seeds were selected for perpendicular cut and place on the SEM-EDS instrument analysis to study their structure and mineral characteristics, when others were selected for germination and early growth experiments (see below).

2.6. SEM-EDS electron microscope analysis

We obtained spectral compositional maps from the different samples of quinoa achenes with a Vega TESCAN Scanning Electron Microscope (SEM) equipped with a Bruker Quantax energy dispersive X-ray (EDS) detector in York University. The beam voltage used for secondary electron imaging (SEI) and backscatter electron imaging (BSE), as well as EDS spectra acquisition, was 10 kV. Therefore, we choose not to coat the achenes seeds samples during the study with the SEM-EDS microscope. During analysis, we studied control quinoa achenes and samples that followed the experimental conditions of 4 h, 8 h, and 16 h of space weathering exposition in a vacuum chamber with plasma and not plasma excitation. Achenes representing each experimental condition analysis were selected (Fig. 1C–E). Spectral compositional map of samples at the pericarp outer surface and internal compositional maps for achene samples cut into transversal sections with the exposition of the embryo were performed. A total of 21 samples representing each experimental condition and control by triplicate were studied. We quantified the EDS spectral data through the P/BZAF QUANTAX analysis strategy (Bruker-Nano, 2011 instrument model). This mention analysis strategy is a standardless, self-calibrating spectrum analysis procedure that uses ZAF matrix correction formulas, enabling simple

processing of the EDS spectra collected for each sample. Since the accuracy of EDS quantification is relatively low, especially with standardless quantification, we use the normalized elemental mass percentages to allow comparison within and between samples for the relative elemental concentrations. Qualitative analysis of the sample elemental composition was possible with these data quantifications.

To ensure that the post-experimental changes were maintained over time, EDS spectrums were repeated by duplicated in a ZEISS SUPRA-55VP field emission SEM (Carl Zeiss NTS GmbH, Germany) scanning electron microscope coupled with an energy dispersive X-ray detector EDS (SiLi model INCA PentaFETx3Oxford Instrument) with the software INCA SUITE V.4.13 in Centro Investigación y Servicios de Microscopía Electrónica (CISME), CONICET-UNT. Working distance 8 mm and spectra acquisition at 20kV. EDS quantification was calculated automatically by the software in basis to standards previously incorporated into the database.

2.7. Seed viability and germination

After each treatment's quinoa achenes (150 samples were selected randomly from control and each experimental condition separately) were placed in 3 Petri dishes (50 in each dish) on wet filter paper with distilled water (Fig. 1F). Petri dishes were placed in a culture chamber at $23 \pm 1^{\circ}\text{C}$ of temperature, $30 \mu\text{mol m}^{-2}\text{s}^{-1}$ of light intensity and 16 h photoperiod. The number of seed germinated was observed and recorded every 2 h. Successful germination was considered when radicle length has a large ≥ 2 mm (Fig. 1G). Germination was followed during the first 12 h because in this period quinoa reach the maximum percentage (100%) according to our previous experience [13]. Germination time courses for all three replicates at a given treatments were combined and fitted using a sigmoidal model in OriginPro 8.0. The hours to 50% germination (t_{50}) was obtained by fitting cumulative germination progress curves. Successful germinated seeds were removed after each count and separated from the aborted seeds. We consider seeds aborted when cotyledons emerge instead of the radicle. The final germination percentage was calculated as follows: $GP = \sum G_i / N$, where i is the hours of germination, counted from the day of sowing, G_i is the number of seeds germinated on day i , and N is the total number of filled seeds. Statistical analyses of the data are expressed as the mean \pm standard deviation (SD) of measurements made on three replicate experiments.

For early growth studies, germinated seeds from control and treated seeds were planted in a germination tray with 5 cm diameter and 10 cm depth holes. Every day, 5 mL of tap water was supplied (one or twice a day) to the cultivation soil. The plants were grown at $26 \pm 1^{\circ}\text{C}$ temperature. After 14 days of cultivation, seedlings were removed, washed and a photographs of the radicle + hypocotyl were taken and measured in length by using the ImageJ software 1.4 g version [35]. Other observations such as aspect, color and presence of ramifications were evaluated.

3. Results

3.1. Effects of different experiments on germination

3.1.1. Effect of high low-pressure simulation

Quinoa achenes subjected to high low-pressure simulation during 4 h, 8 h and 16 h (V4h, V8h and V16h) did not show differences in their final germination in relation to control. All treatments reached near 100% germination (Fig. 2). Considering the parameters t_{50} (time to reach 50% germination) the treatments V4h and V8h were not different from the control (5.5 h and 5.3 and 5.6 respectively), otherwise a big difference was observed in V16h where t_{50} was 7.4 h.

3.1.2. Effect of high low-pressure simulation and laser irradiation

The combination of high low-pressure and simulated plasma (pl) by laser irradiation during different times, V4h + pl, V8h + pl and V16h +

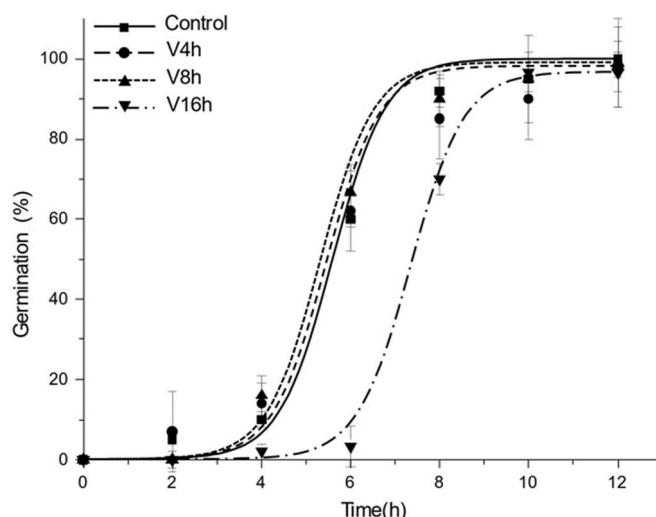


Fig. 2. *Ch. quinoa* (RQ252). Effect of high low-pressure simulation during 4 h (V4h) and 8 h (V8h) and 16 h (V16h) on achenes germination rate and final germination. Each data point was expressed as mean \pm SE ($n = 4$).

pl, did not affect significantly the final germination in relation to control. The maximum germination (90–100%) in treatments and control were reached at 8 h. However the rate of germination was significantly affected with both treatments, t_{50} values were 4.6 h, 4.3 h and 1.9 h for V4h + pl, V8h + pl and V16h + pl respectively, while the control value was 5.6 h (Fig. 3).

3.2. Effects of different experiments on quinoa grain surface and mineral content

The analysis of pericarp by scanning electron microscopy (SEM) and energy dispersive X-Ray spectroscopy (EDS) showed structural changes on the surface of the achenes subjected to the different treatments (Fig. 4).

Ch. quinoa achenes from ecotype RQ252 were white cream, slightly depressed with near circular outline, mean diameter of 1.62 mm, 0.71 mm of high and 1000 seed weight of 2.43 g. Abdelbar [18] and Burrieza et al. [17] described that the quinoa achene is formed by a two layered

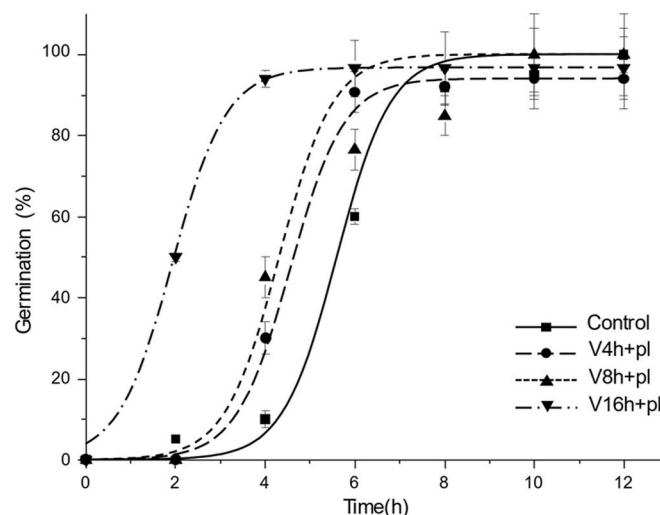


Fig. 3. *Ch. quinoa* (RQ252). Effect of high low-pressure simulation during 4 h (V4h) and 8 h (V8h) and 16 h (V16h) plus laser irradiation simulated plasma (pl) on achenes germination rate and final germination. Each data point was expressed as mean \pm SE ($n = 4$).

pericarp adhered to the seed coat. The pericarp outer layer presents papillose aspect (Fig. 4 A1) whereas the inner layer is discontinuous with tangentially stretched cells. In the mature seeds, the seed coat is formed by two distinguishable layers of cells, the outer one with thick tangential cells walls derived from the exotesta [17] and the inner one with small cells with thickened striped walls, derived from the endotegmen. Between them, the endotegmen and exotegmen appeared as obliterated tissues [18]. The seed contains a peripheral, curved embryo; form by a hypocotyl-radicle axis and two cotyledons, which surrounds the main nutritive tissue of the seed, the perisperm (Fig. 4 A2) rich in starch. Also, micropylar endosperm rich in proteins and lipids surround the radicle tip [17].

SEM analysis revealed significant changes in pericarp surface morphology after V4h and V8h treatments (independently from pl application) with a great loss of turgidity in the outer papillose cells of the pericarp (Fig. 4 B1, C1, D1 and E1). In cross section, no significant changes were observed either in the embryo, nor in the main reserve tissues (perisperm and endosperm), only a slightly folding in the outer layers of the pericarp was detectable which probably responds to the lack of turgidity as a result of the loss of water due to the high low-pressure applied during the treatments (Fig. 4 B2, C2, D2, E2, F2 and G2).

The mineral content of quinoa achenes subjected to the different treatments, only presented significant differences at the pericarp (Table 1).

The SEM-EDS analysis revealed that the pericarp of the quinoa achenes exhibits losses and changes in the mineral contents, particularly in minority elements that in many cases were not detectable (Table 1).

The treatments with V4h and V8h showed a gradual loss of elements, particularly potassium ions in the pericarp, approximately 32% with respect to control and even more in treatments with pl (42%) (Table 1). Conversely an enrichment of potassium in the pericarp tissues was observed in treatments with of V16h, which was reinforced by the application of pl, displaying 35% and 96% of increase in potassium ions respectively relative to control (Table 1, Fig. 4 A1, F3, F4, G3, and F4).

3.3. Effect of different experiments on early growth (radicle and hypocotyl length)

Radicle and hypocotyl lengths were affected negatively by different treatments with an exception in the hypocotyl of the treatment V4h (Table 2) in which this organ increases its length by 23% with respect to control, but without displaying significative differences. The length decrease was stronger in radicle than in hypocotyls (Table 2).

No differences were observed in terms of coloration or branching in the seedlings (Fig. 5).

4. Discussion

It is well known that quinoa presents a wide tolerance to different environment constraints [15,21]. This tolerance is linked to different ecotypes' origin and their genetic identity. The natural quinoa variability is expressed as life-cycle duration, inflorescence types, achenes color, size, weight, saponin, protein and amino acid contents, tolerance to salinity, hydric deficit, and UVB radiation allows quinoa to adapt to diverse environments [21]. Germination process studies allow us to understand the first steps of plant acclimatization in a particular habitat, it is an essential stage for plant development on Earth and eventually in extraterrestrial environments (ISS, Moon habitats and possible Mars).

Seed germination is a process that begins with water uptake and a series of metabolic reactions, such as respiration, that finish with the radicle emergence and seedling development. Water uptake by seeds depends on pressure, temperature, seed coat permeability, salinity, and storage reserves' nature [37].

Some early studies demonstrated that germination and early growth are possible at low atmospheric pressures [38–40] and in space [1]. In

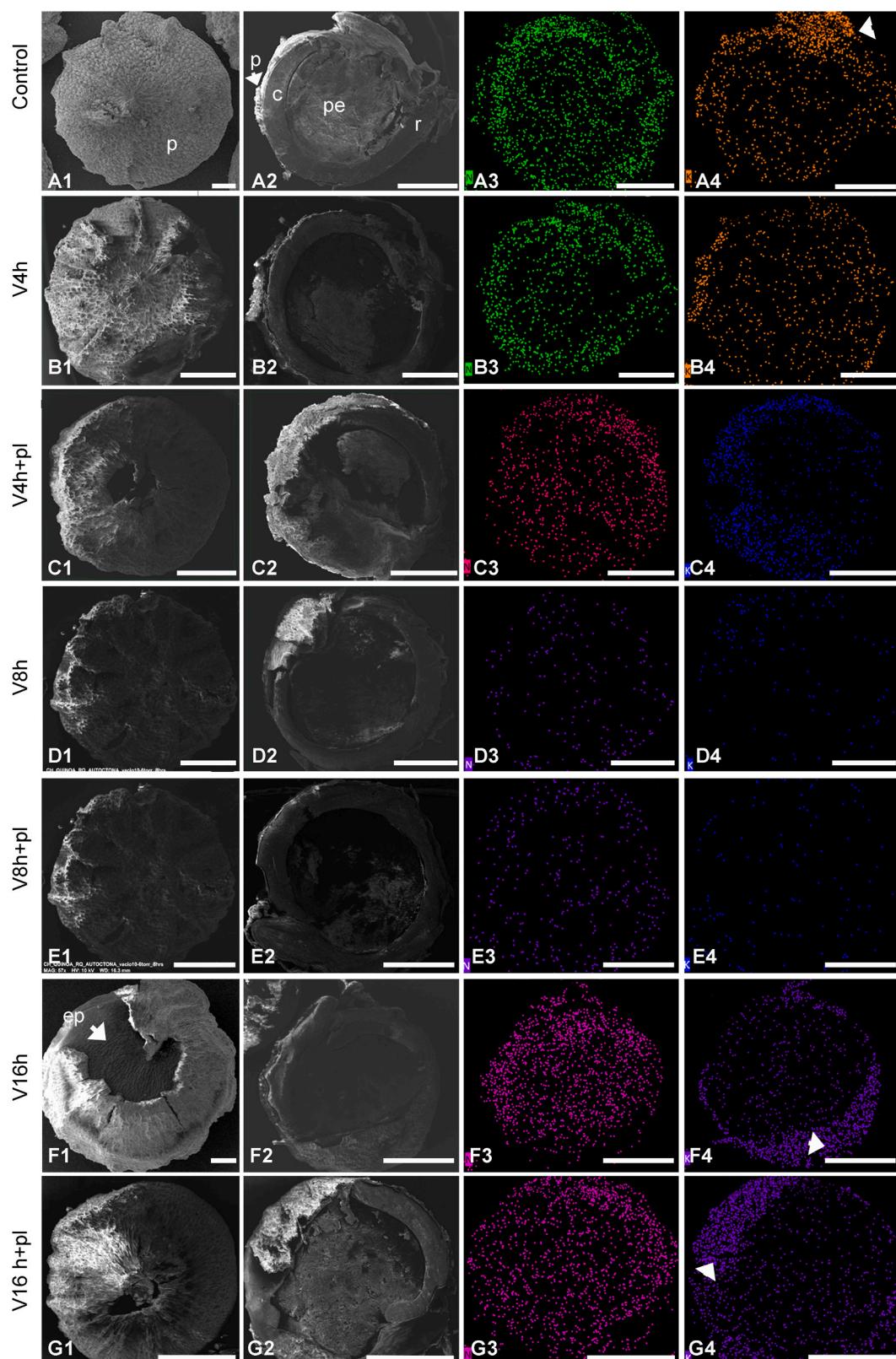


Fig. 4. *C. quinoa* ecotype RQ252 achene control (A1-A4) and subjected to different experiments with high low-pressure simulation during 4 h (V4h; B1-B4), 8 h (V8h; D1-D4) and 16 h (V16h; F1-F4) and with high low-pressure simulation plus laser irradiation plasma (pl) during 4 h (V4h + pl; C1-C4), 8 h (V8h + pl; E1-E4) and 16 h (V16h + pl; G1-G4). Column 1: SEM of the complete fruit showing pericarp (p) and rarely episperm (ep) by rupture of the pericarp. Column 2: SEM of the achene transversal section showing parts of the embryo (co, cotyledon and r, radicle) and reserve substances known as perisperm (pe). Column 3: EDS maps of nitrogen in transversal sections. Column 4: EDS maps of potassium (K) in transversal sections. Arrows indicates accumulation of element to the pointed parts, particularly potassium in the pericarp tissues. Scale bar 200 μ m.

Table 1

Ch. quinoa (RQ252) pericarp elemental composition in atom percentages (SEM-EDS) for quinoa achenes subject to different high low-pressure simulations times (V) and laser irradiation simulated plasma (pl).

%	Control	V4h	V4h + pl	V8h	V8h + pl	V16h	V16h + pl
C	47.47 ± 3.33 (a)	52.58 ± 1.85 (a)	53.96 ± 3.04 (a)	52.14 ± 3.50 (a)	55.03 ± 3.10 (a)	51.35 ± 2.95 (a)	50.43 ± 2.90 (a)
O	44.34 ± 3.95 (a)	41.71 ± 1.70 (a)	41.10 ± 2.90 (a)	41.57 ± 1.51 (a)	40.59 ± 2.31 (a)	40.10 ± 4.90 (a)	35.67 ± 4.60 (a)
Na	0.01 ± 0.01(d) (d)	0 ± 0 (d)	0.11 ± 0.01(a) (d)	0.01 ± 0.01(d) (d)	0.04 ± 0.01(c) (d)	0.09 ± 0.01(b) (b)	0.07 ± 0.01 (b)
Mg	0.46 ± 0.08(c, d) (a)	0.74 ± 0.06(a) (b)	0.60 ± 0.08(b) (c)	0.33 ± 0.03(c) (c)	0.50 ± 0.06 (b,c)	0.36 ± 0.06 (d,e)	0 ± 0 (f)
Al	0 ± 0(d) (c)	0.04 ± 0.01(c) (d)	0 ± 0 (d)	0.13 ± 0.01(a) (d)	0 ± 0 (d)	0.07 ± 0.01(b) (d)	0 ± 0 (d)
Si	0.12 ± 0.03(b) (b)	0.15 ± 0.04(b) (a)	0.28 ± 0.03(a) (a)	0.27 ± 0.03(a) (a)	0.10 ± 0.01(b) (b)	0.10 ± 0.01(b) (b)	0 ± 0 (c)
P	0.16 ± 0.01(a) (c)	0 ± 0 (c)	0 ± 0 (c)	0.07 ± 0.03(b) (c)	0 ± 0 (c)	0 ± 0 (c)	0 ± 0 (c)
S	0.20 ± 0.04(a) (c)	0 ± 0 (c)	0.02 ± 0.01(c) (c)	0.16 ± 0.03(b) (c)	0 ± 0 (c)	0 ± 0 (c)	0 ± 0 (c)
Cl	1.70 ± 0.14(b) (b)	0.56 ± 0.08(c) (c)	0.33 ± 0.04(d) (d)	0.63 ± 0.04(c) (c)	0.33 ± 0.05(d) (d)	0.63 ± 0.04(c) (c)	3.22 ± 0.31(a) (a)
K	5.30 ± 0.42(c) (d)	3.63 ± 0.60(d) (d)	3.10 ± 0.28(d) (c)	3.70 ± 0.14(c) (c)	3.08 ± 0.17(d) (d)	7.15 ± 0.49(b) (b)	10.40 ± 1.98 (a)
Ca	0.27 ± 0.04(a, b) (a)	0.37 ± 0.04(a) (a)	0.31 ± 0.03 (a,b)	0.33 ± 0.04(a) (d)	0.11 ± 0.01(c) (c)	0.18 ± 0.03 (b,c)	0 ± 0 (d)
Fe	0 ± 0(c) (c)	0.15 ± 0.03(b) (c)	0 ± 0 (c)	0.59 ± 0.09(a) (a)	0.58 ± 0.06(a) (c)	0 ± 0 (c)	0 ± 0 (c)

References: Each value was expressed as mean ± SD ($n = 3$). One way ANOVA. Different letters represent significant difference among treatments at $P \leq 0.05$. High low-pressure simulation during 4 h (V4h), 8 h (V8h) and 16 h (V16h); high low-pressure simulation plus laser irradiation plasma during 4 h (V4h + pl), 8 h (V8h + pl) and 16 h (V16h + pl).

Table 2

Ch. quinoa (RQ252). Effect of different experiments on hypocotyls (Hypoc.) and radicle length.

	Hypoc. (mm)	Radicle (mm)
Control	19.8 ± 8.2(a,b)	46.0 ± 12.8(a)
V4h	24.3 ± 8.9(a)	17.4 ± 6.4(b,c)
V8h	13.1 ± 2.5(b,c)	20.2 ± 4.6(b)
V16h	11.1 ± 2.5(c)	21.5 ± 5.7(b)
V4h + pl	16.8 ± 3.4(b,c)	10.0 ± 3.1(c)
V8h + pl	12.7 ± 2.9(c)	22.9 ± 6.2(b)
V16h + pl	12.0 ± 2.0(c)	20.3 ± 6.9(b)

References: Each value was expressed as mean ± SD ($n = 20$). Different letters represent significant difference among treatments at $P \leq 0.05$. High low-pressure simulation during 4 h (V4h), 8 h (V8h) and 16 h (V16h); high low-pressure simulation plus laser irradiation plasma during 4 h (V4h + pl), 8 h (V8h + pl) and 16 h (V16h + pl).

these cases, partial pressure of O₂ was an essential factor due to its relationship with oxidative phosphorylation [41].

Halloy and González [7] studied low-pressure effects on quinoa germination under lab conditions and found an inverse relationship between survival and altitudes increases. Tang et al. [42], demonstrated that dicot and monocot plants can germinate under hypobaria (low pressure at 30 kPa) with the limitation that oxygen partial pressure should be at least 6 kPa, suggesting that oxygen diffusion rate under hypoxic conditions is enhanced. In long-term experiments at low atmospheric pressure in the Skylab vehicle, NASA investigated sprouted and grown of rice plants at reduced atmospheric pressure (near 34 kPa)

[4].

RQ252 achenes were subjected to low pressure during 4, 8 and 16 h, adding a plasma pulse to reproduce solar radiation and –200 °C freezing temperatures.

Subsequently, viability and germination assayed under normal atmosphere pressure (near 101 kPa), the treatments slightly affected the germination rate, V16h delayed germination, while plasma application accelerated it, but did not affect the final germination compared to the control samples. Tepfer et al. [43] demonstrated that *Arabidopsis thaliana* and *Nicotiana tabacum* (tobacco) seeds with and without a dark protection layer could germinate after 1.5 years of exposure to solar and galactic cosmic radiation, temperature fluctuations, and space vacuum at ground simulated experiments and outside the ISS (International Space Station). Twenty-three to 44% of the seeds exposed to total radiation without the dark layer germinated and produced viable plants after return to Earth. Notwithstanding, seeds shielded from solar light (with fewer irradiation conditions) presented delayed germination but achieved total survival rates. Similarly, in our experiment, the combined application of low pressure and cryogenic temperature showed a delay in germination rates, with final germination around 95%. However, when UV simulation through laser-plasma was applied, the germination rate was improved or accelerated. Final germination remained up to 90%, indicating the high resistance of the quinoa ecotype selected for the assay.

Gómez-Ramírez et al. [44] rehearsed two plasma irradiation conditions (atmospheric pressure dielectric barrier discharge -DBD- and a low-pressure radiofrequency -RF-). Doing so, the plasma irradiated produced potassium enrichment in the pericarp and closer zones regions in quinoa achenes resulting in an acceleration of the germination rates of the seeds. This potassium variation probably increases the water uptake through a partially etched of the fruit and seed coat [45,46] or the treated seeds' sterilization produced by the plasma [47]. Other evidence related to the change in the seed coat was found by Dhayal et al. [48] in *Carthamus tinctorium* seed activated by low-pressure plasma.

In relation to water uptake Sigstad and Prado [37] demonstrated that quinoa (Var. Sajama) effectively has a higher rate of water uptake near 10.0×10^{-3} g (H₂O) g⁻¹ seed min⁻¹ during the first 15 min of germination. Probably the modification of the achene surface structure by plasma irradiation alone modified the wettability of the achenes and seeds, as was demonstrated by Bormashenko et al. [49].

In our results, the achenes subjected to different high low-pressure simulation times showed a significant loss of turgidity in the outer papillose cells of the pericarp and a decrease in the potassium content, unlike what was observed by Gómez-Ramírez et al. [44] these effects remained constant even when plasma was added for V4h and V8h experiments; conversely in V16h potassium was increased, and the application of plasma accentuated this increase.

The combination of low pressure plus laser-plasma accelerate germination times, but did not affect final germination, probably due to increased water uptake due to a partially etched fruit pericarp and seed coat as suggested by Gómez-Ramírez et al. [44]. There is much evidence concerning the UV plasma effect on germination improvements, seedling growth, and physiological metabolism in different species like *Chenopodium album*, *Oryza sativa*, *Triticum aestivum*, *Lycopersicon esculentum*, and *Solanum melongena* [45]. When applying laser-plasma UV simulation, it acted as an activation factor in our experiment and improved the germination rate regardless of how long the samples remained at low pressure.

González and Prado [50] found that the quinoa germination rate of 80% at 22 °C was reduced to 22.4% when the temperature dropped to 0 °C. Similar results obtained by Jacobsen et al. [51] and Chilo et al. [52] show the same evidence. Bois et al. [53] reported that the base germination temperatures for ten different quinoa ecotypes varied between –1.9 and 0.2 °C. Low temperatures (–120 °C to –196 °C) can favor DNA and tissue preservation. Nevertheless, this preservation is not enough to stop deterioration over time, as demonstrated by Walters et al. [54] for



Fig. 5. *C. quinoa* (RQ252). Effects of different experiments of high low-pressure simulation during 4 h (V4h) and 8 h (V8h) and 16 h (V16h) without and plus plasma (pl) application on early growth. Scale bar = 5 mm. High low-pressure simulation during 4 h (V4h), 8 h (V8h) and 16 h (V16h); high low-pressure simulation plus laser irradiation plasma during 4 h (V4h + pl), 8 h (V8h + pl) and 16 h (V16h + pl).

lettuce seeds stored for >10 years in liquid nitrogen (between -135 and -196 °C), which showed a reduction in the viability of the seeds.

According to Abdelbar [18], the outer layer of the pericarp of quinoa often fails to maintain shape and loses turgidity in dry fruits but is easily recovered by water imbibition. This turgor recovery is without doubt an essential factor for germination. The potassium enrichment of the pericarp in treatments with V16h + pl could also be associated with the greater germination power and viability of the achenes seeds subjected to this treatment, suggesting that plasma acts as an activator as suggested previously mentioned by Ling et al. [45]. In our study, the cryogenic temperature achieved in the high low-pressure experiments did not reduce germination rates except in V16h experiment, probably as an effect related to seed metabolism by dehydration caused by high low-pressure and temperature effect added to time. However, it is interesting that this effect was reverted, and the laser-plasma accelerated the germination. Thus, our results demonstrate the resistance of quinoa ecotype RQ252 achenes to the experimental conditions evaluated and the maintenance endurance of the viability of the seeds.

Quinoa growth in the open field or controlled environments under normal pressure is a good source of nutrients as fresh sprouts [29,55].

These results indicate the achenes seeds growth viability remains intact after exposure to the extraplanetary condition such as high low-pressure, plasma and cryogenic temperature, pointing out the possibility of obtaining fresh sprouts or establishing quinoa crops in a microgravity environment CELSS or a BLSS system. Furthermore, this observation allows us to point this ecotype as a potential source of high-quality fresh food to astronauts with high nutritional and bioactive components (such as proteins, vitamins, minerals, anthocyanins, and phenolic compounds) and fast growth rates (days).

During the experiment, we demonstrated that low to high-low pressure and low light intensity affect germination, dry matter, and physiological responses of plants and seedlings, modifying plant growth, life cycles, yield, and fruit quality parameters and phytochemicals contents such as carotenoids, chlorophyll, ascorbic acid, triglycerides [56–59]. This work constitutes an initial explorative evaluation of quinoa achenes/seed modifications and viability after exposure to extraplanetary conditions. We selected the ecotype RQ252 due to its high tolerance to extreme Earth conditions. However, evaluating other ecotypes under the same experimental conditions is essential for future research stages, including seed viability studies and post-germination studies to understand how compositional changes affect the development of sprouts and plants, their life cycles, biomass production, and nutritional and bioactive quality.

If heating/vaporization efficiency is the same for both laser-irradiated energy and dust impact energy, irradiation of a 30 mJ pulse laser method corresponds to heating by dust impacts over about 10^8 yr in space [60]. We achieve 12.2 mJ laser pulse irradiation during our experiment, comparable to 103 yr in space. In this way, we proved that the survival of the achene seed in absolute space conditions reaches at least 1000 years, guaranteeing subsequent germination in a period of

equal or lesser times if future space missions are sent with organic material of this type to lunar or Martians orbits for long periods.

Space factors such as microgravity and ionizing radiation can alter many physiological processes directly and indirectly [3]. Therefore ground-based experiments are necessary for understanding how viability, growth efficiency, yield (considering all plant outputs as oxygen, biomass, etc.), and many other factors are affected by exposure and cultivation under simulated space factors.

On this point ground-based facilities for simulation of extra-planetary conditions are valuable and cost-efficient systems for assessing space farming. However, correct responses of plant crops to real microgravity and limited resources can be accurately measured only in natural conditions. Therefore, Carillo et al. [1] suggest that it may be pivotal to validate all the results obtained in ground-based simulators.

After this simulation, quinoa (RQ252 ecotype) achenes showed noticeable structural changes and slight changes in the distribution of minerals, particularly potassium at the pericarp, but present a good tolerance in terms of germination rates and final germination to high low-pressure and simulated plasma application. Under all the mentioned treatments, alone or in combination, achenes germinated and exhibited early growth under terrestrial conditions. The next step for this study's continuity would be to repeat these experiments with and without pericarp and allow the mature plant's growth to evaluate the entire growth cycle and yield (considering all plant outputs).

5. Conclusion

Although the selection of species/cultivars/ecotypes for space exploration can take years, this experiment demonstrates the resistance of quinoa achenes ecotype RQ252 to extra-planetary conditions and the maintenance of the seed's viability, constituting the first approximation to the possible selection of this or other quinoa ecotypes/cultivars for subsequent growth studies in microgravity, short and long-term exploration missions.

Author disclosure statement

No potential competing interest is reported by the authors.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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