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Subsequent inclusion/exclusion criteria to select the best species for an experiment performed on the ISS in a refurbished hardware

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A B S T R A C T

The interest in re-using flown hardware for new and different space biology experiments is increasing. To match the constraints of the flown hardware with the requirements of the new biological system, innovative methodological approaches are necessary. MULTITROP was a successful plant biology experiment that was performed on the ISS to investigate multiple-tropism interactions during the early stage of seedling growth. We used the hardware designed and flown for the IFOAM experiment in 2009. The main challenge was to implement seeds of a crop species in a growth chamber conceived for yeast culture and to grow the seedlings in microgravity condition but activating seed germination on ground before the launch. Our approach was to adapt the biological system to the hardware constraints and also to the experiment timing and the environmental factors expected during the prelaunch, launch and flight operations. We looked for an objective and repeatable method to effectively select the best suited species. Innovatively, we applied the method of inclusion/exclusion criteria to adapt a new biological system to a reused hardware. The list and the consecutive order of the specific inclusive/exclusive criteria turned out to be a valid support to guide the science team in objectively choosing the most suitable species for the experiment. Among the 50 initial food species, the carrot seeds resulted as the best in satisfying all technical requirements and post-flight data confirmed the expectations.

1. Introduction

Routine or easy on-the-ground biological experiments cannot simply be extrapolated in space because many preparatory tasks and precautions are necessary to achieve a scientific goal on the orbital platforms. Technical success of a biological experiment relies on adequate and precise preparation on the ground, mostly involving the production of a specific experiment container and a coordinated teamwork of flight crew, scientists, engineers, managers, and government officials. All this contribute to making space research very expensive in term of costs and of manpower involved.

Reusable hardware can contribute in lowering the cost and the time of payload development that is necessary to perform experiments in space. The re-flight of the same or a refurbished hardware has been often performed to improve a previously launched experiment, and this occurred also for researches on plant biology (e.g. (Kiss et al., 2011)). Otherwise, it is infrequent to re-use a hardware for a biological system and scientific goals that are different from those of the experiment for which it was originally conceived. Recently this approach was applied to educational projects by the Italian Space Agency (ASI) through the YiSS (Youth ISS Science) calls. The initiative gave financial and technical support to experiments to be performed on the International Space Station (ISS) by using a hardware previously designed and employed for a different experiment in microgravity. For the YiSS, the hardware had to be chosen from a list of nine flown experiment containers by Kayser Italia company (Table S1). Kayser Italia was involved in the call to slightly refurbish the hardware and give technical support to the new experiment.

MULTITROP (MULTITROPism: interaction of gravity, nutrient and water stimuli for root orientation in microgravity) was the winner of the YiSS 2017 call and proposed the achievement of a scientific aim in addition to the educational goal. The project was conceived by botanists at the Department of Agricultural Sciences of the University of Naples Federico II and developed by a PhD and two master students from the University of Naples and nine students from the High School ‘Liceo Scientifico Filippo Silvestri’ located in Portici (Italy) tutored by two teachers. The educational aim of MULTITROP was to enhance young people's interest in Space biology.

The scientific goal of MULTITROP was based on the consideration that plants play a key role in the long-term space missions, including those aimed at colonizing Mars or the Moon (Wheeler, 2010; De Micco et al., 2014), and also that practical fulfillment of the

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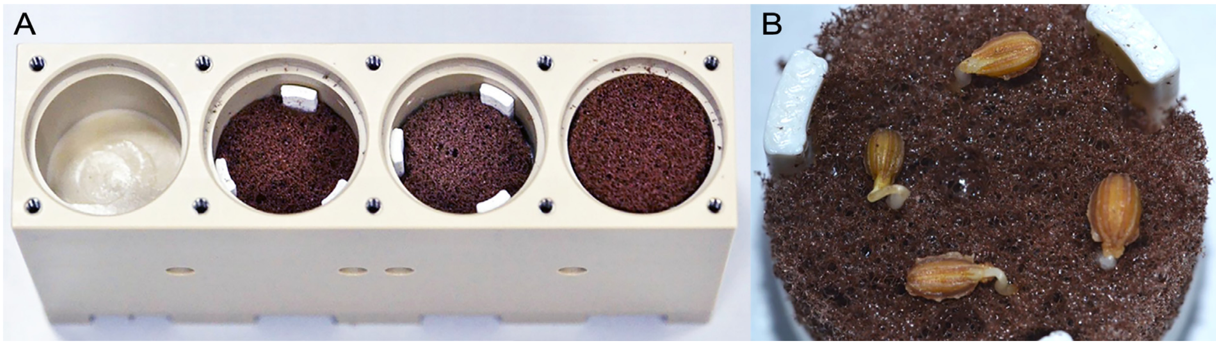

Fig. 1. A) Refurbished YING-B2 hardware showing the four growth chambers at different setup stages. Three growth chambers are implemented with the brown Oasis disks and the 3D-printed holders (white); B) Four carrot seeds germinated and rooting into the Oasis disk.

interplanetary missions relies on minimizing cargo and guaranteeing self-sufficiency to the human crew. The current concept to sustain life in space is the development of artificially closed ecosystems such as MELiSSA (Micro-Ecological Life Support System Alternative), the Bioregenerative Life Support Systems (BLSSs) supported by ESA (Lasseur et al., 2010; Paradisoet al., 2014). In BLSSs, higher plants guarantee O_2 production and CO_2 assimilation, furthermore, they are capable of recycling human wastes and transform them into bioavailable food source (De Micco et al., 2009; Poulet et al., 2016). In addition, they counteract the psychological stress of the cosmonauts by mimicking an earth-like environment.

Plant relevance notwithstanding, their growth in space is challenging. Plants on earth have evolved as sessile organisms and this condition has got them to develop a discreet amount of actions to respond to directional stimuli through movements known as tropisms. It is well known that plants react to several directional stimuli (Gilroy, 2008). It has been proved that organ growth is oriented according to gravity (Su and Masson, 2019; De Micco and Aronne, 2008; De Micco et al., 2006), light (Kiss et al., 2007), nutrients (Izzo et al., 2019), and several other tropistic stimuli (Muthert et al., 2019). Gravitropism is considered dominant on other external stimuli for organ orientation [e.g. (Kiss et al., 2007; Muthert et al., 2019); therefore, experiments in microgravity are necessary to investigate on tropisms different than gravitropism. Many experiments on root gravitropism have been performed on the ISS (Wyatt and Kiss, 2013). Most studies focused on single tropisms, while interactions of multiple tropisms are generally neglected (Muthert et al., 2019). In this context, the scientific aim of MULTITROP was to study the interactions of chemotropism and hydrotropism on root growth orientation of germinating seeds (Aronne et al., 2018). The experiment was successfully performed and both educational and scientific goals were fully achieved (Izzo et al., 2019). Remarkably, a positive root chemotropism was found in microgravity and roots grew preferentially towards disodium phosphate when confronted with both a hydrotropic and chemotropic stimulus (Izzo et al., 2019).

However, during the MULTITROP activities, the research team had to identify and solve an underestimate number of technical problems strictly related to the hardware to be reused (Aronne et al., 2020). The hardest challenge was to adapt the biological system of the MULTITROP experiment to a hardware that was originally designed and used for a research completely different in terms of both scientific aims and type of living organisms.

In this paper we describe the method applied to find the plant species best suited to the hardware constraints and the scientific requirements of the MULTITROP experiment, with the convincement that such a procedure can be further applied to others experiments.

2. Materials and methods

The most common approach for biological experiments in Space is to create a new *ad hoc* payload that fits both scientific requirements and space technical constraints. For MULTITROP, the opposite situation occurred. The adopted strategy was to adapt the biological system to an existing experimental container and to the other technical constraints. The latter are mainly referred to the absence of a temperature control system in the payload (which required to find a biological system weakly reactive to temperature variations) and the difficulties to match biological timing of the experiment with the timeline of the launch activities (from the experiment implementation and payload handover to the payload de-stow from cargo vehicle and experiment deactivation by crew on board of the ISS).

To achieve the scientific goals of the MULTITROP experiment, we needed to implement on ground the dry seeds into a growth chamber with wet substrates, wait for seed germination to occur in microgravity after the launch, stop root development with a chemical fixative on the ISS and have the payload back on ground to analyze root growth orientation. Details of the HW are reported in Aronne et al. (Aronne et al., 2018). Following, we briefly describe the HW system and then mainly focus on the method we used to select the species for the MULTITROP experiment.

2.1. Hardware system

The MULTITROP experiment was launched in a BIOKON passive container previously used to carry the IFOAM experiment during the DAMA mission in 2009. The BIOKON was composed of two volumes: a top vented case containing the batteries pack for autonomous operations of the experiment and a lower sealed case containing the experiment hardware with the biological samples inside.

MULTITROP was performed in two YING-B2 Experiment Units (EUs) specifically designed and used for the YING (Yeast in No gravity) experiment supported by ESA and flown on the ISS in 2009. Each EU (Fig. 1A) consisted of four cylindrical growth chambers (r = 10 mm, h = 16.2 mm) originally designed for the yeast culture. Each growth chamber was connected to a reservoir chamber (r = 9 mm, h = 12.6 mm), originally filled with the chemical fixative.

Considering that not only the scientific aims but also the biological system was different from those of the YING experiment, both the BIOKON and the YING-B2 hardware needed refurbishment to fulfill the scientific requirements of the MULTITROP experiment. Kayser Italia, designed, manufactured and certified for launch the original hardware and was also responsible for the refurbishment and for the new payload integration process, operations and logistics.

The BIOKON configuration used for MULTITROP was different from that used in previous experiments because it had to be self-standing. Refurbishment activities mainly involved a re-design of batteries pack and electronics. For MULTITROP both the electronic and mechanic parts of the YING-B2 had to be interfaced to the self-standing BIOKON container in which the EUs were placed.

The ideal experiment units for the MULTITROP experiment should have had several growth chambers (to allocate the dry seeds), each connected to three reservoir chambers; the first two filled with water and nutrient solution respectively (to soak the two substrate disks and activate seed germination in space) and the third filled with the chemical fixative (to stop the root growth process at the end of the experiment in space). In the YING-B2 configuration, only one reservoir chamber was available for each growth chamber. The reservoir chamber was filled with a chemical fixative to be injected in the growth chamber at the end of the experiment to block root development at the target stage (inflight experiment activation). This decision implied to start the experiment at the launch site and required late access activities. Therefore, the implementation of the MULTITROP experiment included the seed sowing in a wet substrate (biological activation) at the launch site that triggered seed imbibition on ground and seed germination in microgravity.

For the MULTITROP set up, each growth chamber was implemented with a 3D-printed holder to accommodate two disks of Oasis Grower Foam (Growing Solutions, The Netherlands), a phenolic plastic foam, commonly used for soilless cultivations. One disk was imbibed with pure water and the other with nutrient solution. Before implementation, the disks were centrifuged at 9 g to avoid potential leakage at the substrate interface. During the experiment implementation, dry seeds were placed in between the two disks.

To have a number of replicates that was adequate to provide statistical elaboration of the expected results, we required to insert not less than 4 seeds in each growth chamber in order to implement a total of 32 seeds (4 seeds × 4 growth chambers × 2 YING-B2 EUs).

2.2. Method to select seed species

To select the plant species with the seed best adaptable to the technical requirements of the MULTITROP experiment, we applied the method of inclusion/exclusion criteria, that is often used for research in the medical field (Patino and Ferreira, 2018). It consisted in setting a list of eligibility criteria to rule *in* or *out* the species to be used for the research. One inclusion criterion and four subsequent exclusion criteria were used: the first was aimed to put together a list of candidate species, the following four to choose those species best suitable for MULTITROP requirements. The four subsequent exclusion criteria were sorted following a decreasing priority: the species not passing a criterion was automatically excluded from the following ones. Details of each criterion are reported below.

2.2.1. Inclusion criterion: Initial list of candidate species

Among thousands of plant species theoretically usable for carrying out the experiment, we focused only on species of food interest. This decision was based on two considerations: a) the candidate species for cultivation in space must be well integrated into bioregenerative systems which must also provide fresh food for the crew members, in addition to other functions such as air and water regeneration (Wheeler, 2010; De Micco et al., 2014); b) seeds of cultivated plants must be available on the market and be the result of an accurate selection process that guarantees high quality seeds (germination percentage), and uniformity of the seed germination and seedling development rates.

Seed quality of each candidate species (in terms of high germinability) was assessed by the specialized seed companies that purchased the biological material. Only seed sets with germinability equal or higher than 80% were considered for further criteria.

2.2.2. First exclusion criterion: compatibility of the seed size with the growth chamber dimensions

Considering that a considerable increase in seed size might occur during water imbibition at early stage of germination, we evaluated seed size both before and after water uptake. For each species, 10 seeds were analyzed. The measurements were performed by taking pictures of the seeds before and after hydration and subsequently analyzing the images with a digital image analysis software (AnalySIS 5.0, Olympus Soft Imaging Solutions, GmbH, Germany).

2.2.3. Second exclusion criterion: compatibility of the seed germination time with the launch timeline (from experiment setup to microgravity conditions)

For the MULTITROP experiment, seed imbibition had to start on ground because dry seeds had to be sown at launch site. Late access at launch site laboratories allowed us to reduce the period from seed imbibition to arrival in microgravity conditions. Analyzing the launch timetable provided by Kayser Italia (Aronne et al., 2018), we calculated that from the biological activation to the attainment of the microgravity conditions a maximum time of 56 h would elapse. This value resulted from the sum of the following times: 2 h from the beginning of seed imbibition during the disinfection phase and preparation of the growth chambers, 6 h for integration in the BIOKON, 48 h as the maximum interval from the payload delivery to the NASA personnel to arrival in microgravity conditions.

From the biological side, it is well known that seed germination is much affected by environmental temperature (Baskin and Baskin, 2014). In this framework, a critical technical constraint of the MULTITROP experiment was the absence of a temperature controller in the HW setup. To address this issue, the teams involved in the experiment (ASI, Kayser Italia and NASA) estimated with maximum approximation the probable temperature of execution of the experiment. As a result, seed germination tests were performed at 22 ± 2 °C. For each of the 23 species subjected to the second criterion more than 200 seeds were used. Samples were checked twice a day under a dissection microscope. A seed was classified as 'germinated' as soon as the radicle protruded from the seminal tegument. Data on time (number of hours) required to germinate were recorded for each of the about 4600 analyzed seeds.

2.2.4. Third exclusion criterion: compatibility of the post-germination mode of seedling development with the size and set-up of the growth chambers

After seed germination, seedlings can have either a hypogeal or an epigeal development, according to the species. In the first case, the cotyledons do not move from the place where the seed germinates. In the second (most common in nature), the development of the hypocotyl pushes the cotyledons out of the soil. In the epigeal species, the lengthening of the hypocotyl can occur simultaneously or after the initial root growth (Fig. 2). For the MULTITROP experiment, an early development of the hypocotyl was considered detrimental because it would have damaged the Oasis disks. To apply the third criterion, we analyzed timing of root and hypocotyl growth during seedling development of the candidate species. The tests were performed at 22 °C in the dark. For each species 10 seeds were sown in a transparent substrate (agar 0.7% in water). Images of the germinating seeds and developing seedlings were recorded every 30 min and subsequently analyzed with a digital image analysis software (AnalySIS 5.0, Olympus Soft Imaging Solutions, GmbH, Germany) to get data on root and hypocotyl growth rate.

2.2.5. Fourth exclusion criterion: compatibility of root elongation rate with the minimum time required to ensure the activation of the experiment by the astronaut

According to the timeline of the launch, the injection of the chemical fixative to block root growth (activation of the experiment by the crew) could not have occurred before 164 h from the biological activation. The fourth criterion was therefore aimed to compare the species according to the time required by the seedlings to reach the target root elongation. The maximum extension (target stage) of the roots was defined considering that it could not exceed 7 mm, corresponding to the thickness of the individual Oasis disks positioned in the growth chambers.

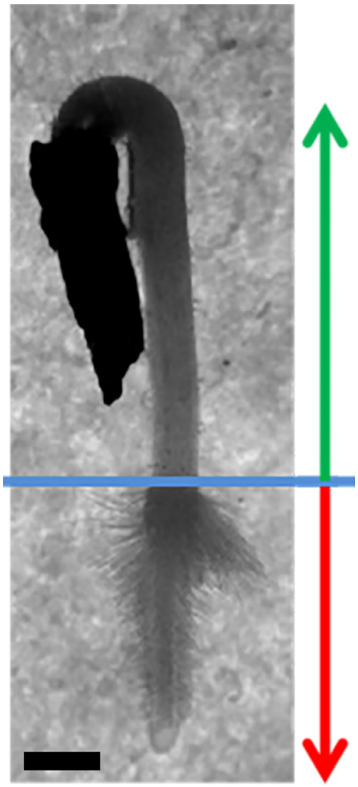

Fig. 2. A seedling at early stage of development. Root and hypocotyl growth direction are marked with red and green arrow respectively. Bar = 1 mm.

Tests were carried out in the dark at 22 °C and placing the seeds in between two Oasis disks. We used both the Petri dishes and the YING-B2 Ground Model, supplied by Kayser Italia. Per each species we tested a total of 100 seeds in the Petri dishes and 16 seeds in the YING-B2. For the preparation of the YING-B2, we followed the protocol prepared for the execution of the assembly operations at the launch site. According to the experimental design, we performed daily destructive samplings aimed to calculate root growth rate from sowing to the target stage.

2.2.6. Fine tuning: cultivar selection

After selecting the best suitable species to be used for the MULTITROP experiment, the fine-tuning phase of the biological implementation began. At this stage, three best seed companies were contacted and invited to contribute to the experiment by suppling a set of high-quality seeds with high germination rate and high uniformity in germination time. Seed germination tests were performed in the dark, at 22 °C, placing the seeds in between two Oasis disks, using both the Petri dishes and the YING-B2 for the setup. We tested three cultivated varieties of carrot seeds, namely *D. carota* 'Berlicum', 'Chantenay', and 'Nantes'. Per each cultivar we tested more than 400 seeds.

3. Results

3.1. Inclusion criterion

Initial list of candidate species to be used for the MULTITROP experiment on the ISS, consisted of 50 food species (Table 1). Among them 41 were dicotyledons and 9 monocotyledons.

3.2. First exclusion criterion: compatibility of the seed size with the growth chamber dimensions

Among the 50 initial candidate species, 18 were excluded with a visual evaluation of the system that showed the evident impossibility to place four seeds in each YING-B2 growth chamber (Fig. 3A, B).

Biometric analyses were therefore performed on the remaining 32 species and results are summarized in Fig. 4. As expected, seeds of all the tested species swollen upon imbibition. However, great variability of the seed swelling was found. This enlargement was minimal in tomato (about 2%) and in garlic (about 9%) and maximum in clover (about 89%) and *Trigonella* (about 95%). Image analysis of the enlarging seed also provided indications on the minimum space needed for a seed to soak, protrude the radicle and start root and hypocotyl development. Results were used to define the threshold value to pass the criterion.

Table 1
List of candidate species for the MULTITROP experiment according to inclusion criterion. Ticks indicate the species that passed the criterion specified in the column. Exclusion criteria: 1) compatibility of the seed size with the growth chamber dimensions after visual (a) and biometric (b) evaluation; 2) compatibility of the seed germination time with the launch timeline; 3) compatibility of the post-germination mode of seedling development with the size and set-up of the growth chambers; 4) compatibility of root elongation rate with the minimum time required to ensure the activation of the experiment by the astronaut.

N	Plant species	Common name	Exclusion criteria 1a	1b	2	3	4
1	*Allium cepa*	Onion	✓	✓			
2	*Allium porrum*	Further	✓	✓	✓		
3	*Allium sativum*	Garlic	✓	✓	✓		
4	*Apium graveolens*	Celery	✓	✓	✓	✓	
5	*Arachis hypogaea*	Peanut					
6	*Avena sativa*	Oat					
7	*Brassica oleracea*	Broccoli	✓	✓			
8	*Capsicum annuum*	Pepper	✓	✓	✓		
9	*Chenopodium quinoa*	Quinoa	✓	✓			
10	*Cicer arietinus*	Chickpea					
11	*Cichorium intybus*	Chicory	✓	✓			
12	*Citrus lemon*	Lemon					
13	*Citrus sinensis*	Orange					
14	*Corylus avellana*	Hazel nut					
15	*Cucumis melo*	Melon					
16	*Cucumis sativus*	Cucumber	✓				
17	*Cucurbita maxima*	Squash					
18	*Cucurbita pepo*	Zucchini					
19	*Daucus carota*	Carrot	✓	✓	✓	✓	✓
20	*Eruca vesicaria*	Arugula	✓	✓			
21	*Fagopyrum esculentum*	Buck wheat	✓				
22	*Foeniculum vulgare*	Fennel	✓	✓	✓	✓	
23	*Glycine* max	Soy	✓				
24	*Helianthus anuus*	Sunflower					
25	*Hordeum vulgare*	Barley	✓				
26	*Lactuca sativa*	Lettuce	✓	✓	✓		
27	*Lens culinaris*	Lentil	✓				
28	*Linum usitatissimum*	Flax	✓	✓			
29	*Lupinus mutabilis*	Lupin					
30	*Medicago sativa*	Alfalfa	✓	✓			
31	*Ocimum basilicum*	Basil	✓	✓			
32	*Oryza sativa*	Rice	✓				
33	*Petroselinum crispum*	Parsley	✓	✓			
34	*Phaseolus vulgaris*	Bean					
35	*Pisum sativum*	Pea	✓				
36	*Prunus armeniaca*	Apricot					
37	*Prunus avium*	Cherry					
38	*Prunus dulcis*	Almond					
39	*Raphanus sativus*	Radish	✓	✓			
40	*Salvia hispanica*	Chia	✓	✓			
41	*Sesamum indicum*	Sesame	✓	✓	✓		
42	*Sinapis alba*	Mustard	✓	✓			
43	*Solanum lycopersicum*	Tomato	✓	✓	✓		
44	*Trifolium pratense*	Clover	✓	✓			
45	*Trigonella foenum-graecum*	Fenugreek	✓	✓			
46	*Triticum aestivum*	Wheat	✓				
47	*Triticum dicoccum*	Hulled wheat					
48	*Vicia faba*	Fava bean					
49	*Vigna angularis*	Azuki	✓				
50	*Zea mays*	Corn					

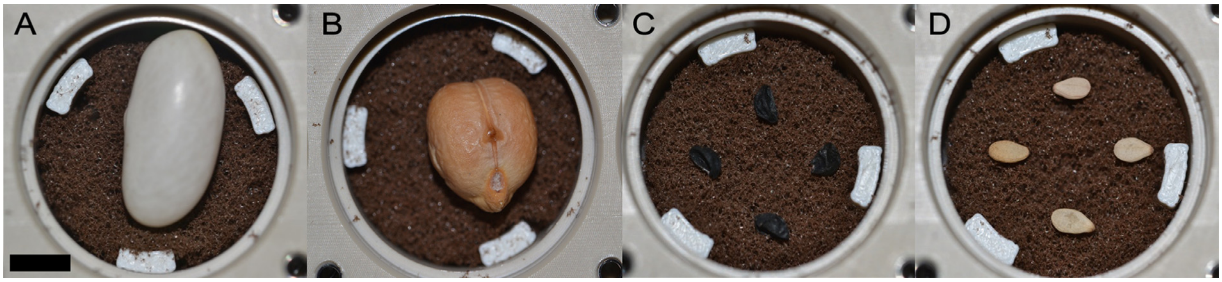

Fig. 3. Top view of the YING-B2 growth chambers with seeds of *Phaseolus vulgaris* (A) and *Cicer arietinus* (B), two of the 18 species excluded by applying the visual evaluation; and seeds of *Allium cepa* (C) and *Sesamum indicum* (D), two of the 23 species that passed the first exclusion criterion. Bar = 5 mm.

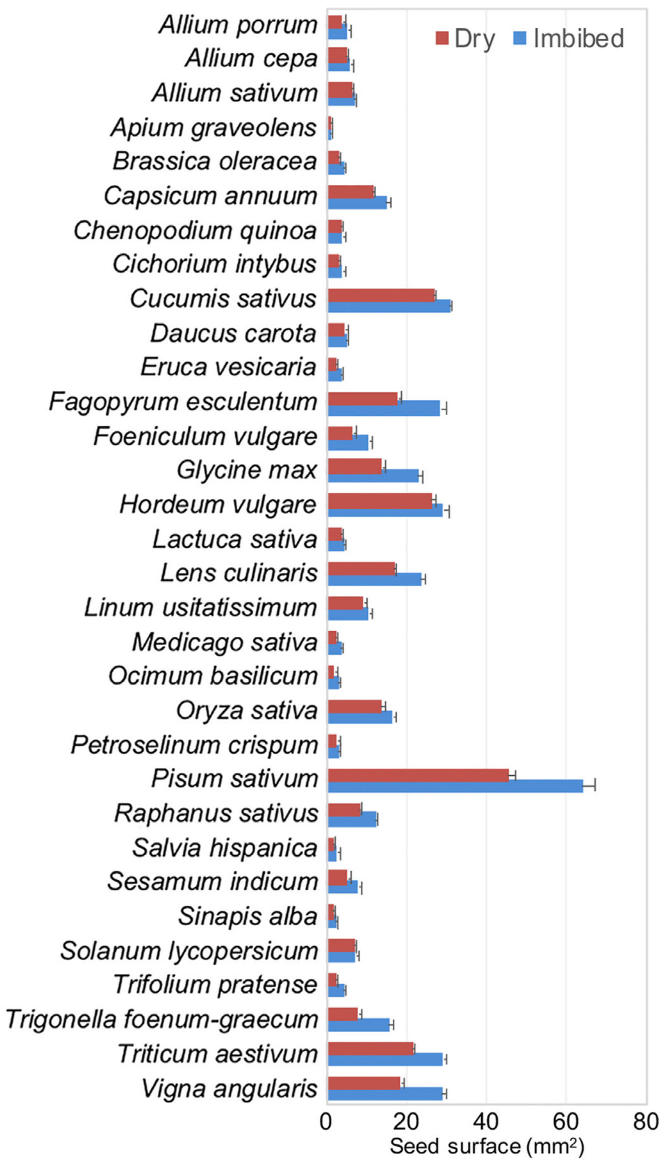

Fig. 4. Seed size before and after imbibition. Data correspond to the projected area of the seed shape on a plane surface. Mean and standard deviation per each species are reported.

Based on the evaluation of seed swelling and movements during early stage of root and hypocotyl development, a surface not less than 4 times the seed size was needed to avoid seed-to-seed interference within the growth chamber Therefore, we calculated the minimum useful surface for seed growth using the formula (1):

$$S_U = S_S \times 4 \quad (1)$$

where S_U = minimum useful surface and S_S = surface of seed calculated with the formula (2):

$$S_S = (A_M/2) \times (A_m/2) \times \pi \quad (2)$$

where A_M and A_m are respectively the major and minor axis of the ellipse resulting from the projection of the seed shape on a plane parallel to that on which it was placed during the measurements.

Finally, taking into account the requirement to insert 4 seeds in each of the four growth chambers per YING-B2, the following ratio was then calculated for each seed (3):

$$S_O/S_U \geq 4 \quad (3)$$

where S_O is the surface of the Oasis disk in each growth chamber.

Considering that the surface of the Oasis disk was equal to 254 mm^2, the maximum admissible seed size resulted to be $S_S \leq 16mm^2$. Therefore, only the species whose imbibed seeds had a surface smaller than this value were considered compatible with the size of the growth chambers (Fig. 3C, D). Applying this threshold to the 32 species investigated, only 23 passed the first selection criterion and were subjected to the second (Table 1).

3.3. Second exclusion criterion: compatibility of the seed germination time with the launch timeline (from experiment setup to microgravity conditions)

The average germination times of the 23 seed species qualified for the second exclusion criterion are shown in (Fig. 5). Data showed that most of the species germinated in less than 36 h: a timeframe too short to guarantee that root development would occur in microgravity conditions.

By applying this criterion, we discarded all but nine species. Seed germination time of the selected species was equal or longer than the required minimum threshold of 56 h.

3.4. Third exclusion criterion: compatibility of the post-germination development mode with the size and set-up of the growth chambers

Image analysis of the seedlings during the post-germination stage, showed that all the species that had passed the second selection criterion were characterized by epigeal development. Species were therefore compared relating the hypocotyl to root elongations. Fig. 6 shows the average lengths of the hypocotyls when the roots of the individual seedlings have reached their maximum length compatible with the size of the growth chambers (7 mm). Data showed that in the dark and at 22 °C, hypocotyl elongation was very variable among the analyzed species. Among all the tested species, only *Foeniculum vulgare, Apium graveolens,* and *Daucus carota* developed a very short hypocotyl (less than 1 mm) at target root elongation; therefore, these three species have been selected for the next criterion.

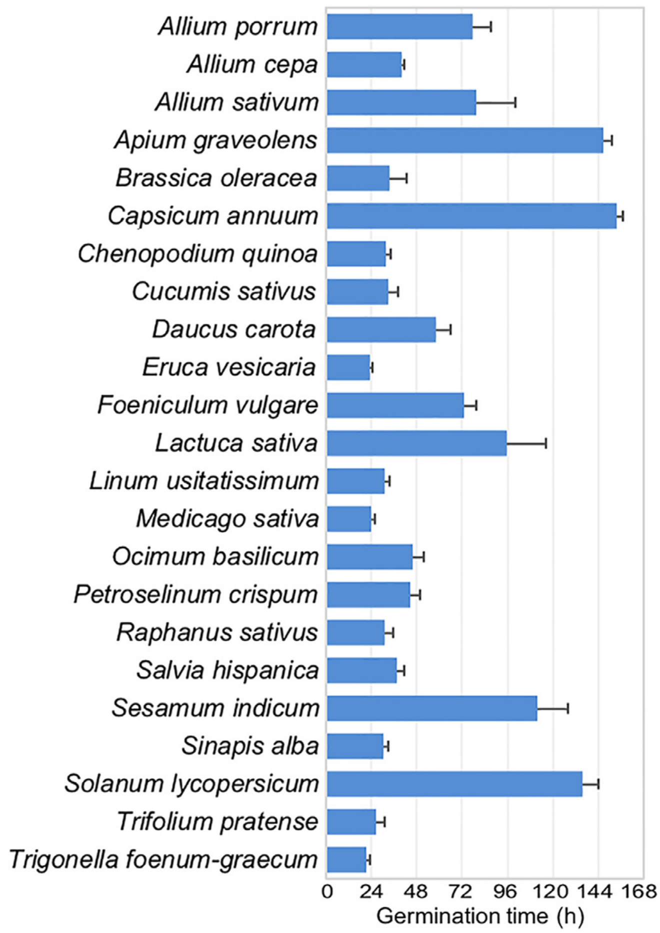

Fig. 5. Germination time (hours from seed imbibition to radicle protrusion). Mean and standard deviation per each species are reported.

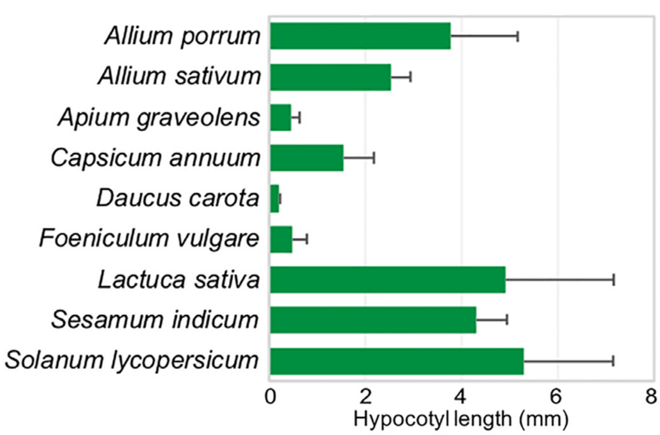

Fig. 6. Hypocotyl length at the time the root reached the target stage (7 mm). Mean and standard deviation per each species are reported.

3.5. *Fourth exclusion criterion: compatibility of root elongation rate with the minimum time required to ensure the activation of the experiment by the astronaut*

The tests aimed to define the root growth rate of *F. vulgare, A. graveolens* and *D. carota,* and showed that each of the three species reached the target stage but with an uneven development trend (Fig. 7). It is evident that both celery seeds (*A. graveolens*) and fennel seeds (*F. vulgaris*) reached the target stage of root elongation in a period of time shorter than that required from the biological activation to the injection of the chemical fixative on board of the ISS (164 h). Among the three species, only carrot seeds (*D. carota*) were compatible with the timing of the launch. For this species, it was confirmed that seed germination starts after the period of time necessary to get the microgravity conditions and that the root reaches the target stage after the minimum time necessary to guarantee the experiment activation by the crew on board. Therefore, we selected the seeds of *D. carota* to perform the MULTITROP experiment.

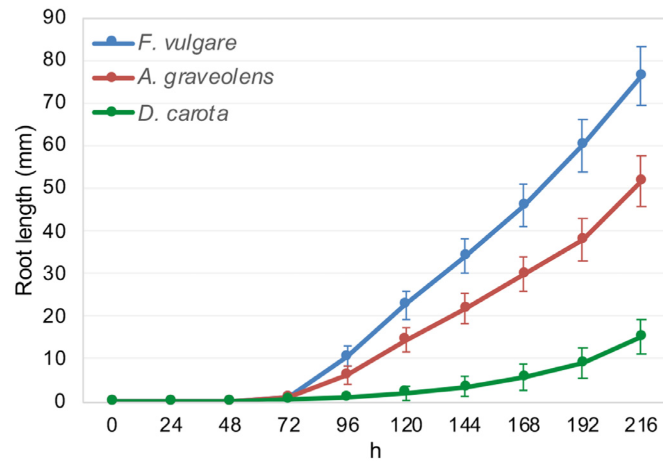

Fig. 7. Root elongation time of *Foeniculum vulgare, Apium graveolens, and Daucus carota*. Dots represent mean and standard deviation per each species.

3.6. *Fine tuning: cultivar selection*

Results of the tests aiming at fine-tuning the selection of the best seeds for the MULTITROP experiment showed that seeds from each of the three analyzed cultivars of *D. carota* germinated within the time-frames compatible with the requirements of the MULTITROP experiment. However, among them, two cultivars showed a more scalar germination and a lower final germination percentage than the other (Fig. 8). Therefore, from the overall evaluation of the data obtained, we selected the seeds of *D. carota* cv. Chantenay to carry out the MULTITROP experiment on board the ISS. They turned out to germinate and grow up to the target stage with no issues (Fig. 1B). A time-lapse video reconstructing the germination and early-stage seedling development of the selected *D. carota* cv. Chantenay is reported in Video S1.

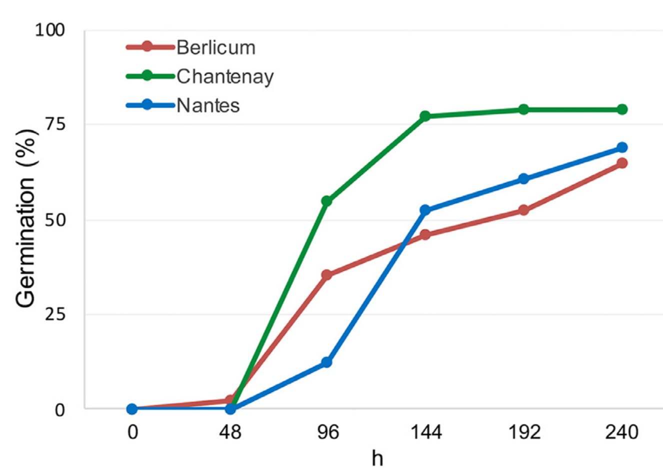

Fig. 8. Germination curves of the three cultivars of *Daucus carota* 'Berlicum', 'Chantenay', and 'Nantes' used for the final selection of the seeds.

4. Discussion

Within the trend of increasing the use of existing experiment hardware, the development of new methodological approaches is necessary. As part of the science team of the MULTITROP experiment, we took the challenge to find an objective and repeatable method for adapting the biological system to an existing hardware (Aronne et al., 2018).

To achieve the scientific goals of the MULTITROP experiment and fulfill the biological requirements, we relied on the adaptation of the biological system (namely the seed species) to the constraints related not only to the hardware characteristics but also to timing and environmental factors during the prelaunch, launch and flight operations. The latter resulted by far the most challenging and our approach was to proceed applying an objective and repeatable method. Defined by this intent, we chose the method of inclusion/exclusion criteria which has been applied to address the problem of objective selection raised in diverse scientific sectors and mainly to systematic literature review process in the medicine subject areas (Patino and Ferreira, 2018; Meline, 2006). In our work, the method of subsequent inclusive/exclusive criteria was applied for the first time to adapt a new biological system to a reused hardware. Scientific results of the MULTITROP experiment in space showed that the concept of this method was effective for the selection of the best suited species (Izzo et al., 2019).

The list and the consecutive order of the specific inclusive/exclusive criteria turned out to be a valid support to guide scientists in objectively choosing the most suitable species for the experiment. Among the 50 initial food species, the carrot seeds resulted as the best to fulfill technical requirements of the refurbished hardware assigned for the space experiment. Post-flight evaluation of the biological results showed that carrot seeds adapted very well to launch and microgravity conditions, maintaining the same germinative capacity observed on Earth (Izzo et al., 2019). Moreover, laboratory analysis of the seedlings developed on the ISS compared with those obtained from the ground control test allowed us to fully achieve the scientific goals highlighting that in microgravity, without the dominant stimulus of gravity, root chemotropism prevails on hydrotropism (Izzo et al., 2019).

One lesson learnt from the MULTITROP experiment was that the adaptation of a hardware specifically designed for an experiment with different scientific goals and biological systems poses a major challenge to researchers. In this process, the hardware itself becomes a constraint to be added to all other limiting factors that must be addressed to get a successful experiment in space.

At the time of proposal submission, the research team was aware of hardware specification and had already planned to use the first two criteria to address the constraints. Nevertheless, during the pre-flight phase, upon the assignment of the MULTITROP experiment to the specific mission (SpaceX CRS-13) a series of new constraints occurred.

The timeline from payload hand-over to de-stowage and experiment deactivation on the ISS (with the steps ranging from a few hours to a few days), combined with temperature uncertainties, gave hard time to the scientific team and required further collaboration with Kayser Italia and ASI teams to obtain as many as possible additional data. In this case, the method of subsequent inclusion/exclusion criteria resulted once more effective. The second set of criteria aimed to address all restrictions related to the timing and environmental conditions during the phases of pre-launch, launch, berthing, de-stowage up to the activation of the chemical fixative injection by the crew. Growth curves obtained by elaborating data from on-ground tests allowed to highlight the risks that a) radicles could protrude after launch but before the berth, hence before reaching the microgravity conditions and b) root growth could reach the target stage before de-stowing from the cargo vehicle, so before the crew could activate the experiment to let the chemical fixative to be injected into the growth chamber (Aronne et al., 2018).

Another objective method synthetized plant characteristics in an algorithm and was proposed to select the plant cultivars that best suits the constrains to grow in space (De Micco et al., 2012; Paradiso et al., 2012). For MULTITROP, the use of the subsequent inclusion/exclusion criteria in the selection of the most suitable seed species resulted spot-on. This method was valuable also to go beyond the species level and to select the most suitable cultivar within the last candidate species. The first criterion of considering agronomic/food species turned out to be useful also to easily find a wide assortment of high-quality seed stocks on the market.

Overall, the MULTITROP experiment showed that refurbished HW can be successfully used for experiments in Space with scientific aims different from those of the experiment previously flown. In our case, the use of an objective method to adapt the biological system to the assigned hardware and other technical and environmental constraints resulted successful. However, to plan experiments using a reused hardware, researchers should always have, as early as possible, the opportunity to evaluate together with the HW details also all other specific environmental conditions expected to occur during the pre-flight and flight operations. For instance, in retrospect, the fine adjustments of the biological system to the technical requirements would have been much easier within a framework of controlled temperature conditions.

5. Conclusions

Considering that experiments performed in microgravity are demanding in terms of costs and time, and that the development, approval and production of new hardware play a major role, the use of refurbished hardware is a trend to be supported. The MULTITROP experiment showed that, applying an objective and rigorous methodological approach, the use of a refurbished hardware to achieve scientific goals different from the original is possible. Therefore, reused hardware can be proposed as an affordable alternative for researchers who want to perform flight experiments in microgravity with fast approval procedures.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lssr.2020.07.002.

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