Aerotecnica Missili & Spazio
https://doi.org/10.1007/s42496-020-00040-8

ORIGINAL ARTICLE

Solutions to Overcome Technical Constraints and Achieve Scientific Goals of the Multi-trop Experiment

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Received: 26 March 2020 / Revised: 26 March 2020 / Accepted: 18 April 2020

Abstract
Sustainable development in space is based on affordable initiatives. Reuse of hardware is a strategy increasingly adopted to reduce the experiment costs, but its success relies on adapting the biological systems to experiment units. MULTI-TROP won the Youth ISS Science (YiSS) call promoted by Agenzia Spaziale Italiana (ASI) in 2017 and was performed on the ISS during the VITA mission. Kayser Italia provided to ASI the industrial support services for the new payload integration process, operations and logistics. The experiment was aimed to study hydrotropism and chemotropism in roots of seeds germinating in microgravity. The experiment unit (EU) was selected within a set of previously flown EUs designed by Kayser Italia. Use of a hardware designed for a different experiment was a challenge and technical constraints got complicated by the interaction with environmental conditions and timing of pre-flight and in-flight operations. Several constraints had to be solved to achieve MULTI-TROP scientific goals. Biological tests allowed an accurate selection of the plant species on the basis of the hardware design and environmental conditions expected during the experiment. In addition, arrangements of the experimental setup were necessary. Overall, the experiment was successfully performed and the scientific goals were fully achieved.

Keywords Microgravity · Plant biology · Refurbished hardware · Root tropisms

1 Introduction

Sustainable development in Space is based on affordable initiatives and efforts are needed to reduce the costs of experiments performed in microgravity. Reuse of hardware is a strategy increasingly adopted, but its success relies on the ability to adapt biological systems to experiment units. In this context, researchers face technical constraints of hardware originally designed for satisfying the requirements of a different experiment.

In 2017, ASI promoted the YiSS program, that provided access to the Space resources thanks to a bilateral agreement with NASA. YiSS was a call for educational and scientific experiments to be performed on the ISS during the VITA mission (Expedition 52/53) with the astronaut Paolo Nespoli, using hardware belonging to ASI and used for previous experiments in microgravity. The hardware provided to perform the experiment on the ISS was designed, developed and flight certified by Kayser Italia (KI) that was also responsible for its new refurbishment and for the new payload integration process, operations and logistics.

The "MULTI-TROPism: interaction of gravity, nutrient and water stimuli for root orientation in microgravity" (MULTI-TROP) proposal was selected among others. Activities were distributed in a period of about 28 months (from September 2016 to December 2018) and organized in four phases: (a) pre-submission phase; (b) pre-flight phase; (c) in-flight phase; (d) post-flight phase.

The educational aim of MULTI-TROP was to enhance young people's interest in Space biology. The experiment was conceived by scientists at the Department of Agricultural Sciences of the University of Naples Federico II in collaboration with a PhD and two Master students from the same Department and nine students from the High School

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Published online: 28 April 2020

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In addition to the educational objectives, the MULTI-TROP aimed to disentangle the role of gravity from two other stimuli for root orientation: hydrotropism and chemotropism. Considering that gravitropism is a dominant over other tropisms [1], the relative importance of water and nutrient solution in root growth orientation was investigated by performing the experiment in microgravity. The experimental setup foresaw the placement of seeds in between two disks of the same inert substrate imbibed either with pure water or with nutrient solution.

One of the requirements of the YiSS call was the use of a hardware (HW) to be chosen among a list of nine available options provided by the company Kayser Italia. After a careful evaluation of the technical description of each HW option, the Principal Investigator (PI) considered the YING-B2 Experiment Units (EUs) and the BIOKON container as the most suitable ones to accomplish the scientific goals of the MULTI-TROP experiment.

It was immediately clear that a set of critical constraints had to be tackled during the pre-flight phase to adapt the biological system to the HW characteristics without altering the scientific goals of the experiment.

During the pre-flight phase, several activities were needful and included hardware refurbishment, selection of seed species/cultivars, substrate type and nutrient solution. Successively, experiment implementation, biological activation and execution in microgravity were performed during the in-flight phase. Finally, the post-flight phase included a ground reference experiment and the processing of plant samples returned from Space.

2 Materials and Methods

2.1 HW Characteristics and Constraints

MULTI-TROP was performed in a BIOKON container equipped with two YING-B2 EUs previously flown for the YING experiment in 2009. Both the BIOKON and the YING-B2 units have been designed, manufactured and certified for launch by Kayser Italia which is a SME operating in the space sector for more than 30 years.

The BIOKON is a passive container which provides a dedicated environment for the execution of life science experiments in microgravity and it is 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 two YING-B2 experiments units with the biological samples inside.

The BIOKON used for MULTI-TROP was flown in 2009 for the DAMA mission carrying the IFOAM experiment. The configuration used for MULTI-TROP was completely different from the one used in previous experiments, mainly in terms of battery type and electronics. Indeed, the hardware was composed of heaters, thermal sensors, optical sensors and a camera, requiring high energy rechargeable batteries for operating.

For the YING experiment, the EUs were interfaced to the BIOLAB facility and, therefore, both their electronic and mechanic interfaces were not suitable for the MULTI-TROP experiment, where the EUs were interfaced only to the self-standing BIOKON container in which these ones were placed.

Both the BIOKON and the YING-B2 hardware needed refurbishment for fulfilling the scientific requirements of the MULTI-TROP experiment. The main technical constraint and challenges were related to the fact that biological activation would have occurred at the implementation phase (placing the dry seeds between the two wet substrates) and most of the experiment would have run during the launch phase. The reason behind this constraint is that YING-B2 EUs have only one reservoir chamber per each culture chamber (Fig. 1) and the latter had to be filled with the chemical fixative (activation of the chemical solution was driven by the pre-loaded timeline). For this experiment, it was preferred to fill the reservoir chamber with the chemical fixative required for "blocking" the root growth at experiment completion, also considering the medium-long re-entry of biological samples on Earth (1 month). The first consequence of this choice was the need to perform the biological and hardware integration at the launch site, a few hours before the SpX launch.

2.2 Methodological Approach

Experiments performed in space typically define the scientific requirements first and then develop HW that fits both scientific requirements and space technical constraints.

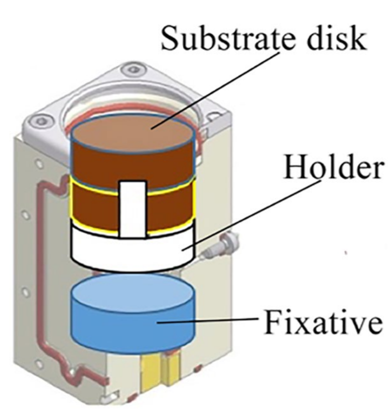

Fig. 1 Experimental setup of MULTI-TROP experiment into YING-B2 hardware

To perform the MULTI-TROP experiment it was necessary to adapt the biological system to the HW and other technical requirements. The small volume of the culture chambers within each EU, the absence of a temperature control system in the payload, and the difficulties to match biological timing of the experiment with the timeline of the launch activities were critical constraints to be considered.

Biological activation of the experiment had to occur at the launch site during the experiment implementation. However, although seed hydration had to start immediately, to fulfil scientific requirements, the radicle protrusion and root development had to occur in microgravity conditions.

During the pre-flight phase, ASI and Kayser Italia provided to the scientific team: (a) an extensive description of the HW characteristics, (b) training lessons with the HW itself in view of the implementation/integration activities at the launch site, (c) the estimated timeline from sample integration into the EU at the launch site to payload arrival on the ISS, (d) the expected temperature variation throughout the period from biological implementation at the launch site to experiment activation (root growth fixing) by the crew on the ISS.

These parameters were all used as key information for selecting the best inert substrate and the species of the seeds to be used for the experiment.

The experiment design consisted of two substrate disks to be soaked one with distilled water and the other with a nutrient solution. The most suitable inert substrate was selected according to the following requirements: 3D porous material, highly water absorbing and with a very limited liquid interface exchange.

To test the chemotropic response of roots, the composition of the nutrient solution was chosen according to scientific literature reports [2].

The biological system was selected applying subsequent criteria, considering the main constraints of the HW and of the flight operations. Several candidate species were considered. Seeds used for the tests were bought on the market as certified seeds from specialized companies. Seeds for final tests and launch experiment were purchased by the FRANCHI-Sementi seed company, that offered the professional expertise to guarantee high quality of the seeds in terms of high and uniform germinability. Germination tests were preliminarily performed in Petri dishes layered with filter paper. Subsequently, all tests were carried out in the YING-B2 ground reference units (identical to the flight ones), assembled according to the protocols defined by the team for the experiment biological implementation at the launch site.

3 Results

3.1 HW Refurbishment

The refurbishment of the HW was performed by Kayser Italia according to the experiment scientific requirements. Refurbishment activities involved a re-design of batteries pack and electronics of the BIOKON container, a re-design of the YING-B2 electronics for their power supply, a new firmware (FW) for commanding the YING-B2 according to the defined experiment timeline, the mechanical accommodation of the YING-B2 inside the BIOKON and the realization of specific tools for adaptation of the experiment samples (seeds and substrates) to the YING-B2 culture chambers.

The new battery type was selected also according to Space Safety requirements and a battery pack, including an electronic circuit protection, was designed, manufactured and tested by KI against possible leakage and launch loads vibrations. Then the YING-B2 EUs electronics was adapted for receiving power from this battery pack and the microcontroller was re-programmed to load a new timeline requested for the activation of the eight culture chambers at BIOKON manual switch-on. Finally, an interface plate was designed and realized to accommodate the two YING-B2 EUs inside the BIOKON.

A tool for correct insertion and then retrieval of the MULTI-TROP biological samples inside (/from) the culture chambers was needed because of the risks of damaging the substrate and the roots during experiment handling. In addition, to ensure complete hydration of the substrate with the fixative solution, it was necessary to reduce the volume of the culture chamber so that it corresponded to the volume of the fixative reservoir. Therefore, the research team and Kayser Italia designed and 3D printed ad hoc sample baskets (Fig. 1) for fulfilling these contingencies.

3.2 Substrate Selection

More than ten different inert substrates commonly used for plant production were tested to evaluate their performance in terms of adaptability to biological and technical requirements of MULTI-TROP experiment. Particular attention has been paid to avoid leakage of the two substrate disks. Thus, centrifuge tests were performed to simulate the hypergravity conditions expected at launch. Among the tested substrates, Oasis® Grower Foam, a phenolic plastic foam, proved to be the best to meet the biological and technical requirements. This substrate, in fact, retains a sufficient liquid content to carry out the experiment and thus the germination of the seeds and their development, even in conditions of hypergravity.

3.3 Species Selection

Although thousands of seed species could have been considered as a candidate for the experiment, we focused on food species to be used for plant cultivation in Space.

A further criterion for species selection was the compatibility of the seed size with the culture chamber volume. However, it has been also considered that to obtain an appropriate number of replicates to satisfy the scientific requirements (total number of seeds to be used for the experiment), four seeds per culture chamber had to be used. A maximum value of volume occupied by both dry and imbibed seed was, therefore, calculated on the basis of culture chamber size. This requirement significantly reduced the number of candidate species.

Successively, we performed specific laboratory tests (including time-lapse video recording) for each of the candidate species. Images were used to evaluate seed germination in dark conditions and root/hypocotyl elongation dynamics. In this context, the impossibility of controlling the environmental conditions during the experiment on the ISS did complicate the species selection. Scientists were forced to look for a biological system weakly reactive to temperature variations. The tests performed were aimed to evaluate the effect of temperature on seed germination and root development as well as the timing of radicle protrusion and root growth rate. At this stage, the analyses went beyond the level of species, testing also different cultivars. All data were statistically elaborated, and results showed that the seed set of *Daucus carota* L. cv Chantenay by FRANCHI-Sementi was the most suitable cultivar to perform the MULTI-TROP experiment in the YING-B2 EU. Selected seeds were also used to setup the Experiment Simulation Test that confirmed the expected outcomes as shown by the tests previously carried out.

3.4 Launch Experiment and Post-Flight Operations

The experiment was prepared in the laboratories at the Space Station Processing Facility at Kennedy Space Center due to the scientific constraints abovementioned. The activities were meticulously scheduled by the team before arriving at the launch site to have all the needed equipment and a dedicated laboratory assigned. The MULTI-TROP team worked following a timeline and scientific and technical protocols consolidated during the Experiment Simulation Tests performed in Italy. The late access implementation of the YING-B2 EUs inside the BIOKON occurred one day prior to launch. The experiment implementation included both biological activities (seeds placement inside each culture chamber equipped with the two types of substrates, and filling of fixative reservoir with RNA Later) and engineering activities (YING-B2 EUs leak test for proving the correct fluid containment, timeline loading on YING-B2 electronics, accommodation of the battery pack and of the YING-B2 EUs inside the BIOKON, and hermetic closure of the container and placement inside its transportation bag). Once integrated, the payload was handed over to the NASA Cargo Mission Contract (CMC) for installation inside the Dragon capsule. SpX-13 launched on December 15th and berthed to the ISS on December 17th. The BIOKON was immediately de-stowed from Dragon according to scientific requirements. Activation of the fixative injection was accomplished by the crew member Scott Tingle on December 21st and, once completed, the BIOKON was re-stowed in its transportation bag till Dragon departure. Dragon left the Station and splashed down in the Pacific Ocean on January 13th. The BIOKON was then transferred to JSC laboratories at controlled temperature (between 2 and 6 °C) as per science requirement and shipped back to Italy for samples retrieval. All the related logistics were managed by Kayser Italia.

4 Discussion

Reuse of hardware is a strategy increasingly adopted to perform experiments in microgravity, but efforts are needed to adapt biological systems to experiment units. The hardware provided to perform the MULTI-TROP experiment on the ISS were already designed by Kayser Italia for a different experiment. The use of refurbished hardware resulted in overall positive. Scientists were ready to adapt the biological system to the HW characteristics. Before submission, the scientific team was informed of all HW details and had envisioned solutions for the constraints (compatibility between growth chamber volume and seed size, the necessity to select seedlings with late hypocotyl development, the requirement of late access to the launch site for experiment implementation, etc.).

However, specific constraints of the HW got complicated by the interaction with environmental conditions and timing of pre-flight and in-flight operations. During the pre-flight phase, the assignment to the specific mission and consequently the definition of all the timing and environmental conditions of the pre-launch, launch, berthing, de-stowing phases up to the injection of chemical fixative by the crew (on-orbit operations), generated a series of further constraints.

The refurbishment of the hardware (BIOKON and YING-B2) was driven by the scientific requisites, mainly the need to adapt the samples to the culture chamber for avoiding the risk of damage when retrieving them from the EU once returned to Earth, and the uncertainty—during the first steps of the project development – on the requirement to have or not a battery pack with enough stored energy for activating the experiment timeline during the SpX launch phase (up to berthing).

The first constraint was solved with the ad-hoc design and realization of 3D-printed baskets, which also needed to be compatible with the biological sample and, therefore, required some material compatibility test; the second constraint required a feasibility study of two different batteries pack options with the involvement of different Safety approaches. The choice was then driven by the germination performance of the selected seed species.

Although most research focuses on single tropisms, in natural circumstances the plant roots respond to several tropic signals [3]. MULTI-TROP aimed to evaluate hydrotropism and chemotropism interaction for root orientation in microgravity.

The study of root tropisms is often complicated by the dominant effect of gravitropism over other secondary tropisms including hydrotropism and chemotropism [4]. The possibility of carrying out experiments on the ISS in the absence of gravity stimulus allows scientists to exclude the effects of gravitropism and further investigate a wide range of plant tropisms and their interactions. Thanks to a bilateral agreement between ASI and NASA, the MULTI-TROP experiment had the possibility of carrying out an experiment in microgravity. However, scientists have faced several other technical constraints.

As regards substrates selection, Oasis® Grower Foam, a phenolic plastic foam, was chosen for the experiment. Mainly the choice was based on the water retention capacity of the substrates exposed to levels of hypergravity expected for the launch. Oasis substrate, in fact, retains a sufficient amount of water/nutrient solution to imbibe seeds and support their development, even in conditions of hypergravity.

One of the main scientific requirements was that the root protrusion occurred after the launch, in microgravity. Furthermore, the seedlings should have reached the target stage after de-stowing from the cargo vehicle. Adaptation of the biological system to the constraints related to the prelaunch-launch operations resulted by far the most challenging. The timeline from payload hand-over to de-stowage and experiment deactivation on the ISS combined with temperature uncertainties, required much dedication from the scientific team and further collaboration with Kayser Italia and ASI teams to obtain as many as possible additional data.

Among others, temperature control turned out to be critical to achieving scientific goals. Impossibility to run the experiment within a specific range of requested temperatures was clearly stated at the beginning of the pre-flight phase. However, temperature range turned out to be extremely wide and useless to fine-tune seed selection and the timeline for experiment deactivation. The ideal seeds for MULTI-TROP had to germinate all together, not earlier than three days after payload handover (minimum time interval to guarantee germination in microgravity). Moreover, their radicles had to reach the target elongation not earlier than six and a half days from payload handover (minimum time interval for crew availability to deactivation). For most of the seed species, temperature changes of even a few degrees would have resulted in a complete mismatching of these requirements. After numerous and extensive tests, the carrot seeds turned out to adapt quite well to the wide range of environmental conditions expected for the experiment. Overall, the use of the subsequent criteria in the species selection resulted spot-on. This method resulted valuable also to go beyond the species level and to select the most suitable cultivar within the last candidate species. In addition, considering agronomic/food species turned out to be useful also to easily find a wide assortment of high-quality seed stocks on the market. 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 Concluding Remarks

Refurbished HWs can be successfully used for experiments in Space even when their scientific aims are far away from those of the original experiment. However, for the scientific team the challenges to accomplish the mission are not comparable with those commonly experienced with experiments for which a dedicated HW is developed. To plan a new experiment with a refurbished HW, researchers should be able to know as soon as possible not only the HW details but also all other specific environmental conditions expected to occur during the pre-flight and in-flight operations. Within this scenario, to limit biological constraints, the possibility to control common environmental parameters (such as temperature) should be taken into consideration.

Acknowledgements We acknowledge all members of the MULTI-TROP scientific and industry teams for their valuable contribution. We are thankful to students and tutors of the Liceo Statale Filippo Silvestri, Portici (NA) for their enthusiastic support in MULTI-TROP activities.

Funding MULTITROP research has been financially supported by the agreement between ASI and the University of Naples Federico II, n. 2017-016-H.0. ASI coordinated the program and provided the access to the ISS and to the onboard resources thanks to the Memorandum of Understanding between ASI and NASA for the design, development, operation, and utilization of three mini pressurized logistic modules for the ISS.

Compliance with Ethical Standards

Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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