First Successful Space Seed-to-Seed Plant Growth Experiment in the SVET-2 Space Greenhouse in 1997

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1. Introduction

The effect of microgravity on plant growth is an important research area since plants could eventually be a major contributor to biological life support systems (BLSS) in future long term space missions. On space stations orbiting the Earth, Moon, or other planets, and in spacecrafts making long journeys, supplementing the food that is carried along from the Earth with food that can be grown in space, would be a great weight-saving benefit. In addition plants can provide a service now performed by sophisticated equipment to scrub the carbon dioxide in the atmosphere expelled when the crew breathes. At present the oxygen necessary for breathing has to be replaced by onboard supplies carried from the Earth. Plants that use carbon dioxide in their metabolism and expel oxygen as a waste product can potentially take on the task of regenerating the atmosphere and eliminating excess humidity from the environment. And, not to be underestimated, is the up-lifting psychological effect of caring for and nurturing a garden, so far away from the greenery of the Earth. With the goal of studying this important link of a future BLSS in mind a Russian-Bulgarian group started working in 1984 on the project and created the first Space Greenhouse (SG) named SVET. Greenhouse modules were designed and tested on Earth by Bulgarian scientists from the Space Biotechnology Department of the Space Research Institute, Bulgarian Academy of Sciences.

2. Brief description of the early experiments in SVET SG

Some unique onboard experiments in the field of fundamental gravitational biology have been conducted in the SVET SG. The first major advance was made in June 1990 when the equipment was launched to the MIR Orbital Station (OS) inside the Krystal module and the first space experiment began [1]. The first fresh vegetables (radishes and Chinese cabbage) were produced in space during this experiment. Although plants grew during the 54-day period, a delay in the stages of development of the space plants was observed [2]. The fresh 23- and 54-day and dried 29-day plants returned to Earth were less of the size of the ground control plants that had been grown under the same lighting conditions as onboard MIR OS. Physiological and chemical analyses showed that the space plants were exposed to significant moisture and nutrient stress [3]

In 1994, NASA, the U.S. Space Agency, and the Russian Space Agency signed an agreement to conduct a number of joint SHUTTLE-MIR missions. According to this agreement a number of experiments on the “Greenhouse” Project were planned by the Utah State University (USU) and the Institute of BioMedical Problems (IBMP), Moscow, that were carried out in the SVET SG equipment [4]. Wheat, as an important agricultural crop and primary candidate for a future plant-based BLSS was used in the beginning. To achieve the ultimate goal, growing up of plants “from seed to seed” in microgravity, a series of flight and earth experiments were conducted, the most important being: a 3-month space experiment on MIR OS in 1995 termed “Greenhouse 2a” and a 6-month one in 1996-1997, termed “Greenhouse 2b” when mature wheat plants were harvested.

The “Greenhouse 2a” experiment was conducted on MIR OS between Aug. 10 and Nov. 9, 1995. Seeds of *Super-dwarf* wheat plants were planted in the SVET-2 SG[5]. Plants were sampled at 5 stages: on the 14th, 19th, 35th, 53rd and 74th day from planting and final harvest was on the 90th day. The plants were disoriented since, due to the Mylar mirror, the light was coming from all directions. The most interesting results were that the plants remained vegetative throughout the experiment, no flower formation was observed in any of the samples that were studied and some mature plants had as many as 18 leaves instead of the usual 8 leaves plus an ear.

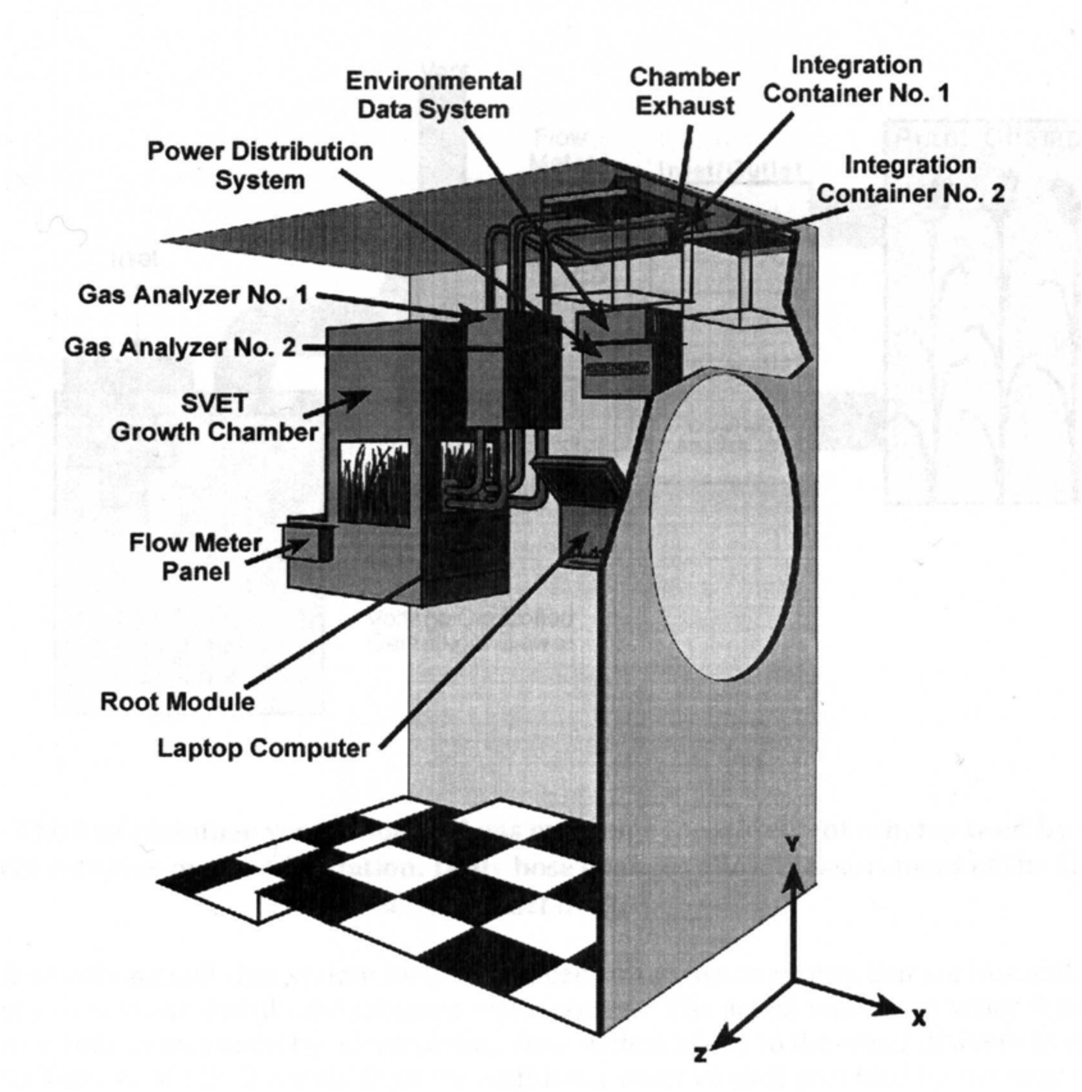
The “Greenhouse 2b” experiments were conducted on MIR-NASA-3 program from Aug. 10, 1996 to Jan. 17, 1997. Healthy plants of *Super-dwarf* wheat were grown through a complete life cycle [6]. The seeds were sown on 10 August 1996 by astronaut Shannon Lucid and plants were sampled at 4 stages: on the 43rd, 61st, 74th and 85th day. U.S. astronaut John Blaha harvested the first crop of healthy plants on 6 December 1996 and said he collected about 400 seeds! Later we were disappointed - it turned out there were 297 heads but not a single seed (all the heads were sterile). Our plants produced far more biomass and additional drying of the biological material showed that the absolute content of the dry material in the plant biomass was 95.6% - that is far more than in any other plant experiments. A second crop was planted with seeds brought from Earth a few hours after the harvest, and when the Shuttle Atlantis docked with MIR OS in mid-January 1997 (on 42nd day) that crop was harvested green, frozen in liquid nitrogen, and returned to Earth for investigation.

Ground studies were initiated to replicate the conditions on MIR OS to try to isolate the cause of the plant sterility. There is a list of about a dozen things that could have caused the sterility of the heads, only one of which is microgravity. Prof. Frank Salisbury from USU – the principal investigator of the study believes the likely culprit for the plants heads sterility to be the gas, *ethylene*, in the cabin atmosphere of MIR OS. Ethylene, a gaseous plant hormone, is known to produce male sterility (lack of valuable pollen) in wheat, and the preliminary observations revealed a lack of pollen in well-formed but unopened anthers in the wheat flowers (which were arrested in their development). There was an excessive amount of tillering (branching) in the plants (hence the many heads), and this is a well-known symptom of excess ethylene. Various species of fungi produce ethylene, and there is much fungus growth in MIR and even in SVET-2 SG (apparently not directly harmful to vegetative growth and head formation of the wheat). Thus, a strong circumstantial case exists for ethylene as the cause of the lack of seed formation. Ethylene is not normally measured in the cabin atmospheres of spacecraft because it is harmless to humans until concentrations reach about 85 % in the atmosphere, but plants are sensitive to ethylene in the parts-per-billion range.

The scientists from NASA reached a decision to make an attempt with another plant species, *Brassica Rapa* plants, in the next “seed-to-seed” experiments conducted on MIR OS in 1997.

3. Equipment used in 1997 experiment

The basic SVET SG equipment including all life support systems necessary to provide normal plant growth was used in these experiments but it was upgraded and extended under Bulgarian-American contract. A new generation SVET-2 SG with considerably improved technical characteristics was developed to satisfy the increased requirements of the experiments [7]. A new brighter Illumination Unit and updated Root Modules (RM) were developed, delivered to the station and mounted in the SVET Growth Chamber existing onboard the MIR OS (Fig. 1).



The RM were charged with a new kind of “substrate, the so-called “turface”. It was not dressed beforehand with nutritives and mineral salts as the “Balkanine” used in the “GH-2” [8]. But 1 gram of special Osmocote pellets was to be added to each plant row as a nutritive supply. The Gas Exchange Measurement System (GEMS) designed and built in USU (under the direction of Dr. Gail Bingham) was added to the basic SVET equipment to extend the range of environmental parameters that were monitored [9]. It included the Gas

Analyser System with two infrared Gas Analysers that provide all the measurements of H_2O and CO_2 concentrations and differences and the Environmental Data System with sensors for soil moisture, air and leaf temperature, light, and cabin air pressure. GEMS was controlled by the Laptop Computer, collecting all data on disk. Controlling the moisture within the RM has always been a problem of utmost importance (the Flow Meter Panel was added). To solve this problem USU developed sophisticated system including sixteen soil moisture sensors inserted into the RM to measure distributed water content both on the surface and in depth of the substrate. But even though that was sophisticated and provides indisputable possibilities to monitor the moisture distribution in the substrate volume the rather simpler measurements of the two Bulgarian-design sensors were the ones that were used to control the moisture level.

4. Description of the 1997 Experiments

These experiments were again addressed to the problem of “seed-to-seed” cycling in microgravity. They were planned by the Louisiana State University (LSU), IBMP and USU and were carried out between 27 May and 31 September 1997 under the direction of Dr. Mary Musgrave from LSU on the MIR-NASA-5 Program. The species *Brassica rapa* which derive from the economically important mustard family and have a very short life cycle were used in the experiments. The experiments were carried out by the astronaut Michael Foale (M.F.) who was launched on MIR OS on May 15 by the STS-84 mission of the joint Russian-American project MIR-SHUTTLE [10]. Along with Foale aboard Atlantis were *Brassica rapa* seeds and associated experiment hardware. Hardware transported included all supplies required to collect biological samples, equipment for pollination and operation with the seeds as well as Bulgarian equipment for SVET-2 SG (three RM and new lamps for the Illumination Unit).

The goal of the experiments was to study a plant’s entire life cycle in microgravity and better understand the effects of microgravity on plant reproduction. It was approached with the hypothesis that the microgravity environment caused a reduction in the storage reserves available to support reproductive events. The plan was for three successive plantings of *Brassica rapa*, beginning with planting of 52 seeds launched from the ground on STS-84 - the so-called “Earth seeds” (E1 seeds). These plants take about 14 days to flower after planting and the plan was for Foale to spend seven days to pollinate them. After 45 days plants were to be harvested. Seeds produced in space from this first planting on MIR OS were to be collected, dried and used in the second growth cycle. This second set of seeds was called “Space 1” (S1 seeds). Seeds generated in space from the second planting on MIR OS, the S2 seeds were to be collected and planted in the third planting in space, along with Earth seeds and S1 seeds. The experimental data would aid in determining the effects of space flight on plant growth, reproduction, metabolism, and production. By studying the chemical, biochemical, and structural changes in plant tissues, we hoped to understand how processes such as photosynthesis, respiration, transpiration, stomatal conductance, and water use were affected by the space station environment.

From May 21 to May 26, 1997 the greenhouse hardware was assembled on board the MIR OS and prepared for the experiment operations. After that "Program 1" was run to test the SVET-2 SG hardware. On May 29 M.F. started "Program 2" on the Control Unit adding water for initial substrate moistening. This program was performed two times to provide sufficient water to the two RM cells K1,2 of the SVET-2 SG. The seeds were planted on May 31. They were in cellophane strips on a sticky tape about a millimetre in size. He placed them into the wick, about 13 per row. After planting the seeds M.F. started "Program 3" for long vegetation. The Illumination Unit was switched to a program in a cycle of 23 hours on and one hour off.

The first sprouts were visible on June 2. As the seeds started to germinate, about 50% of them grew along the wick because M.F. had placed them fairly deep down into the wicks. When they finally popped up above the wick he teased them up with tweezers and the light, through phototropic action, drew the plants upwards, toward the lamps. Overall, about 80% of the seeds germinated. On June 6 when the plants had grown about three or four centimetres M.F. placed the leaf bags over. They allowed to measure CO_2 and humidity levels of the air entering and exiting each bag. In a matter of a week or so the plants started to grow one or two primary leaves, and than flower buds. After about four weeks, when the plants were about five centimetres, the buds produced a plethora of yellow flowers, which had pollen on the stamens. On June 16 the leaf bags were removed and four plants were harvested and fixed for ground analysis.

Pollination procedures were started on the next day (June 17). There is only a day's or two days' window in this very rapid growth cycle when the flower is ripe to give pollen. That is why pollination was carried out over a period of about a week. Throughout the week before June 18, only cell K1 was yielding mature plants for pollination. On June 23 M.F. reported that cell K2 started producing flowers and should be pollinated an additional week. He also reported that seed pods, known as siliques, in cell K1 appeared to be "lumpy" which he believed was indicative of seed development.

After finishing the pollination procedures the leaf bags were put back on in order to measure CO_2 over the plants again. The second harvest and subsequent fixation was scheduled to occur five days after the first harvest but it was postponed to allow more time for pollination activities.

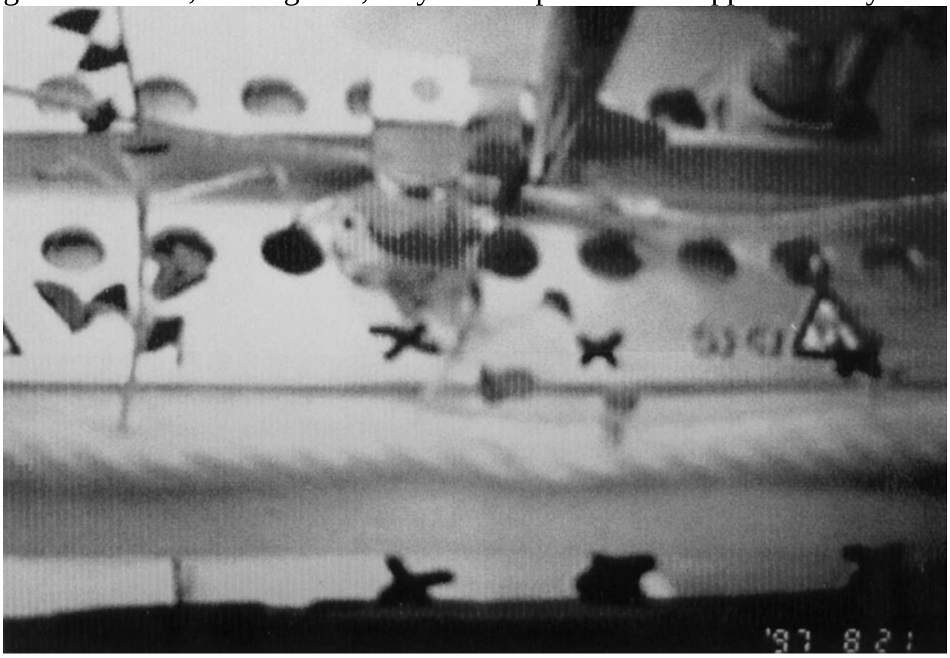
Experiment operations continued normally until the space accident of June 25 during the docking of a supply ship Progress 234 that collided with the MIR OS causing subsequent Spektr depressurisation. Almost all science on board MIR OS was affected. Due to power constraints, normal lighting operations for the experiment were interrupted for a few days and plants experienced 72 hours of darkness. The Krystall module was totally unpowered. Then, to save the experiment, M.F. powered the SVET-2 SG from the base block using a long extension cord. It was a pretty hard environment for that experiment. At that time to assess the effect on the plants and advise M.F. on future operations the scientists from LSU created the same conditions for the control plants growing on Earth. The ground-based laboratory plants exhibited reduced seed weight and had a higher percentage of undeveloped seeds than plants that continued in the light. However, it is not believed that the irregular lighting schedule seriously impacted the study. Daily observations and photography sessions continued despite the power constraints. Unfortunately the leaf bags were stored in Spektr at the time of the collision. So they were not usable and the experiment had to go on without gas-exchange measurements.

On Earth the cycle is about four weeks. It took longer in space, because the conditions were harder on MIR OS at that time. The temperatures were not quite so constant, they were down even in the $5^{o}C$ range, CO_2 concentrations ranged 15-20 times above normal levels on Earth and it slowed down things. After six weeks, some pretty long seed pods (just like pea pods) grew in the place where the flowers were. They seemed to be full of seeds.

Seeds (S1 seeds) were collected just before the final harvest, which occurred on

July 21. Seed pods were placed into little vials with desiccant, while the rest of the plants were put into formaldehyde, so they could be studied later on Earth. Space produced S1 seeds were a total of 15 or 20. They were tiny, half a millimetre in size. They were smaller than Earth seeds and not as strong. The preliminary report said that the seeds had similar structures to Earth seeds and that they looked viable. Unfortunately the S1 seeds were so weak and flimsy that only a few of them were worth even planting.

The “second planting” was performed on July 23, 1997. Six or seven of the S1 seeds were replanted together with Earth seeds so that there were half space seeds and the other half, original Earth seeds in the first row. The Earth seeds sprouted and were reported to be approximately 1 cm tall on July 30. Some of the S1 seeds sprouted too, but by that time they had not appeared above the wick material. Both Earth and S1 seeds continued to grow and later, on August 6, they were reported to be approximately 2.5 to 3.0 cm tall.



The pollination procedures for the plants germinated from Earth produced seeds were started on August 9 and were performed daily for eight days thereafter due to various stages that the plants were at. Pollination for the S1 seed germinated plants would be scheduled as these plants matured. The first fixation of samples from the second harvest was completed on August 14. The Earth plants produced siliques and on August 27 they were 3-5 cm long. Both the space plants and the Earth plants that did not produce siliques were collected and placed into a chemical preservative solution on August 29. On September 3, 1997, the Earth plant seed pods appeared healthy and were reported to be 5-6 cm long and 6 mm wide. They were harvested on September 13. The final harvest for the second planting (S1,2 seeds) was performed on September 16.

Only four of the space produced seeds germinated, out of the six or seven that were planted. There were 4 space plants on orbit - a total of four space seeds matured into “space plants” but M.F. thinks only two of them actually ended up producing a viable plant that grew up (see Fig.2). These second generation plants were smaller than the first. A cause for this is likely to be the small size of the S1 seeds. In the conditions of this hard environment in SVET-2 SG with difficulties in substrate moistening and the uncertainty of how the seed gets the nutrient through the wick it is supposed that the seed that is bigger is always favoured to do better because how much built-in carbohydrate material that seed has, determines how far it gets going. If the environment was more favourable and the soil - very nutritious, then it would not matter too much, how strong the seed was. Irrespective of this result, the germination of the space seeds and subsequent plant maturation were major experimental milestones since these events had never before occurred on-orbit.

The third planting was performed on September 17 with the seeds collected from the September 13 harvest (S1,2 seeds). The Greenhouse experiment operation concluded with the final harvest for the third planting performed on September 30 and the hardware was subsequently disassembled. The Greenhouse hardware and all preserved plant samples were returned to Earth on STS-86.

A total of three successive generations of plants were grown and harvested during the MIR-NASA-5 mission. On-orbit seed germination of seeds and maturation of plants that were themselves grown in space has never been accomplished before and this was a great success of the science.

5. Some environmental conditions during the 1997 experiment

One of the vital parameters for plant development is the substrate moisture SM1,2, measured in the two separate cells K1 and K2 of RM and maintained within defined (set up by the astronauts) threshold levels (SMT) by the Control Unit (CU) of SVET-2 SG [11]. On Fig.3 are shown the substrate moisture (SM2) and temperature (ST2) values in the second cell K2 of RM as well as the necessary water consumption (WC2). The data has been received through the telemetry system during the first month of the 1997 experiments. During the first two days from the start of the experiment (29 and 30 May 1997) the astronauts run Program 2 for initial substrate moistening of RM twice, each time supplying 30 water doses (relying on our experience of 1996).

When starting Program 3, SMT was set to 50 % to provide a good and regular substrate moistening in the whole RM volume (close to the full soil moisture capacity when the water distribution velocity is highest). Such a moisture level creates favourable conditions for better water movement and good aeration of the root zone at the same time. In space metrology, a method has been adopted for calibration of the substrate moisture measurement sensors SM1,2 accepting as 100% moisture level the moisture of a volume filled with substrate and flooded. For that reason, the full soil moisture capacity has different values for different media (substrates) and varies between 45% and 65%. During the initial period of moistening when evaporation is relatively small (only from the surface of RM and without plant transpiration) the American sensors have indicated moistening of the whole volume and the SMT was reduced by 5% (to 45%). During the first days of germination the seeds placed in the wicks (connected to the pipes of the hydrosystem) were pretty well moistened.

When Program 3 was restarted during the 6^{th} day of the experiment the necessary threshold of 45% was not set up and the result was an abundant water supply in the substrate volume. This raised the water content in RM to such a level that during the following week it was not necessary to supply even a dose of water (there was no value measured under 45%). On the 14^{th} day SMT was raised to 50%, the automated system reacted immediately and supplied continuously water doses, maintaining SM2 regularly in these limits, compensating the evaporation. On the 25^{th} day the SMT was reduced again to 45% to dry the plants and mature the seeds, but on the 27^{th} day the experiment was stopped (temporarily) and the TMS information - definitively due to the collision.

From the measured substrate temperature values (ST2) it is clear that the temperatures maintained on MIR OS have been comparatively high during the first 2 weeks – within 27-28^{o}C, and they have returned to normal during the next two weeks (22-23^{o}C). The substrate temperatures being in direct dependence on the environmental air onboard the MIR OS are only 1-2^{o}C lower (due to the evaporation).

6. Conclusion

After the failure of the idea of producing wheat seeds in space during the "Greenhouse 2" experiment the success of the 1997 experiment stimulated the scientists to continue conducting wheat plant experiments aimed at carrying out a complete life cycle ("seed-to-seed") under microgravity.

As a third experiment ("Greenhouse 3") in the consecution of wheat plant experiments in February 1998 the American scientists made an unsuccessful attempt to grow *Super-dwarf* wheat on MIR OS in another greenhouse equipment ("Astroculture"). Unfortunately the equipment failed and the experiment was interrupted.

The next experiment ("Greenhouse 4") was carried out in the SVET-2 SG on MIR OS between November 1998 and February 1999 by the Russian 26 crew (astronaut S. Avdeev). Healthy plants of wheat (a variety of *Apogey)* were grown through a complete life cycle. This variety has been developed by scientists of USU specially for greenhouses and is distinguished for it's ability to form seeds in high ethylene concentrations. The *Apogey* wheat reaches average height which is important for the conditions of space flight. The seeds were sown on 18 November 1998. Only 8 of them germinated because of problems with the program for substrate moistening. After solving these problems the seeds were sown again on 30 November. In the beginning of December they started to germinate and grew good sprouts. Later, about 15 January, plants started to form ears and on February 26 the life cycle was completed and the plants were harvested. The first wheat seeds were produced under microgravity conditions in this experiment. Some of the plants were dried for chemical and biochemical analysis.

The next wheat plant experiment ("Greenhouse 5") was started onboard the MIR OS in the SVET-2 SG equipment by the Russian 27 crew. On 3 March 1999 the astronauts soaked 40 seeds (30 of them were Earth produced and the rest of 10 - space produced). The goal was already sprouting seeds to be planted in SVET-2 SG. All the seeds germinated and on 9 March were sown in the RM. The program for initial substrate moistening passed and the crew completed successfully the 3-month experiment.

Reliable equipment and biotechnology were created and considerable experience in conducting of plant experiments was gained in the 1990's. Certainly, these experiments will be continued in the next 21st Century on the International Space Station (ISS): Russia is interested in a new updated version of SVET-3 SG; NASA is developing two suites of equipment - the Gravitational Biology Facility (Plant Research Unit) and Centrifuge Facility; ESA - Biopack and SGH. ESA and NASA signed an agreement for joint use of all life science facilities on ISS.

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