DEVELOPMENT OF A SYSTEM TO SUBMIT SUGARCANE PLANTS IN REAL MICROGRAVITY USING THE VSB30 SOUNCING ROCKET

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ABSTRACT

Studies at the SSI station showed that some plant species had problems in development. Furthermore, others studies indicated that microgravity affects cell-growth and differentiation. However, as of yet, no coherent explanation has been provided for these observations. Our group has been using sugarcane as a plant model, due to its available genetic information and economic importance for Brazil. Considering these aspects, the aim of this work is to identify molecular messengers produced in response to real microgravity conditions using the VSB30, a Brazilian sounding rocket. The results presented here correspond to the development of a system for sugarcane to be used in the VSB30 rocket.

INTRODUCTION

Plants are sessile organisms that evolved to grow on earth under a 1 g gravitational force. Under these conditions it has been observed that stems grow upwards and roots downwards. This physiological stimulus is known as graviotropism: negative for roots and positive for stems. During plant development, a complex signal network regulates cell differentiation and tissue differentiation by gene regulation and protein modification [1]. Studies at the SSI station revealed that some species had problems in plant development, in pollen production, or setting seeds such as maize and wheat [2, 3, 4]. Furthermore, it has been observed for Brassica rapa that plantlets had a similar development at SSI; however, the chloroplast morphology had some difference [5]. Metabolic modification was also observed in the Arabidopsis plant cell when the sounding rocket was used [6]. In other experiments with analysis performed at the molecular level, many genes were up-regulated when a hypercentrifuge was used for simulated microgravity conditions [7, 8]. These experiments were done using Arabidopsis thaliana cells. The results suggested that the gravitational signal may be perceived at the plasma membrane and then translated by different pathways related to stress response in plants.

Sugarcane (Saccharum spp.) is a major crop for biofuel production, which is currently based mostly on the conversion of the sucrose accumulated in stems into ethanol. Brazil is responsible for 34.6% of world production, followed by India, China, and Thailand. Furthermore, the monoploid genome size of sugarcane is estimated to be about 930 Mbp, which is comparable to sorghum and roughly twice as large as the rice genome [9]. Although a comprehensive genome sequence is not available for sugarcane yet, transcriptome...
studies have been reported [10, 11]. The Sugarcane Expressed Sequence Tag (SUCEST-FAPESP) database contains almost 250k ESTs sequences [11], and the DFCI database (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=s_officinarum) currently contains 282k ESTs, representing 42k contigs (release 3.0), facilitating functional genetics studies. Since 2002, our group has been using sugarcane as a plant model, in DNA repair and understanding the response to abiotic stress (oxidative, drought). Considering these aspects, the aim of this work is to employ molecular tools to identify the messengers (mRNA and proteins) produced in response to real microgravity conditions using the VSB30, a Brazilian sounding rocket. The results presented here correspond to the first step of this work in order to submit sugarcane plants to real microgravity using the VSB30 rocket.

RESULTS
Development of a system for the VSB30 flight
The sugarcane experiment was called VGP (VSB30 Gravity Plant). As our aim was to analyze the sugarcane plants at the molecular level, it was important not to stress the plants, so this experiment had a late access (4 hours before launching). Considering this, the VSB30 sounding rocket had two opposite windows that allowed introducing the experiment without opening the payload. The other aspect to be considered was that the payload recuperation was on sea. Consequently, the VGP experiment was composed of two hermetic boxes in anodized aluminum (Fig. 1). The size of these boxes was 169 x 125 x 300 mm. The box called 00 had 20 sugarcane plants, and box 01 had 14 sugarcane plants. Furthermore, each box had two data-loggers systems in order to measure the temperature and humidity (Fig. 2). These two systems provided the information of the temperature and humidity of the plants during the flight.

**Figure 1 – VGP box** – a photo showing the top and bottom of the aluminium box

![Figure 1](image1)

**Figure 2- Data logger system.**
Each box had two data logger systems to measure the temperature and humidity every 5 seconds. This logger was made by Novus (Brazil).

![Figure 2](image2)

Another aspect that was considered that may affect the plant response was vibration that happened during the VSB30 flight. Then, it was important to fix plants in a way that they were tightly held so as not to fall or break. Thus, an aluminum plate with holes was designed, where
it was possible to use plastic cables to tie these plans securely (Fig. 3).

Figure 3 – Securing plants for flight
A photo showing how sugarcane plants were fixed by plastic cable on the aluminium plate.

As the payload would be on the sea, it was important to consider the possibility of sea water in the box. Then, to reduce this problem, these two boxes were hermetic as they had two o’rings to assure the box seal. Then, all the systems had to be qualified for the VSB30 flight. So, all systems were tested first for temperature. The data-loggers were placed in a hood where the temperature was kept high. The idea was to test whether all electronic components and batteries would be working after the temperature elevation. After this test, everything was determined to be satisfactory. The other test was for vibration. For this test the data-logger was analyzed and then the boxes. These elements were placed separately in a shaker that vibrated at three axes (X, Y and Z). All systems worked properly. The next step was to use helium gas to determine whether the boxes were airtight. As these boxes had two o’rings, it was observed that the system was well-sealed. This aspect was very important as payload recuperation was on the sea. The final test performed was the EDA test - a dynamic-assay using the vehicle. In all tests that were done, it was observed that the VGP system worked properly, and then it was qualified for flight. Having the system qualified, the next phase was to determine the plant-age to be used in this experiment. Sugarcane plants were tested at age 10, 15 and 20 days old. These plants were put inside the box-system, fixed with the plastic cable at the aluminum plate, and then transferred to a shaker that would vibrate in the three axes, simulating the same vibration level as the VSB30 rocket. The results showed that plants that were 10-15 days old worked better. Plants that were 20 days old suffered in the vibration test. Furthermore, a biological control for vibration was also done in order to minimize the interference that may happen during flight. On December 12, 2010, at CLA (Brazil), the VSB30 was launched. The apogee was 241.9km and the sugarcane plants were submitted to microgravity conditions for 6:00 min. After the recuperation, the plants were isolated for molecular analysis according to the specific protocol for RNA or proteomic analysis. Thus, the results, presented here, confirmed that the system developed for sugarcane plants worked-well, allowing us to submit whole plants to real microgravity conditions.

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