The Effect of Hypergravity on the Germination and Growth of *Eruca Sativa Mill*

[1]MARLISE ARAÚJO DOS SANTOS, THAIS RUSSOMANO, [1]FLAVIA NATHIELY SILVEIRA FACHEL, [2]EDUARDO CASSEL, [3]LEANDRO VIEIRA ASTARITA

[1]Joan Vernikos Aerospace Pharmacy Laboratory, Microgravity Centre FENG/PUCRS- BRAZIL,
[2]Unitary Operation Laboratory – FENG/ PUCRS- BRAZIL,
[3]Laboratory of Plant Biotechnology, Bioscience Institute – PUCRS- BRAZIL

marliseas@hotmail.com, E.cassel@pucrs.br, astarita@pucrs.br

Abstract: Many environmental factors such as soil, temperature and gravity influence plant development. The aim of this research was to evaluate the effects of hypergravity on *Eruca sativa* Mill. (Rocket plant). A centrifuge was used to perform hypergravity experiments. Rocket plant seeds were placed on germination paper followed by the addition of water, and subsequently subjected to intermittent hypergravity (8h hypergravity followed by 16h rest), repeated over four days. Total germination and size of seedling were evaluated before and after being cultivated in soil in a natural environment. Results showed that root growth was greater in the centrifuge group than the control group. The growth of shoots after transference to soil was also found to be higher than the control group. Additionally, the centrifuge group of rocket seeds germinated 1% more and had a material mass of almost 20% more than the control group.

Key words: Hypergravity, Eruca Sativa Mill.

Introduction

One of the main problems faced by food, pharmaceutical and perfume companies working with vegetal materials is the maintenance of access to good quality and quantity of supply in order to keep continuous production of their products. The quality of vegetal material produced depends on cultivation conditions as well as the specific germination time of each species. The environment interferes with the physiological mechanisms of plants (Arimura et al., 2005) and consequently on the substances they produce. Among these environmental factors are soil, temperature, altitude, luminosity and gravity (Leite, 2009; Martins-Ramos et al., 2010). Gravity is one of the most important cues and plants respond to it by growing shoots upwards (negative gravitropism) and roots downwards in the direction of gravitational pull (positive gravitropism). Gravitropism is a coordinated response composed of four sequential processes: gravity perception, signal formation, intracellular and intercellular transduction and transmission of the signal, and asymmetric cell elongation between the upper and lower sides of the responding organism (Morita et al. 2007). Researchers have been investigating the application of different technologies to plant growth aimed at producing greater volumes of vegetal material in shorter time periods, whilst maintaining a good quality of plant. Rocket plant (*Eruca sativa* Mill. or *Eruca vesicaria* L.) is widely distributed all over the world, is usually consumed fresh (leafs or sprouts) and is known for its typical spicy taste. It contains a number of health promoting agents including carotenoids, vitamin C, fibers, flavonoids, and glucosinolates. Rocket plant has been used in traditional pharmacopoeia for various purposes: antiphlogistic, astringent, depurative, diuretic, digestive, emollient, tonic, stimulant, laxative, and rubefacient (Barillari et al., 2005). All of these aspects, in addition to its short germination time, stimulated the investigation of the effect of hypergravity on *Eruca sativa* Mill. Therefore, the aim of this research is to evaluate the effects of hypergravity on the germination and growth of *Eruca sativa* Mill.

Material and Methods

Hypergravity conditions

A small centrifuge was built at the Microgravity Centre/PUCRS in order to perform hypergravity experiments. It consists of two main structures and an electromechanical motor system. The base structure is made from carbon steel (120mm height, 350mm length, 230mm width) and holds the electromechanical motor system within. Connected over this via a rotary shaft, is a round plastic formation with a diameter of 660mm (Figure 1) and this holds the plant containers. This structure can carry 12 sample holders of 65mm in diameter for each test (Figure 2). Each sample holder allows the plant container to lean during centrifuge tests to ensure that the acceleration is applied to the desired axis.

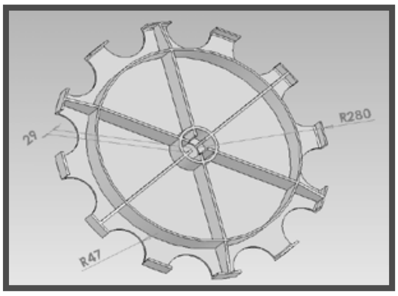

Figure 1. Schematic view of the round structure used to support plant containers (n=12)

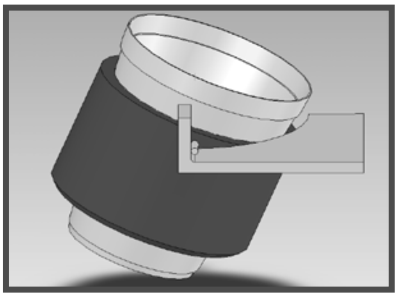

Figure 2. Schematic view of the plant container.

The electromechanical system consists of one electric motor that is connected to a reduction pulleys and gears system capable of delivering a continuous rotation up to 200rpm on the samples support structure. Rotation of the system is controlled by a DC power supply that is directly connected to the DC motor. The desired rotation in revolution per minutes (rpm) is established by changing the voltage of the power supply and measuring the rotation frequency with a contactless optical tachometer. A Digital Timer is used to control the schedule for turning the system on and off, by programming it for a period of 4 cycles of 16 hours off and 8 hours on.

For this study the centrifuge was set to run for a determined rotation frequency that would result in an acceleration equivalent to +7Gz. To calculate the rotation frequency for the desired acceleration the following procedure was used.

Using the schematic of acceleration in the centrifuge (Figure 3) and all the known parameters:

Length of the arm (Length): 0.33m

Desired Resultant Acceleration (a_r): +7Gz

Earth's Gravity Acceleration (aG_z): +1Gz

Then the parameters to be determined:

Centripetal Acceleration (a_{cent})

Resultant Sample Angle (α)

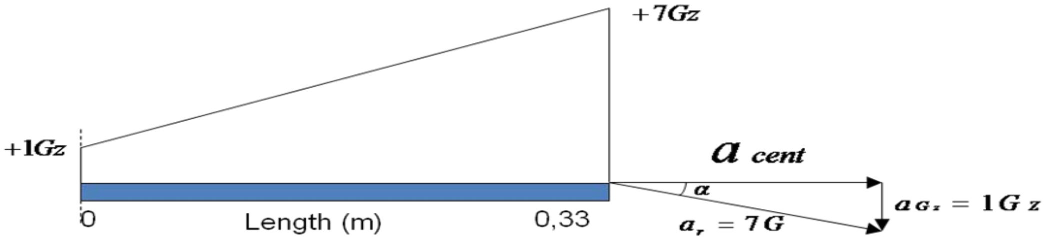

Figure 3. Schematic of acceleration of the sample in the centrifuge.

The α angle can be determined using a rectangle triangle formed by the a_r as hypotenuse and the aG_z as the opposite side and the Equation 1.

$$\sin\alpha = \frac{a_{G_z}}{a_r} \quad \text{Equation 1}$$

Applying the values a_r = 7 and aG_z =1 on the Equation 1:

$$\sin\alpha = \frac{1}{7} = 0.143$$

It is then possible to have the α angle using the Equation 2:

$$\alpha = \text{arc sin}(\sin\alpha) \quad \text{Equation 2}$$

Resulting in:

$$\alpha = 8.21°$$

The α angle is then used to determine the value of a_{cent} by applying the cosine relation, Equation 3:

$$\cos\alpha = \frac{a_{cent}}{a_r} \quad \text{Equation 3}$$

Applying the values $a_r = 7$ and $\alpha = 8.21°$ on the Equation 3:

$$\cos 8.21° = \frac{a_{cent}}{7}$$

$$a_{cent} = 0.989 \times 7$$

$$a_{cent} = 6.923G$$

To determine the rotational frequency of the centrifuge needed to produce calculated a_{cent} the value was converted to meters per square second (m/s²) multiplying it by the Earth´s Gravity 9.81m/s²:

$$a_{cent} = 6.923 \times 9.81$$

$$a_{cent} = 67.914 \text{ m/s}^2$$

The a_{cent} can be described in the Equation 4, where ω is the rotational speed in radians per second (rad/s):

$$a_{cent} = \omega^2 \times Length \qquad \text{Equation 4}$$

It is then possible to determine ω, using:

$$\omega = \sqrt{\frac{a_{cent}}{Length}}$$

$$\omega = \sqrt{\frac{67.914}{0.33}}$$

$$\omega = 14.346 \text{ rad/s}$$

Then convert this value to frequency f in revolutions per minute (rpm):

$$f = \omega \times \frac{60}{2\pi}$$

$$f = 14.346 \times \frac{60}{2\pi}$$

$$f = 137 \text{ rpm}$$

Hypergravity experiment

Eruca sativa Mill (Rocket plant) seeds from Isla Pak (Batch:18708/10), were submitted to hypergravity experiments. The centrifuge described above was used to simulate hypergravity. Three rolls of germination paper, previously humidified with water and seeded with fifteen seeds each (forty five seeds in total), were placed in each of six of the twelve recipient containers. The hydration of the seeds was maintained by the addition of 80mL of distillated water to each container, and subsequently, each was covered by a plastic film containing holes to allow air exchange, yet reducing the loss of water by evaporation. The experiment was performed in an intermittent form, 8h in a centrifuge rotating at a +7Gz velocity speed, followed by 16h of rest, repeated over a four day period. The same procedure and number of samples were used to prepare a control group. At the end of the allotted time the number of germinated seeds were evaluated for both groups, and all seeds then removed from the germination paper and measured individually for the total size of seedling (shoot + root). The results of the experimental and control groups were compared by applying Student's *t*-test on the average, using an SPSS statistics program.

Natural environmental cultivation

After being submitted to hypergravity simulation in an intermittent form over four days, the seedlings from both groups were transferred to plant pots containing soil and kept outside on a terrace for continued cultivation in a natural environment. Water was added to each to keep the soil humidity at 80%.

Results and Discussion

Many studies about the effect of hypergravity on plants have been done (Kasahara et al., 1995; Soga et al., 1999; Hoson and Soga, 2003). According to Kasahara *et al*., (1995) gravitational forces greater than +1G have been useful to study the influence of gravity on the growth of plants. The stems of the seedlings from the centrifuge group were verified by this present study as being less flexible than those of the control group. These findings are in agreement with experiments conducted by Hoson et al. (2002), who demonstrated that the hypergravity produced by centrifugation increased the stiffness of the cellular wall due to the gravitational force. Research performed by Tamaoki et al. (2009) has shown that the content of matrix and cellulosic polysaccharides in unit length increases in shoots when under hypergravity conditions. Some authors have also mentioned that hypergravity increased the amount of cell walls per unit length of shoot in radish, cucumber, cress, azuki bean, and maize seedlings (Wakabayashi et al., 2005). These observations could explain the weight difference observed between the two groups in this experiment, whereby the total weight of *Eruca sativa Mill* seedlings submitted to hypergravity simulation was 25.72g, as compared to 20.89g for the control group, an increased vegetable mass of almost 20%.

Upon analysis of the shoots and roots of the *Eruca sativa Mill* seedlings, it was seen that growth was greater in the centrifuge group than the control group ($p<0.001$) (Figure 4 and 5). When subsequently being transferred to the pot containing soil, the aerial part of the plant grew more in the experimental group than the control group, probably due to the greater root development during the hypergravity simulation. These results are very important, considering that the active substances of

Eruca sativa Mill. are presents in its the aerial part. (Leite, 2009).Additionally, it is important to mention the germination of the Rocket plant seeds in the centrifuge group was 1% more than for the control group.

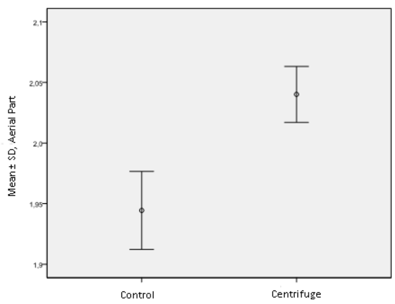

Figure 4. Growth of Aerial Part

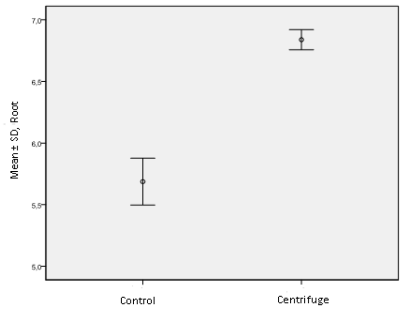

Figure 5. Growth of Root

Conclusion

The results suggest that hypergravity simulation increases the germination and growth of *Eruca sativa* Mill. The roots of those seeds subjected to hypergravity simulation developed more than those in Earth's gravity, but in addition, after continued cultivation in a natural soil environment, an increase in growth rate for the experimental group plant shoots as compared to the control group would suggest that the effect of the hypergravity simulation on the rocket plants is ongoing and will consequently produce a greater volume of vegetal material for use by industry. Further qualitative analysis must be conducted to evaluate if any modifications to the secondary metabolites occur for plants subjected to hypergravity simulation. More studies are also necessary in order to increase understanding of the influence of hypergravity on plants.

References

Arimura G., Kost C., Boland, W. (2005). Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta – Molecular and Cell Biology of Lipids* 1734, 91–111.

Barillari, J., Canistro, D., Paolini, M., Ferroni , F. , Pedulli, G.F.; Iori, R., Valgimigli, L. (2005). Direct Antioxidant Activity of Purified Glucoerucin, the Dietary Secondary Metabolite Contained in Rocket (*Eruca sativa* Mill.) Seeds and Sprouts. *Journal of Agricultural and food chemistry*, 53, 2475-2482.

Hoson, T., Soga, K., Ryuiji, M., Mizue, S., Yukiko, N., Kazuyuki, W., Seiichiro, K. (2002). Stimulation of Elongation Growth and Cell Wall Loosening in Rice Coleoptiles under Microgavity Conditions in Space" *Plant Cell Physiology*, *43* (9), 1067-1071.

Hoson, T. & Soga, K. (2003). New aspects of gravity responses in plant cells. *International Review of Cytology*, *229*, 209-244.

Leite, J.P.V. (2009). *Fitoterapia*: *Bases científicas e tecnológicas*. São Paulo: Atheneu.

Kasahara, H., Shiwa, M., Takeuchi, Y. & Yamada, M. (1995). Effects of hypergravity on elongation growth in radish and cucumber hypocotyls. *Journal of Plant Research, 108* (1), 59-64.

Martins-Ramos, D., Bortoluzzi, R.L.C., Mantovani, A. (2010). Plantas medicinais de um remascente de Floresta Ombrófila Mista Altomontana, Urupema, Santa Catarina, Brasil. *Revista brasileira de Plantas Medicinais.de Botucatu*, *12* (3), 380-397.

Morita, T. M., Saito, C., Nakano, A.; T asaka, M. (2007). endodermal-amyloplant less 1 is a novel allele of Short-Root. *Advances in Space Research*, *39*, 1127-1133.

Soga, K.i, Wakabayashi, K., Hoson, T. & Kamisaka, S. (1999). Hypergravity Increases the Molecular Mass of Xyloglucans by Decreasing Xyloglucan-Degrading Activity in Azuki Bean Epicotyls. *Plant Cell Physiology*, *40* (6), 581-585.

Tamaoki, D., Karahara, I., Schreiber, L., Wakasugi, T., Yamada, K. & Kamisake, S. (2006). Effects of hypergravity conditions on elongation growth and lignin formation in the inflorescence stem of Arabidosis thaliana. *Journal of Plant Research*, *119*, 79-84.

Wakabayashi, K.; Nakano, S.; Soga, K.; Hoson, T. (2009). Cell wall-bound peroxidase activity and lignin formation in azuki bean epicotyls grown under hypergravity condition. *Journal of Plant Physiology*, *166* (9), 947-54.