Future Plants: Effects of Gibberellic Acid and Brassinolide on Plants in a Simulated Microgravity Environment

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Abstract

Living cells exposed to the microgravity of space for limited time show changes in function and structure. This suggests that alterations in cell metabolism, immune cell function, cell division, and cell attachment have occurred. Among plant hormones, brassinosteroids are structurally the most similar to animal steroid hormones. Like its animal counterparts, it regulates the expression at numerous genes, impacts the activity of complex metabolic pathways, contributes to the regulation of cell division and differentiation, and helps control overall developmental programs leading to morphogenesis. The addition of brassinolide can help reverse or prevent the alterations of plants exposed to microgravity and space. A rotating clinostat was prepared to expose plants to a simulated microgravity environment. Brassinolide was added to determine whether it could prevent the alterations of microgravity. Also tested was a control group, and gibberillic acid as a standard. Over the past decades, scientists have discovered that space flights have wide-ranging effects on living systems. Through millions of years of evolution, most terrestrial organisms have adapted to function optimally in the presence of a constant gravitation field, but little is known about the effects of the absence of gravity, and even more so, how to prevent it.

Purpose

The purpose of the project is to determine the change in growth of *Glycine max* in microgravity compared to control. Also considered was if plant hormones such as Gibberelic acid and brassinolide will promote plant growth in microgravity. Understanding the behavior of plant organisms in microgravity will aid our understanding of systems for long duration space flight and also to gain a better understanding of how plants adapt to changes in gravity, which could lead to improved growth of plants in space and on Earth.

Problem

Does microgravity affect the growth of *Glycine max*?

Does the addition of brassinolide and Gibberelic acid combat microgravity's strain on plants?

Review of Literature

Gravity is an all-pervasive force that exerts its influence on all organisms on the planet. As a consequence, gravitaxic (or geotaxic) responses are found in all organisms. However, gravitaxic perception and the pathways which mediate appropriate behavioral responses are perhaps the least understood (http://www.informatics.ed.ac.uk.html).

Zero g infers that gravity disappears in Earth's orbit and weightlessness implies that weight goes away. On the contrary, gravity is still present in Earth's orbit. In a typical Space Shuttle orbit gravity is the gravitational pull of Earth and is still more that 90 percent the pull at Earth's surface. Weight doesn't go away in orbit, but the ability to measure it does. The term microgravity can be interpreted in a number of ways depending upon the context. The prefix micro is derived from the original Greek micros, meaning "small". Another common usage of micro is found in quantitative systems of measurement, such as the metric system, where micro means one part in a million. Earth creates a gravitational field that acts to attract an object with a force inversely proportional to the square of the distance between the center of the object and the center of Earth. When measured at the surface of Earth, the acceleration of an object acted upon

only by Earth's gravity is generally referred to as one g or one Earth gravity. Microgravity is an acceleration of gravity at Earth's surface (Dotto). Microgravity is also the state in which gravity is reduced to almost negligible levels, such as during space flight (http://spaceflight.nasa.gov/history.html).

Human, plant and animal cells exposed to the microgravity of space for only a few short days show changes in function and structure. The data suggests that alterations in cell metabolism, immune cell function, cell division, and cell attachment have occurred in space. Scientists have reported that after nine days in space, human immune cells failed to differentiate into mature effector cells. The results of investigations into how the stress of space flight can alter normal metabolic activities and important aspects of immune cell function may indicate the body's inability to produce mature and fully differentiated cells in space. This may lead to health problems on long-term space flights, including impaired healing abilities and increased risk of infection (http://www.lifesci.arc.nasa.gov/lis2/Chapters1_3/Introduction.html).

Brassinosteroids also known as "BRs" comprise a class of over 60 polyhydroxylated sterol derivatives that appear to be distributed throughout the plant kingdom. Among plant hormones, BRs are structurally the most similar to animal steroid hormones, which have well-known functions in regulating embryonic and post-embryonic development and adult homeostasis. Like their animal counterparts, BRs regulate the expression of numerous genes; impact the activity of complex metabolic pathways. Contribute to the regulation of cell division and differentiation, and help control overall developmental programs leading to morphogenesis. They are also involved in regulating processes more specific to plant growth including photomorphogenesis and skotomorphogenesis, and cell expansion in the presence of a potentially growth-limiting cell wall (http://www.bioone.org/perlserv/?request=get-document&issn=1543-8120&volume=026&issue=01&page=0001).

In the early 1980s, USDA scientists showed that BRs could increase yields of radishes, lettuce, beans, peppers, and potatoes. However, subsequent results under field conditions were disappointing because inconsistent results were obtained. For this reason testing was phased out

in the United States. More recently large-scale field trials in China over a six-year period have shown that 24-epibrassinolide, an alternative to brassinolide, increased the production of agronomic and horticultural crops (including wheat, corn, tobacco, watermelon and cucumber). However, once again depending on cultural conditions, method of application, and other factors, the results varied (http://www.chm.bris.ac.uk/motm/brassinolide/brassinolidec.html).

The most common brassinosteroid is brassinolide. It is the first and most active of the brassinosteroids. It is a naturally occurring plant steroid that promotes growth, increases yields for grain and fruit crops, and makes plants more resistant to drought and cold weather. Brassinolide was first isolated from rapeseed plant pollen (*Brassica napus* L.). Its molecular formula is C28H48O6. Plants treated with brassinolide can produce yields that would be unable to attain by using standard practices. Specific effects on plants by brassinolide includes: promoting shoot elongation and strongly increasing root growth and development. Crops treated with the steroid aren't harmed by it nor are humans (Phytochemistry 17). In addition to increasing yields, brassinolide can improve the freezing tolerances of the plants, and prevent premature fruit drop. Brassinolide used in the present invention is a crystalline substance soluble in organic solvents such as ethanol and acetone (Nature new Biol. 239). Brassinolide is usually employed in the form of a liquid formulation as a stock solution, an emulsifiable concentrate or a solid (powder or granular) formulation as a water-dispersible agent. Theses formulations are diluted with a sufficient amount of water to have given concentration of brassinolide. It is also possible to prepare a brassinolide paste based on lanolin to apply brassinolide directly to a specific part of a plant (http://3e.plantphys.net/article.php?ch=e&id=201).

A great deal can be learned about the effects of gravity, especially changes in the fixed gravity vector, here on Earth. This is accomplished just by turning specimens on their side or upside down. However, because gravity is always present on Earth, it is more difficult to stuffy the effects of microgravity or gravity levels less than 1 g while here on the surface. One answer is clinorotation. The idea behind clinorotation is if a specimen continually rotates without generating a centrifugal force, it cannot detect the direction of the gravity vector (Wyatt). Scientists had invented a device called a clinostat that mimics near-zero-gravity environments. By slowly rotating an organism vertically and horizontally, the clinostat hinders the organism

from fixing on gravity. It then grows almost as if there were no gravity at all (Carlson). With a three dimensional clinostat, rotation of the wheel assembly in one direction will cancel out Earth's gravity. Spinning the wheel at various speeds will generate centrifugal forces that imitate the gravity of the moon, space, or any g level between zero and one. Correctly choosing the wheel's rotation speed and placing specimens at different distances from the center can simulate any gravitational force. If the wheel is tumbles while spinning, the Earth's gravity averages essentially to zero, so the only force the specimen consistently experiences is the acceleration from the spinning (Wyatt).

The level of similarity of behaviors between the clinostat and actual space flight has been shown to be very high for various systems. "Thus, the clinostat creates a vector-averaged gravity environment, which is a first approximation to the microgravity of space." Obviously while earthbound, the clinostat mimics one aspect of microgravity – the diminished gravitational vector (http://www.physiol.AZ.com).

Over the past decades, scientists have discovered that space flight has wide-ranging effects on living systems. Through millions of years of evolution, most terrestrial organisms have adapted to function optimally in the presence of a constant gravitational field. The Earth's gravitational force generally pulls body fluids toward the lower extremities. The body works against this force to maintain proper fluid distribution. In space, the absence of gravity results in an upward redistribution of fluids. The body interprets this as an overall increase of fluid volume, signaling organ systems, such as heart and kidneys, to adjust their function accordingly. Mechanical loading of the body is nearly eliminated in the microgravity of space flight. This sets in motion a cascade of changes that affect practically every system in the body to some extent (http://lifesci.arc.nasa.gov/lis2/Chapters1_3/Introduction.html).

Hypotheses

A. The hypothesis is that if *Glycine max* are exposed to microgravity, then the plants will have less plant growth, leaf growth, stem circumference, and chlorophyll production. The plants' ability to detect the presence of gravity will be decreased causing shortened and stunted growth. According to research, plants on a rotating clinostat may try to respond to the continually changing gravity vector by inhibiting their growth on all sides of the plant.

B. Through research, it is also hypothesized that plant growth hormones such as gibberelic acid and brassinolide exposed to plants in microgravity will show increased growth and germination rates.

Experimental Design

Independent Variable- Microgravity created by the clinostat, and addition of brassinolide and gibberellic acid

Dependent Variable- Growth of the plants

Control- Plants grown without addition of brassinolide and gibberellic acid on and off clinostat.

Constants- same type of soil, same size of peat disks, same type of seeds, and same depth each seed is planted, same type of hosiery used, same amount of water given to each plant, same type of water used, same distance each plant is placed from the center of the clinostat, same temperature, same amount of light

Quantitative Measure – Length of plants in centimeters, diameter of stems in millimeters, and chlorophyll production in mg/mL

Materials (Clinostat Construction)

97 x 18 cm board, 13 x 12 cm board, (2) 11 x 4 cm boards, (2) 19 x 8 cm board, 9 x 11 cm board, 80 x 14 cm board, 30 x 5 cm board, 8 x 13 cm board, 1 1/8 inch bearing, 2 meters of speaker wiring, 2 metal L braces, 41 screws, 34 cm diameter Motorized Ferris Wheel Erector Set, Dayco v-belt Super II (1/2 inch fan belt), ½ inch Maurey Sheave pulley, 11.25 cm diameter slip joint from Ragdons, 3M097 Dayton gear motor 17 rpm, 12 volt DC motor from Radio Shack, 1.5-12 volt 300 mA AC-DC regulator from Radio Shack

Materials

(Plant Growth)

5 liters tap water, centimeter ruler, 105 *Glycine max* seeds, 2 meters of picture frame wire, 6 packages of 10 knee-hi hosiery, 60 cc syringe, 105 peat disks, 2 grow lights, 1 liter of Gibberellic Acid (0.1% solution), 50 grams of Brassinolide

Procedure I: (Clinostat)

1. Gather materials needed for clinostat construction.
2. Build Erector Set according to directions provided.
3. Attach two boards to middle 1/3 of baseboard.
4. Attach metal L braces to the two boards.
5. Drill openings to connect to Erector set.

6. Insert wooden dowel through the drill openings.
7. Attach slip joint to wooden support lined up with the hole drilled.
8. Attach small wooden platform on top of wooden dowel.
9. Attach 2 electrical brushes on small wooden platform to line up and rub against slip joint.
10. Attach high speed DC motor to side of erector set lined up to turn the erector set wheel.
11. Attach slow DC motor to support boards.
12. Attach wooden dowel through drilled holes.
13. Attach Maurey Sheave pulley onto wooden dowel.
14. Attach rubber fan belt on pulley and axel of slow DC motor.

Procedure II *(Glycine max)*

1. Gather materials.
2. Build Clinostat—see Clinostat Procedure.
3. Insert soybean seed 3 cm deep into each peat disk.
4. Cut hosiery into 15 cm sections.
5. Place each peat disk including seed into 10 cm of cut hosiery.
6. Tie both ends of hosiery.
7. Repeat for all 105 seeds.

8. Label 20 hosiery pods Control.
9. Label 20 hosiery pods Brassinolide.
10. Label 20 hosiery pods Gibberellic Acid.
11. Label 15 hosiery pods Control (Clinostat).
12. Label 15 hosiery pods Brassinolide (Clinostat).

13. Label 15 hosiery pods Gibberellic Acid (Clinostat).
14. Attach plants marked Clinostat 20 cm from the center of the clinostat alternating between each group using picture frame wire.
15. Place remaining plants into plastic trays.
16. Place a grow light above clinostat and plastic trays.
17. Using a syringe, daily inject 10 cc of water 2 cm deep into each control hosiery pod.
18. Using a syringe, daily inject 10 cc of water containing brassinolide at 0.9 ppm 2cm deep into each brassinolide hosiery pod.
19. Using a syringe, daily inject 10 cc of water containing 0.1% gibberellic acid 2 cm deep into each gibberellic hosiery pod.
20. Record germination date for each plant.
21. Record growth of each plant every 2 days after germination.

Data Table1: Table of *Glycine max* Growth in Centimeters

Retests	Control		Brassinolide		Gibberellic Acid	
	Control	Clinostat	Control	Clinostat	Control	Clinostat
1						
2	31.3		32.1		1.0	1.3
3	21.2		29.4	4.1	10.2	1.7
4	3.4		2		21.1	
5			35.2	2.6	4.9	2.6
6	12.8	1.6	36.3	.8		
7			36.4		7.5	2.4
8	11.6	1.7	5.6	.9	21.1	2.1
9	13.0		2.8	3.6	37.1	
10		0.5	37.1	1.3		
11	29.4	0.3	36.9	1.6	6.1	1.8
12			28.2		29.6	
13	28.1	1.9	17.4	3.4		
14	3.5		4.3	1.6	3.5	2.2
15	0.7		37.1		9.2	
16	6.4		24.1	4.1	1.6	2.4
17	28.6		30.9		1.8	
18			34.1		.8	
19			34.3		5.4	
20	2.9		32.6		41.9	
Mean	14.8	1.2	32.7	2.4	4.7	2.1
Median	12.8	1.6	26.5	2.1	12.2	2.2
Mode			32.4	2.1	6.1	2.4
Range	30.6	1.6	37.1	3.3	21.1	1.3
Std. Dev.	11.5	0.74	35.1	1.31	41.1	0.36

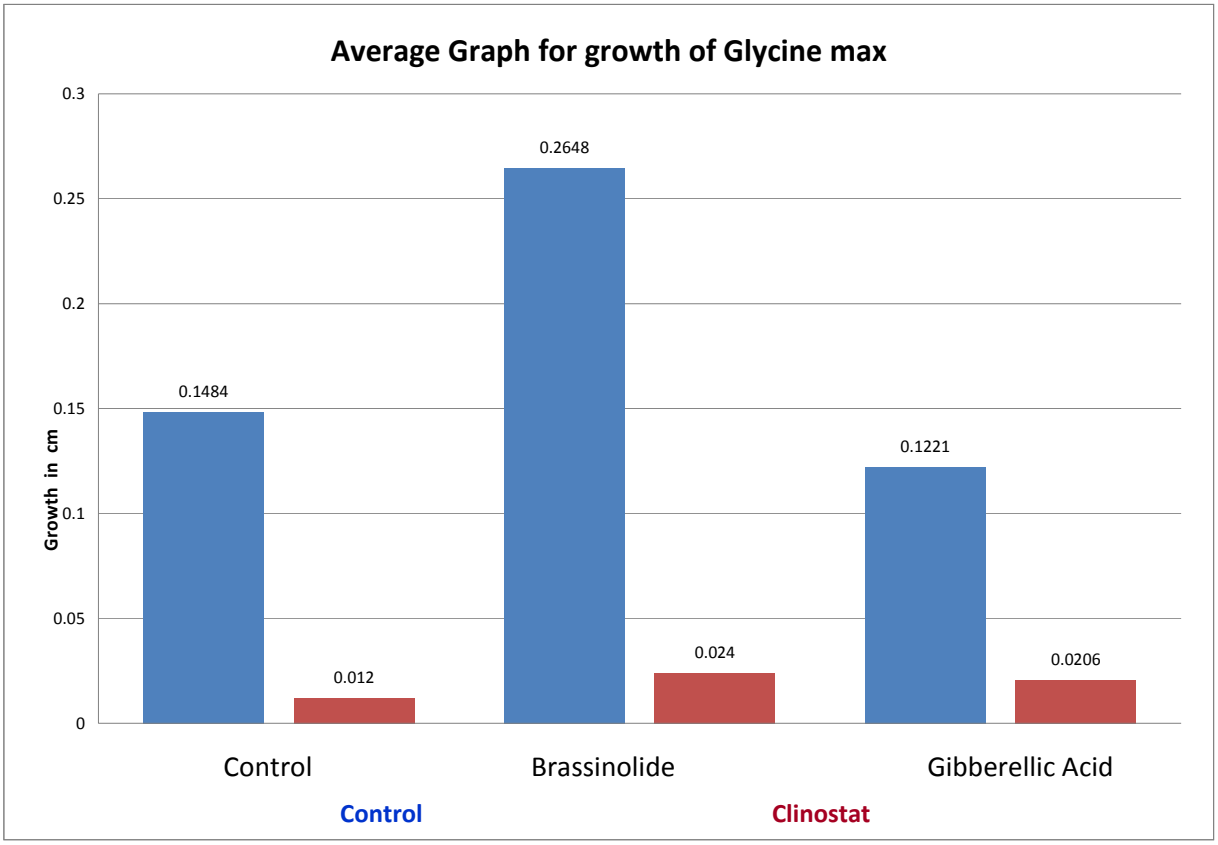
Data Table 2: Table of *Glycine max* Average Leaf Length (mm) and Stem Diameter (mm) Top and Bottom of Plant

	Control			Gibberellic Acid			Brassinolide		
Retests	Leaf Length	Stem Top	Stem Bottom	Leaf Length	Stem Top	Stem Bottom	Leaf Length	Stem Top	Stem Bottom
1	24.15	1.79	1.10	58.28	2.06	0.47	33.06	2.51	1.13
2	28.53	1.90	0.91	18.46	2.08	0.67	37.83	2.58	1.26
3	24.07	2.58	1.10	15.18	1.80	0.64	37.76	2.32	1.28
4	17.87	2.18	0.34	15.74	1.85	0.62	27.54	2.74	1.15
Mean	23.66	2.11	0.86	26.92	1.95	0.60	34.05	2.54	1.21
Median	24.11	2.04	1.00	17.10	1.96	0.63	35.41	2.55	1.21
Mode			1.10						
Std. Dev.	4.4	0.35	0.36	21.0	0.14	0.09	4.9	0.17	0.08

Data Table 3: Table of Chlorophyll Concentration (mg/mL) Measured by UV-VIS Spectroscopy at a 652 nm Wavelength

Retests	Control Plants		Gibberellic Acid		Bassinolide	
	Conc. mg/mL	Absorbance	Conc. mg/mL	Absorbance	Conc. mg/mL	Absorbance
1	0.0013	0.046	0.0013	0.044	0.0014	0.048
2	0.0013	0.046	0.0013	0.045	0.0014	0.047
3	0.0013	0.046	0.0013	0.046	0.0014	0.049
Mean	0.0013	0.046	0.0013	0.045	0.0014	0.048
Median	0.0013	0.046	0.0013	0.045	0.0014	0.048
Mode	0.0013	0.046	0.0013		0.0014	
Std. Dev.	0.000	0.000		0.001		0.001

Calculation: C = A/ϵl *where c = concentration, A = absorbance, ϵ = absorption coefficient for chlorophyll (34.5 mL/mgxcm) and l = path length of cuvette*



Tukey HSD			
	Normal Gravity	Clinostat	
Control to Brassinolide	P<0.0118	P<0.0835	
Control to Gibberellic Acid	P<0.5698	P<0.0216	
Brassinolide to Gibberellic Acid	P<0.0019	P<0.4984	
ANOVA(Unpaired t-tests)			
	Control	Brassinolide	Gibberellic Acid
Normal Gravity to Clinostat	P<0.0191	P<0.0001	P<0.0409

Results

In comparison to plants on and off the clinostat, the plants grown off the clinostat showed greater growth and germination rates. The addition of brassinolide greatly increased growth and germination rates in the plants grown off the clinostat, and somewhat in plants grown on the clinostat. Control on and off the clinostat showed the least amount of growth and germination rates. Tukey HSD tests were done and showed that only the comparison between Control and Gibberellic Acid was not significant at normal gravity. On the clinostat the only comparison that was significant was control to Gibberellic Acid.

Observations

Possible errors in this project could be the placement of the clinostat and plants in the basement. Also since plants are not accustomed to 24 hours of constant light then possibly the grow lights should not have been on the entire test period.

It was observed that plants on the clinostat grew at significantly lower rates, and their stems were much thinner and weak. Plants exposed to brassinolide on and off the clinostat showed increased growth and germination rates, more so that gibberellic acid.

Conclusion

"The Clinostat has been used by biologists for over a hundred years to study how organisms might adapt to the microgravity environment and what effects the force of gravity has on plant and animal development and behavior. As humans venture out to eventually colonize space, information concerning adaptation to this novel physical environment will assume increasing educational as well as scientific value."(http://www.physiology.arizona.edu/people/gruener/clinostat/index.html)

The hypothesis states that the plants affected by the clinostat will not grow in normal fashion. The plant's ability to detect the presence and direction of gravity will be decreased by the action of the clinostat. As a result, the plants will experience stunted growth. Previous experimentation and research shows that the addition of brassinolide will increase the growth and germination rates for plants on and off the clinostat. Both hypotheses were accepted.

After being introduced to the clinostat, the plants affected by microgravity were stunted in growth. Brassinolide increased growth rates on the clinostat minutely by not near as great as off the clinostat.

Future Study

This project could be continued in the following ways:

-use different species of plants.

-Vary the distance of the plants from the pivot of the wheel to determine plant growth at different microgravities.

-Determine maximum and minimum gravitational fields for plant growth

-Conduct experiment in climate controlled chamber

-Vary the speed at which the clinostat rotates

-Adjust the amount of plant hormones given to plants

-Use different plant hormones to determine their effects on plants in microgravity.

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