Zinnia Germination and Lunar Soil Amendment

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Germination testing was performed to determine the best method for germinating zinnias. This method will be used to attempt to germinate the zinnia seeds produced in space. It was found that seed shape may be critically important in determining whether a seed will germinate or not. The ability of compost and worm castings to remediate lunar regolith simulant for plant growth was tested. It was found that neither treatment effectively improves plant growth in lunar regolith simulant. A potential method of improving lunar regolith simulant by mixing it with arcillite was discovered.

Nomenclature

K	=	kinetin
GA	=	Gibberellic acid
nH2O	=	no soaking treatment
sH2O	=	water soak
100K	=	kinetin, 100mg/L concentration
500GA	=	gibberellic acid, 500mg/L concentration
Arc	=	arcillite
Fert	=	fertilizer
Comp	=	mushroom compost
Worm	=	worm castings
DAP	=	Days after planting

I. Introduction

Growing plants in extraterrestrial environments presents many challenges. Microgravity environments cause water stress, as fluids behave differently in microgravity. Air circulation is also a challenge in such environments. On extraterrestrial worlds, such as the Moon and Mars, these stresses are reduced, but other challenges for plant growth are present. Since it will be impossible to ship large quantities of water or media over such long distances, it would be preferable for the plants to grow in lunar or Martian regolith. However, these regoliths are often far from ideal substrates in which to grow plants, as they tend to compact together when wet. This makes it difficult for the plant roots to grow through the media, and stunts plant growth.

Among the plants grown so far in the VEGGIE unit, the zinnias suffered the most from the effects of water stress. This was primarily due to a lack of air circulation in VEGGIE during growth, as a result of the fan unit being turned off. Since there was no airflow, water did not evaporate off the plants, nor were the zinnias able to transpire. Since plants rely on transpiration to move water through their vascular systems, this caused severe stress on the plants, and helped promote the growth of the fungus that killed many of the zinnias. Under Scott Kelly's care, the surviving zinnias grew and flowered. The resulting seeds were brought back to earth. Unfortunately, attempts to germinate the seeds produced in flight have been unsuccessful so far. The goal of this project was to germinate at least one zinnia seed produced during the flight of VEG-01C.[1]

If humans intend to establish a lunar colony, it will be difficult, and expensive, to ship fresh produce, or plant growth substrates to the moon. Thus it would be preferable for future lunar colonists to be able to grow crops in lunar regolith. However, prior research has shown that lunar regolith simulant, which is considered to be a close approximation of the soil texture of actual lunar regolith, is an extremely poor substrate for growing plants. Many plants germinate poorly, or fail to germinate at all in lunar regolith. They also experience stunted development, and some die before reaching maturity.[2] This is most likely due, in part, to the structure of the lunar regolith simulant. When uniformly wetted *in situ*, the lunar regolith simulant grains compact together, forming a solid structure that is

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nearly impenetrable to plant roots. Amending this structure, so that it would be more acceptable to plants, was the secondary project goal.

II. Zinnia Germination Testing: Phase One

In order to successfully germinate zinnia seeds produced during the flight of VEG-01C, a rigorous seed decontamination and treatment protocol needed to be established. Seed treatments are one of the best methods of increasing the germination rate of seeds, with plant hormones being one of the more effective treatment types. Cytokinins and gibberellic acid are the two plant hormone classes that are most effective at promoting germination.[3] Hydrogen peroxide has been also been investigated for both its decontamination and germination promoting properties on zinnias.[4]

A. Materials and Methods

In this experiment kinetin (K) – a cytokinins, gibberellic acid (GA), and hydrogen peroxide were tested to determine their effectiveness in promoting germination in 'Profusion' zinnias. 'Profusion' zinnia seeds produced during other tests were used during the initial testing. As there was a limited number of zinnia seeds produced during the flight and ground tests, there were not enough to waste on large amounts of germination testing. Seeds that were produced in analogous controlled environment testing were utilized.

The initial tests used six different treatments: K(50mg/L), GA (100mg/L), GA (100mg/L) +K (50mg/L), 3% hydrogen peroxide (H_2O_2), a water soak (sH_2O), and no treatment (nH_2O). The last two treatments served as controls. Seeds were soaked in each of the treatments for 20 min., except for in the hydrogen peroxide treatment.

In the initial test of the hydrogen peroxide, a twenty minute soak in the 3% solution resulted in a 16% germination rate, whereas the water soak resulted in a 52% germination rate. This initial hydrogen peroxide testing was performed without the no treatment control. It was thought that the length of the treatment caused the lowered germination rate in the hydrogen peroxide treatment. The soak time was reduced to ten minutes in hydrogen peroxide, followed by ten minutes in a water rinse for the next round of testing. This increased the germination rate to 40%, and this procedure was followed for all of the subsequent testing.

The plant hormone solutions were prepared by dissolving the appropriate amount of K and GA in tap water. Kinetin did not dissolve well, as it was extremely hydrophobic. Therefore, it was suspended in the water, then stirred at 70°C for an hour and a half, until all of the kinetin had dissolved into the solution. During the later rounds of testing, higher concentration solutions were also prepared. These were GA, at 500mg/L (500GA), and K, at 100mg/L (100K). The kinetin solution was prepared using 0.5% Tween-20. Even in the Tween-20 solution, the kinetin did not dissolve well, so this solution was stirred at 70°C for roughly two hours, until all of the kinetin had gone into the solution. All of the solutions, except the 100mg/L K, were stored at 4°C. The 100mg/L kinetin solution was stored at room temperature, as it would have precipitated out of solution at 4°C.

Two sets of seed were used during the germination testing, and three tests of all the treatments were conducted on each of the seed sets. Twenty five seeds were tested in each treatment.

Table 1: Seed Set Production Conditions

Seed Set	Day Temperature	Night Temperature	Day Humidity	Night Humidity	CO_2
1	22°C	22°C	40%	40%	2000
2	26°C	24°C	70%	75%	1200

The first set of seeds had been generated during a test of the PoNDS system, were relatively immature, and had not been dried or exposed to cold storage. The flowers formed under the conditions given in Table 1.

The second set of seeds were much older, and been produced during testing in 2013. This set of seed had been in dry storage for several years, although they had not been stored particularly well. The second set of seed was also produced under slight different conditions than the first, most notably lower CO_2 and higher humidity.

All of the seeds were placed on top of two layers of germination paper, which had been cut to fit snugly into the large, circular petri dish. One layer of germination paper was placed in the lid of the dish as well. The germination paper was kept wet during the germination process.

B. Results

The results from the first set of seeds showed that some sort of aqueous soak definitively increased the consistency and percent of germination in the zinnia seeds. Although a lack of any treatment occasionally resulted in a high germination rate, it also resulted in some of the lowest germination rates seen in any of the treatments. As seen in Figure 1, in different rounds, different treatments had higher values. However, it can be seen that in two of the three rounds, kinetin gave the best result of any of the treatments. The hydrogen peroxide and GA+K treatments also gave high germination rates. The rates of the different treatments relative to one another were also similar across the different rounds of treatments.

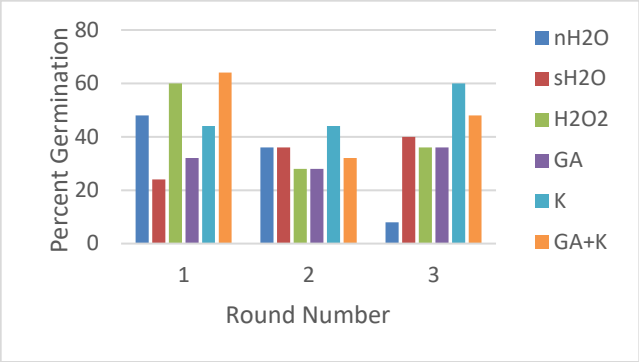

Figure 1: Seed Germination Rates for Seed Set 1. *Seed germination rates for each treatment during three rounds of germination testing.*

For the second seed set the results were similar, although this seed set showed far more variability in what treatments generated the highest germination rate. The water soak treatment, no soaking treatment at all, GA, and GA+K all generated high germination rates during different rounds of treatments. The germination rates of different treatments relative to each other were also more variable than for the first seed set. The overall germination rates were lower as well. Whereas the first seed set showed germination rates that often reached above 20%, and several germination rates in excess of 40%, the second seed set had notably lower germination rates. As shown in Figure 2, the germination rates for the second seed set were substantially lower – typically right around 20%, and with only one germination rate reaching above 40%.

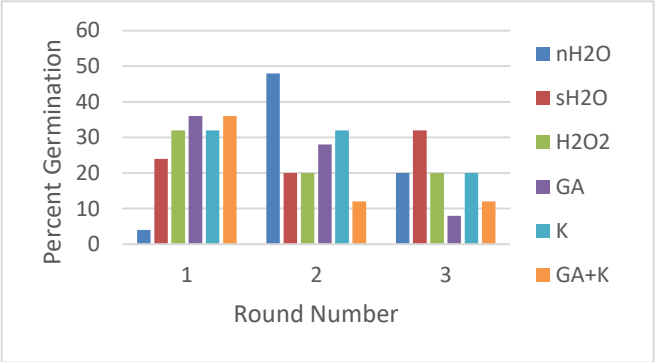

Figure 2: Seed Germination Rates for Seed Set 2. *Seed germination rates for each treatment during three rounds of germination testing.*

During the last two rounds of testing on the second seed set, treatments with higher levels of kinetin and GA were also tested. Since the kinetin (100mg/L) was suspended in 0.5% Tween-20, it was also necessary to test the 0.5% Tween-20 solution, to ensure it did not have a strong effect on germination. The results of this testing are presented in Figure 4. They showed that the GA (500 mg/L) treatment gave the strongest increase in germination rate. Although it appears from the two tests analyzed that 0.5% Tween-20 increases the germination rate, when the third data point was included in the analysis of the water control, this advantage is reduced. Unfortunately, the variability of the data collected was quite high, with 100K outperforming 500GA in the second round of treatment. Since three data points were not collected, a definitive answer as to which treatment served to promote germination the best is not possible.

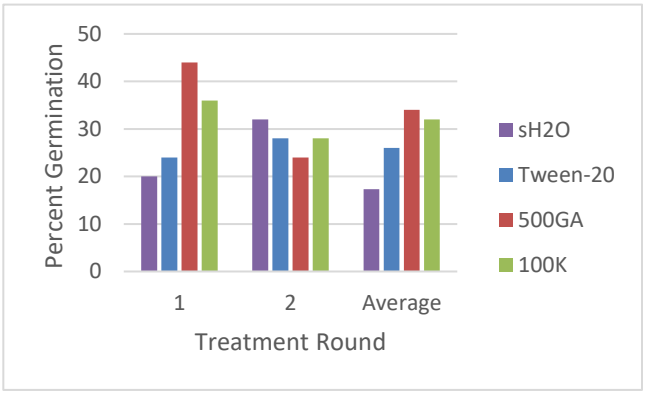

Figure 3: Seed Germination Rates. *Seed germination rates after the application of increased concentrations of plant hormones.*

The average germination rates for the two treatments also showed the lowered germination rates seen for the second seed set, as well as the general trend for each treatment. As seen in Figure 4, for both treatments kinetin had the highest germination rate, although it was less noticeable for seed set 2. The other germination rates were roughly similar across the two seed sets, except for the hydrogen peroxide and GA+K treatments. The hydrogen peroxide treatment performed well in both sets of seed, but in set two, it performed worse than the water soak control, and better than the GA+K treatment. For seed set one, this trend was reversed.

The standard deviations are presented in Table 2, below. The standard deviation was the highest for the seeds that had not been treated, and was lower for the seeds that had been treated with various aqueous solutions.

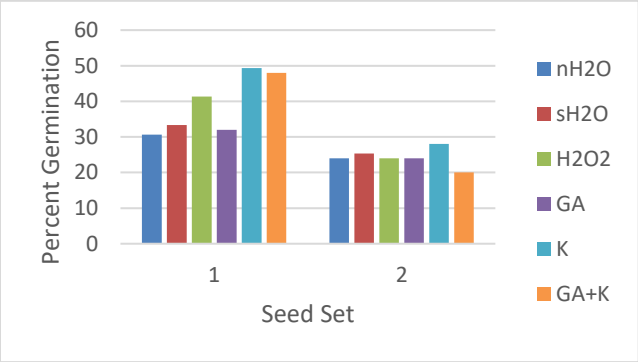

Figure 4: Average Seed Germination Rates.
Average seed germination rates for each of the original six treatments in two sets of seed.

Table 2: Standard Deviations for Set One and Set Two of Seeds.

Set One	nH2O	sH2O	H2O2	GA	K	GA+K
Av.	30.66667	33.33333	41.33333	32	49.33333	48
S.D.	20.52641	8.326664	16.65333	4	9.237604	16
Av. + S.D.	51.19307	41.66	57.98666	36	58.57094	64
Av. - S.D.	10.14026	25.00667	24.68001	28	40.09573	32
Set Two	nH2O	sH2O	H2O2	GA	K	GA+K
Av.	24	25.33333	24	24	28	20
S.D.	22.27106	6.110101	6.928203	14.42221	6.928203	13.85641
Av. + S.D.	46.27106	31.44343	30.9282	38.42221	34.9282	33.85641
Av. - S.D.	1.728943	19.22323	17.0718	9.577795	21.0718	6.143594

C. Analysis

The kinetin treatment was thought to be the best treatment out of those tested, based on its superior performance in increasing germination rate, and on its lower standard deviation. This indicated that kinetin increased the germination rate, and that it did so consistently. The lower standard deviation of gibberellic acid also indicated that it, although less effective than the kinetin, also increased germination rates consistently.

Unfortunately, the kinetin has continued to present problems due to its low solubility. Precipitation has been observed in many of the tubes of kinetin, indicating that the true concentration may not be exactly 50mg/L or 100mg/L. As expected, this deposition has been more severe in the tubes containing the 100 mg/L kinetin solution.

Hydrogen peroxide also proved decently effective during the germination trials. Since it is also an excellent sterilizing solution it was retained for the next phase of the experiment.

III. Zinnia Germination Testing: Phase Two

Before attempting to germinate the seeds produced during flight, the ground control seeds were tested. They were the closest analogue to the flight seeds, but were less valuable, and were more plentiful. Ground control seeds are produced in an identical VEGGIE unit to the one on the ISS, and are produced under identical climactic conditions to the plants on the ISS. The only major difference is the lack of gravity – and in this case, the lack of airflow and subsequent fungal growth that the plants on the ISS experienced when the ventilation fan lost power.

A. Materials and Methods

For these tests, the seed treatments were refined, based on the knowledge gained from the previous testing. During the initial trial, the seeds were soaked in 3% hydrogen peroxide for ten minutes, and then soaked in either GA, K, 500GA, or 100K for ten minutes. Three replicates were used, with five seeds per replicate, for a total of sixty seeds.

During the second trial, these treatments were changed to increase the probability of preventing contamination, and to further prevent any negative effect of the hydrogen peroxide on the seeds. The seeds were soaked in 70% ethanol for five minutes, then soaked in hydrogen peroxide for five minutes. They were then soaked in either GA, 500GA, 100K, or a specially prepared extract of tomato for ten minutes. Only one replicate was performed, with six seeds per treatment, for a total of twenty four seeds.

The tomato extract was taken from a supermarket tomato, in which the seeds had begun prematurely germinating. Only the gel surrounding the seeds, and the seeds themselves were extracted, and stored.

During the second trial, the seeds were selected based on their type. Three types were placed in each treatment. One, was termed 'petal', and was thought to come from the ray florets. The second was termed 'tiny', and was thought to come from the normal florets in the center of the floral head. The last was termed 'flat and plump' in contrast to another type of seed not utilized in the experiment, which was termed 'flat and thin'. Four 'petal' seeds, one 'tiny' seed, and one 'flat and plump' seed were placed in each treatment. Figures 5 shows these seed types.

Figure 5: Five Seed Types. *From left to right, 'papery', 'flat and thin', 'flat and plump', 'tiny', and 'petal' seed types.*

All of these treatments were placed in small square plastic petri dishes, on top of two layers of germination paper, which had been cut to fit snugly into the dish. One layer of germination paper was also placed in the lid of the dish.

B. Results

During the first trial, heavy contamination was observed on most of the seeds, after a time interval of roughly four to five days. Out of the original sixty seeds, only one even began to germinate. This seed was in the 100K treatment in the third replicate, and was transplanted, as seen in Figure 6. For the one treatment that experienced partial germinate, the rate was 20%, for every other treatment there was a 0% germination rate.

Figure 6: Transplanted Seedling. *Seedling transplanted into arcillite from 100K treatment.*

The second trial was more successful. One seed germinated in each treatment, except for the 100K treatment, which had zero germination.

The one seed that germinated in each of the three treatments that experienced germination was of the type described as 'flat and plump'. These three treatments (GA, 500GA, and Tomato) had a germination rate of 16.7%. Pictures of the three treatments that germinated can be seen in Figures 7-9 on the following page.

Figure 7: Seed Germination. *One seed germinated in the GA treatment.*

Figure 8: Seed Germination. *One seed germinated in the 500GA treatment.*

Figure 9: Seed Germination. *One seed germinated in the Tomato treatment.*

C. Analysis and Further Testing

One of the most important results of this testing was that it showed that seed shape may be even more important than seed treatment. Whereas 75% of the 'flat and plump' seeds germinated across all three treatments, none of the 'petal' or 'tiny' seeds germinated. In previous trials, this tendency had been noted anecdotally, but had not been rigorously observed, as the amount of flight seeds is limited – one cannot select the 'best type' of seed, because it may not exist.

In fact, very few 'flat and plump' seeds have been observed in the flight seed set. This means that germination of the flight seeds may be impossible, as many of the other seed types may not even contain viable embryos. Without a living embryo, the seed treatments have nothing to stimulate into germination, as a baby plant does not exist within the seed.

However, germination of the flight seeds using several seed treatments will still be attempted, as occasionally some of the other seed types do seem to germinate.

IV. Lunar Regolith Amendment

The compact structure of wet lunar regolith simulant is one of the biggest challenges to plant growth in that substrate. Prior research carried out by the author had indicated that compost might improve the suitability of Martian regolith simulant for plant growth. Compost is often added to terrestrial soils to improve their structure, and it was hypothesized that it might also improve the soil structure of the lunar regolith simulant as well.

A. Materials and Methods

Four different treatments were used for the experiment, as outlined in Table 3. There were three 1" pots for each treatment, in each of which four 'Tokyo Bekana' Chinese cabbage and four carrot seeds were planted. The carrots were harvested and photographed, but were not measured as they had not yet produced carrots and did not have large leaves. The amendments were added to the lunar simulant, and to arcillite, as a control, for a total of 24 pots. Once the seeds had germinated, the excess seeds were thinned so that there was only one plant of each species in each pot. The pots were set in a tray, and watered with tap water from below. They were germinated, and grown until ten days after planting under white LEDs. The plants were then moved under red/blue LEDs, and rotated frequently to maintain a relatively even light distribution among the plants.

Table 3: Soil Amendments.

TREATMENT	LABEL	AMOUNT INCOPORATED
Fertilizer (Nutricote 18-6-8)	+fert	2g
Mushroom Compost	+comp	6g
Worm Castings	+worm	6g
Nothing	null	0g

The height (from base to meristem), SPAD and number of leaves were recorded 12, 14, and 19 days after planting. On the twenty-first day after planting (DAP), these measurements were recorded, as were two measurements of plant diameter. The diameter was measured from leaf tip to leaf tip. The plants were harvested on DAP 22, with a few plants harvested on DAP 23.

The harvest procedure was as follows: soil was removed from the pot as a solid root ball unit, and the entire plant was photographed, as seen in Figure 10. Then the soil was washed from the roots. The leafy portion of the plant was then cut off at the base, and the roots and shoots were then patted dry and weighed immediately. Shoots and roots were then bagged separately in paper bags, and dried at 70°C for 72 hr. They were then reweighed to obtain the dry weights.

The rough area of the plant was calculated as follows:

$$(((Diameter\ 1 + Diameter\ 2)/2)^2)\ \pi$$

Figure 10: Root Ball. *Root ball of plant grown in lunar regolith simulant, removed from its pot.*

B. Results

For each treatment, the values for each of the three pots in a treatment were averaged for each measurement type. The average values are reported below.

Unfortunately, unlike in the Martian regolith simulant, there was no evidence that the addition of compost or worm castings improved the growth of plants in lunar regolith simulant. In fact, in many cases, it seemed to decrease the growth of the plants in comparison to the null treatment.

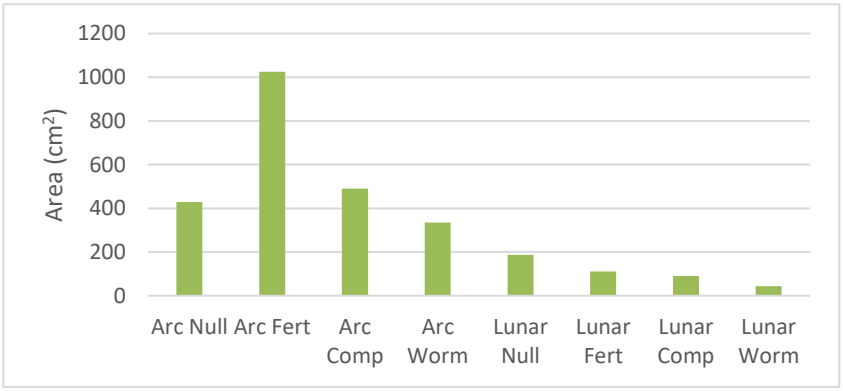

Figure 12: Area. *Rough average area measures of 'Tokyo Bekana' cabbage for different treatment regimens.*

The arcillite control also performed poorly when amended with compost or worm castings. This trend can quickly be seen from the rough area of the plant calculated from the two diameter measurements, and presented in Figure 12. The arcillite treatment treated with fertilizer performed the best, as would be expected, and the arcillite treated with compost performed marginally better than arcillite that had not been treated with anything. However, the worm castings decreased the plant area as compared to the null treatment in both arcillite and lunar simulant. In the lunar simulant, both fertilizer and compost had reduced area as compared to the null treatment.

These trends were less pronounced according to other measures, most notably SPAD. In general, the lunar simulant treatments scored extremely well in these measures, with only the arcillite fertilizer treatment comparing favorably to the lunar simulant treatments. This trend, however, was most likely due to the stunted size of the plants growing in the lunar simulant. Although they had a higher density of chlorophyll, they had far less leaf area than the arcillite treatments. This is demonstrated most easily by the lunar worm treatment. As can be seen in Figure 13, the lunar worm treatment had the highest SPAD value. However, a quick glance at the final area measure of the plant reveals that it also had the smallest area.

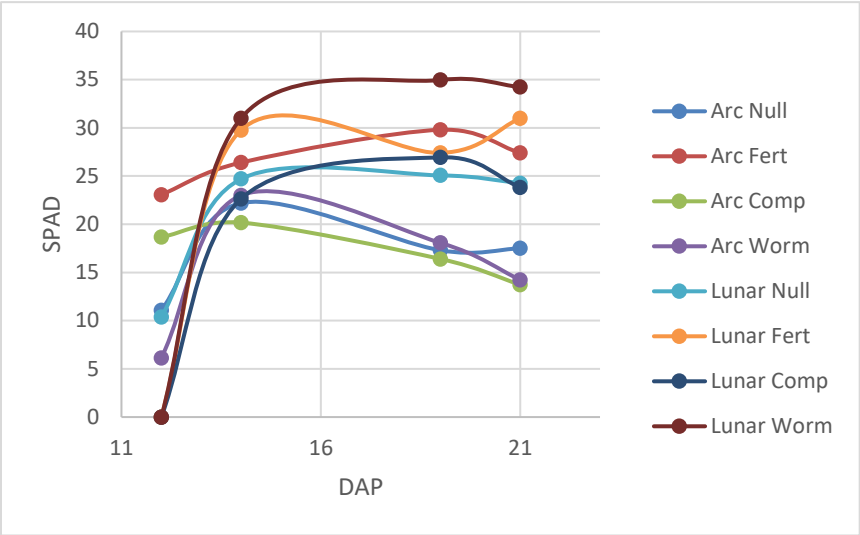

Figure 13: SPAD. *A measure of the chlorophyll level in the leaves of the plants under various treatments over the course of their growth.*

The height of the plants, as seen in Figure 14, was more variable. Both of the compost treatments produced extremely tall plants. The fertilizer produced shorter plants – in the case of the arcillite substrate, the fertilizer treatment actually produced a shorter plant than the null treatment. Both of the worm castings treatments produced short plants.

The wet and dry weights of each treatment were similar to each other, although there were some differences. The compost and worm casting treatments had lower values than the null treatment in the arcillite substrate for fresh weight. In dry height, their values were higher. This anomaly was only present for the shoot weight values, and not for the root weights. Unfortunately, the analytical scale was not available, thus some of the weights, particularly of the roots were below the detection limit of the scale. These were recorded as a weight of zero.

The weights recorded are shown in Figures 15 and 16. They closely resembled the distribution seen in the area values, with the fertilizer treatment in arcillite substrate producing the largest plants and roots of any of the treatments. All of the arcillite treatments, even the one to which worm castings had been added, outperformed all of the lunar treatments. This was true for both wet and dry weights, for both shoots and roots.

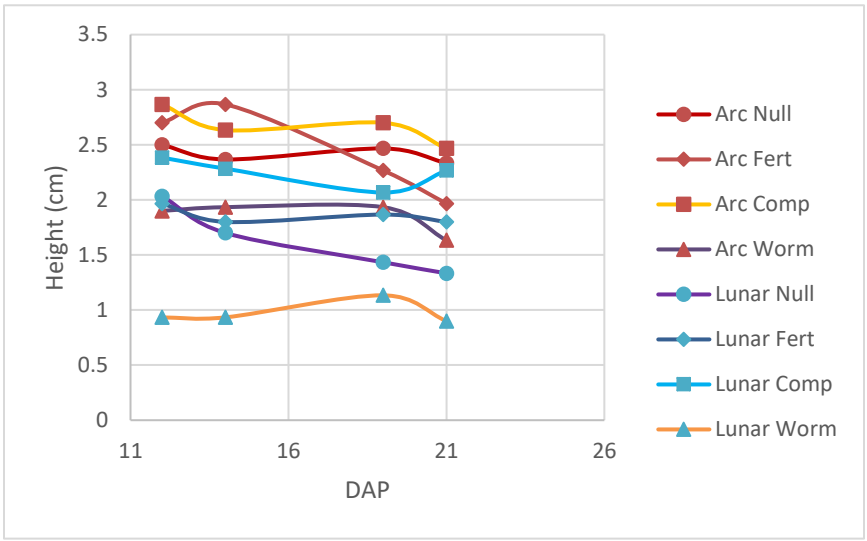

Figure 14: Height. *Height of the plants under various treatments over the course of their growth.*

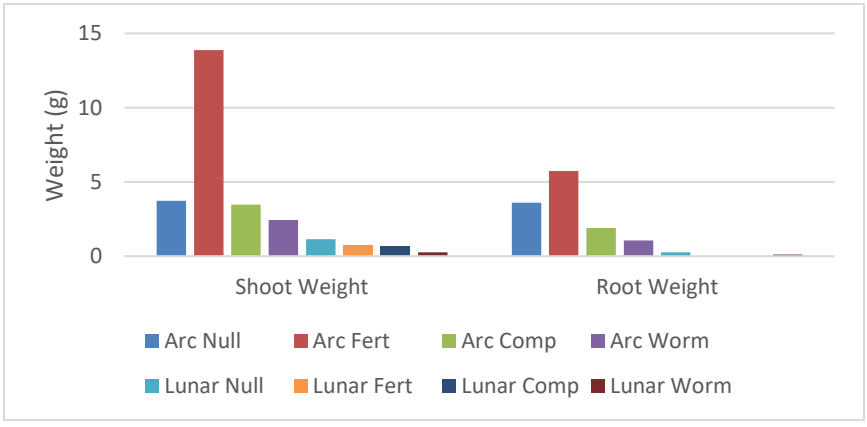

Figure 15: Wet Weight. *Wet weights at harvest.*

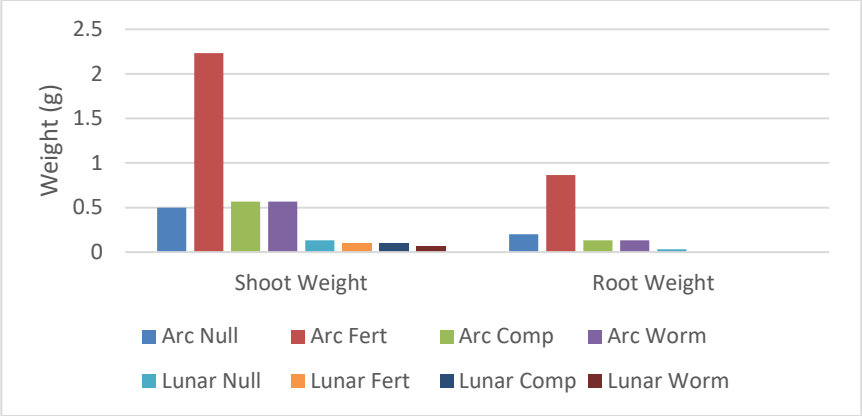

Figure 16: Dry Weights. *Dry weights after harvest.*

C. Analysis and Further Testing

The hypothesis that amending lunar regolith simulant with compost or worm castings was rejected. Although compost did increase plant height, the weight of the plant, and its leaf area, are far more important measures of how much edible biomass a plant can produce. It was also determined that fertilizer was the most effective method of increasing the area and weight of plants grown in arcillite, as opposed to compost or worm castings. As expected, the fertilizer also marginally increased the SPAD value of plants grown in arcillite.

As there was a great deal of arcillite and lunar regolith simulant left over after this experiment, it was decided to reuse it, as a 50-50 mixture. If a colony is established on the moon, it is not unreasonable to think that astronauts might first use a system similar to VEGGIE, in which case, waste arcillite would be produced from the pillows. This arcillite could then be combined with the lunar regolith to form a 50-50 mixture.

In the follow-up experiment, three treatments were used: arcillite, 50-50 arcillite-lunar regolith simulant, and 50-50 arcillite-lunar regolith simulant bound with starch from packing peanuts. Five grams of T70 18-6-8 fertilizer were added to each 4" pot. Three pots of 'Tokyo Bekana' cabbage were grown for each treatment, with four plants per pot.

So far, this experiment has been far more successful. The cabbages grown in the 50-50 mixtures look far healthier than those grown in the lunar simulant treatments in previous experiments. Although they look slightly smaller than the arcillite cabbages, the lunar cabbages still appear healthy. Data collection for this experiment has not yet concluded, but will involve similar measurements to the first experiment, with the addition of weight and diameter data collection at DAP 14 and 21.

V. Conclusion

It was found that kinetin and high concentrations of gibberellin help improve germination in zinnias. Hydrogen peroxide may also help improve zinnia germination. However, it appears that seed shape and size is perhaps the most important determiner of whether a seed will germinate or not. Unfortunately, this is not particularly helpful in germinating seed produced in space, as there are limited amounts of such seed.

Amending lunar regolith simulant with compost or worm castings is rather ineffective, and provides less nutrition to the plant than fertilizer does. However, mixing arcillite in with the lunar simulant may actually prove effective at partially, or fully remediating lunar simulant for plant growth.

Acknowledgments

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