American Journal of Life Sciences
2021; 9(1): 11-18
http://www.sciencepublishinggroup.com/j/ajls
doi: 10.11648/j.ajls.20210901.13
ISSN: 2328-5702 (Print); ISSN: 2328-5737 (Online)

Effects of Clinorotation on the Enzyme Activities and Morphology of *Zea mays* Seedlings

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To cite this article:
Alexander Oseghale Orukpe, Geofrey Obinna Anoliefo, Beckley Ikhajiagbe. Effects of Clinorotation on the Enzyme Activities and Morphology of *Zea mays* Seedlings. *American Journal of Life Sciences*. Vol. 9, No. 1, 2021, pp. 11-18. doi: 10.11648/j.ajls.20210901.13

Received: January 5, 2021; **Accepted**: January 13, 2021; **Published**: March 22, 2021

Abstract: For sustainable space exploration there is need for torrential food supply. Apart from food in storage, constant production is vital as this could also serve as a source of Oxygen when activated during space expedition. However, the impact of gravity in crop development is significant. This study therefore, investigated the morphological and physiological responses of Maize (Zea *mays)* seedlings subjected to clinorotation. Agar Agar was used as source of nutrient for the developing seedlings. The Agar was prepared by measuring 1 – 1.5 g and made up to 100 ml with tap water. Seeds were collected in the seed bank of the Space-Earth Environment Research Laboratory, Benin City. Three sets of petri dishes were prepared and marked; one for normal surface, one rotated at 90°C and another set for clinorotation. Those for clinorotation were rotated at three different times; with 1, 2, and 3 rpm for 6 hrs respectively. The plumule, radicle and Enzyme activities were measured and analysed after four days. Results showed significant difference in germination parameters as occasioned by microgravity. Where as clinorotation enhanced radicule length (1.8-2.1cm), effect on plumule was minimal ($p>0.05$). Significant increase in CAT activity in the plumule was reported (7.59mol/sec) in the clinorotated (1rpm) seed compared to the control (2.56mol/sec). MDA activity in both radicle and plumule were higher than that of the control ($p<0.05$). Microscopic study of the cells was carried out using a binocular microscope (Labo) with a camera and result showed that the normal surface sample cells were arranged concentrically with spaces, while that of clinorotated seeds were arranged concentrically but highly packed with little spaces for the plumule. For the radicle, the normal surface sample cells were scattered within the plant with more spaces, while that of clinorotated were mostly clustered throughout.

Keywords: EA: Enzyme Activities, MDA: Malondealdehyde, Clinorotation

1. Introduction

Human population growth rate has not been successfully controlled with all forms of family planning. There could be a time when the earth is carrying capacity could be over shot both in terms of space and life sustainability. Space scientists therefore thought it wise to explore other planets for habitation through colonization, mineral exploitation and possible technology testing [70, 72-74].

Space exploration however is limited by a number of limitations especially unsustainable food supply. The production of food to meet the nutritional needs of astronaut is important, this will also enhance O_2 supply via plant cultivation [43-44, 46, 57].

The astronauts need to be able to cycle their source of energy for water, oxygen and ready to eat food for example carrot, cucumber, watermelon, maize, cabbage. The most limiting factor in growing these crops in Space is microgravity. The gravitational force in space is almost zero. For plant to grow successfully, they must first germinate and then develop to form seedlings. We are looking at the mechanism of survival of plant in space either on board or the grand [26-27, 48, 51-55].

Reported that when a plant is subjected to clinostat effect, the cells are dispersed compare to the one the earth surface which is concentric in arrangement. For this we believe the

plant to development this great differences, the plants could either be transformed and use for bioremediation because there are more vacuoles on the planted subjected to clinostat effect, moreso, earlier study shows that the maize that have been subjected to clinostat effect and transferred to the field produced bigger cubs and more seeds. We also believe it help for crop improvement that further help to address Zero hunger as the SDGs two which is now a worldwide pursuit [39-40, 67, 69].

Colonizing Space will depend on the ability to routinely provide for the metabolic needs (oxygen, water, and food) of crew with minimal re-supply from Earth. Throughout history, manned exploration missions have often succeeded or failed according to the degree to which nutrition was considered [6, 28, 31, 62-63].

Gravity is the force of attraction between bodies. It is proportional to the mass of the objects, and inversely proportional to the square root of the distance separating those masses [7-8, 19, 56]. Just as any other planet, Earth has its own gravitational field, which applies a force described as an acceleration of 9.81 meters per second squared (expressed as 1 g as well) to every object on the surface of the planet. Microgravity is the equivalent of 1 μg (1x10-6 g), but the word is used to describe any other acceleration corresponding to gravity below 1 g on Earth's surface [25, 68].

Many plant space biology experiments have shown abnormalities such as chromosomal breakage failure to produce seed, altered or nonviable embryos, alterations in the cell wall composition and properties, increased breakdown of xyloglucans changes in polar auxin transport, or other morphological abnormalities. Most plant space experiments last less than 18 days. Prior to the present study, plants had only been successfully grown from seed to seed during the course of two experiments, each of which showed developmental alterations [9, 23, 32, 41-42, 60, 66].

Microgravity environment affects plants especially through its physiological activities, the movement of the water and nutrients round the cells lead to high enzyme activities. More so, there is high rate of cell division and also the plants tend to always lengthen [51, 64-65, 75].

Gravity can produce effects related to displacement (motion) and/or deformation. The consequences of having weight are related to internal stress induced over an object manifested as tensile or compressive normal loads [30, 71]. Research about Microgravity effects has improved our knowledge about the importance of gravitational forces on the materials development field, as well as biological field. In order to explore the opportunities of long-term space travel, technologies for growing vegetables and harvest food in space must improve. Understanding how different plants react in a reduced or almost nullified gravity environment can help future development of those technologies [18, 35, 55, 58]. On the other hand, the production of materials could be completely different if the whole process is under the influence of Microgravity forces. Understanding if those differences are beneficial or harmful for the final results is going to be crucial in the development of procedures for material production in space [45, 47, 49-50].

Maize seed was selected because it has short germination period of three to four days and also it is easy to prepare before eating. Catalase and Malondealdehyde enzymes were selected because they are stress enzymes, they help the plant to survive or manage stress.

Some work had been done by some scientists in the simulation of microgravity environment and its effect on plant development [13, 33, 50]. The first successful seed-to-seed experiment in microgravity was reported by, who used *Arabidopsis thaliana*. They observed some viable seeds, but most seeds had nonviable embryos. The second successful experiment was performed with *Brassica rapa* [17, 34, 48, 59].

To understand the behaviour of plant in space environment, the factor that define the physiological state of plant is the microgravity in space which is different from what is obtainable on earth. This study is to investigate the activities expressed by Catalase and MDA in maize seed subjected to clinorotation as these enzymes helps plants to manage stress.

2. Materials and Methods

2.1. Site Location

The experiment was carried out at the Space-Earth Environment Research Laboratory, University of Benin operated by Centre for Atmospheric Research of the National Space Research and Development Agency (NASRDA). The study was conducted between November 2018 and May 2019.

2.2. Preparation of Agar

The Agar was prepared by weighing 1 - 1.5g in 100 ml of tap water. It was Stirred when boiling for uniform dissolution [68]. After a clear solution was observed, it was allowed to cool for about 5 – 8 mins after pouring it on the petri dishes of 30 mls each, sowing followed immediately.

2.3. Sowing

Sowing was done after the Agar was observed to be cool by inoculating the petri dishes with maize seeds, 9 seeds per plate. Three petri dishes were inoculated, one for Microgravity experimentation (clinorotation), one for earth gravity experimentation, one extra petri dish in case of any damage to other two petri dishes.

2.4. Clinorotation

Three different petri dishes inoculated with an average of seven (7) maize seeds per petri dish, which was then mounted on the clinostat at 1, 2, 3 rpm respectively with a specific time interval of 6hrs. (2-D, one Dimension clinostat, UN, New York, USA).

2.5. Enzyme Analysis

2.5.1. Catalase Extraction/Analysis

One gram of the tissue was excised, 10ml PO_4 buffer pH7.0 was used to grind it and form solution.

Table 1. *Catalase extraction and analysis composing the reagents used* [24].

Reagent	Bulk (ml)	Standard (ml)	Test (ml)
0.05MPO_4 buffer, pH 7.0	0.5	1.0	0.4
0.2MH_2O_2	0.5	-	0.5
5MH_2SO_4	0.2	0.2	0.2
0.01MKMnO_4	0.5	0.5	0.5

The solution was properly mixed by inversion and absorbance was taken at 480nm for 60 Sec., the result was recorded. See Table 1 for the composition of the solution.

Enzyme activities was calculated using the formula below:

$$\text{Catalase Activities (CatA)} = \frac{\Delta\text{Abs} \times \text{V}}{\text{M} \times \text{Vol.} \times \text{W}} \quad (1)$$

▲Abs = Change in absorbance= Standard – Test, V = Volume of reaction mixture = 1.7ml

Vol = Volume of aliquot = 0.1mls, m = molar extinction of Hydrogen Peroxide = $40m^{-1}cm^{-1}$, W = weight of sample in the aliquot

Total Volume of the Crude extract is 2ml [15].

2.5.2. Malondialdehyde Extraction/Analysis

Extraction was done by excising 1g of the tissue of the seedling in 10ml PO_4 buffer pH 7.0 and was properly ground and the supernatant collected.

Table 2. *Malondialdehyde extraction and analysis, composition of the reagents used.*

Reagent	Bulk (ml)	Test (ml)
Water	0.5	-
TCA-TBA-HCL	1.0	1.0

The mixture was well mixed, boiled for 15mins in water bath, cooled and centrifuged for 10mins at 400rpm and absorbance of 1ml of the supernatant was taking at 535nm. (See Table for the composition of the solution). [1]

$$\text{MDA Activities} = \frac{\text{Abs} \times \text{V}}{\text{M} \times \text{Vol}} \quad (2)$$

Abs = Absorbance

V = Volume of reaction mixture = 1.5ml

M = Molar extinction coefficient = $1.56x10^5$cm

Vol = Volume of aliquot = 0.5ml

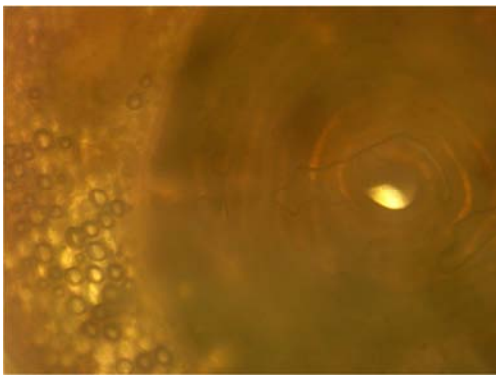
Volume of crude extract 2mls

2.6. Cell Examination

This was done using binocular microscope. A slice of the seedlings was taken and placed on the microscope slide and was viewed at x10 magnification. The photograph was taken through a laptop connected to the microscope [24].

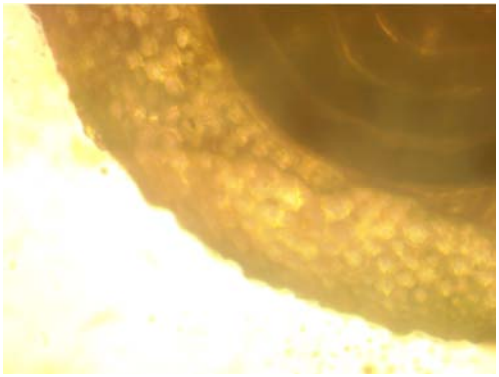
3. Statistical Analysis, Results and Discussion

3.1. Growth Observation



Mag. x10

Figure 1. *Cell (Stem) distribution in a plant under gravitational influence.*



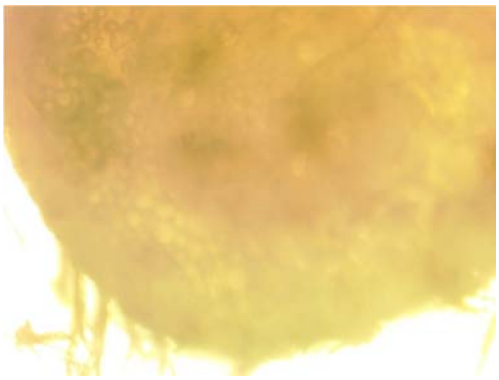
Mag. x10

Figure 2. *Cell (Stem) distribution in a plant under microgravity influence.*

Figure 1 and Figure 2 show significant difference in their cell arrangement, Figure 1 has cells dispersed or scattered while Figure 4 has it cells packed and arranged in a concentric manner as a result of centrifugal force generated from the rotation of the clinostat. This shows that the cells were affected by the simulated microgravity environment.

Mag. x10

Figure 3. *Cell (Root) distribution in a plant under Gravitational influence.*



Mag. x10

Figure 4. *Cell (Root) distribution in a plant under microgravity influence.*

Figures 3 & 4 show a significant difference as the cells in Figure 3 were dispersed but not clustered while the cells in Figure 4 were dispersed and clustered.

3.2. Morphological Examination

The growth of the root and the plumule were observed and recorded (Table 3).

Plumule length of seedlings placed on bench surface and no clinorotation was 1.3 cm. This was not statistically significantly different (P > 0.05) from the seedlings that were exposed to clinorotation

Table 3. *Measurements taken between plant under the influence of gravity and microgravity.*

	Plumule length (cm)	Radicle length (cm)
Flat surface	1.3^{a}	1.5^{a}
90^{o} turned	1.6^{a}	2.1^{a}
3 rpm	1.8^{a}	5.6^{b}
2rpm	2.1^{a}	6.3^{b}
1rpm	1.3^{a}	4.5^{ab}
F statistic	2.736	2.016
p-value	0.535	0.0127

Means on the same column or same row with similar alphabetic superscripts do not differ from one another (p>0.05).

Figures 5 and 6 show the growth difference between gravity influence and microgravity influence.

Figure 5. *Growth of a clinorotated seedlings of maize.*

Figure 6. *Growth of seedlings of maize under gravity.*

The above Figures 5 & 6 shows the radicle and the plumule emergence and elongation of seeds. Figure 5 shows the growth of seeds that was clinrorotated and longer in plumule and radicle compare to Figure 6 which is under gravitational force influence within the same period of time.

Catalase activity was higher in the clinorotated plumule than in the radicle as well as in those not clinorotated When seedlings were rotated at 2 rpm for 6 hrs, CAT was 7.59 mol/sec compared to 2.56 mol/sec when placed on a flat surface. However, activities of CAT in the radicle were higher in the unrotated seedlings than when exposed to clinorotation.

Table 4. *Catalase responses of the shoot and root of the test plant exposed to clinorotation.*

	Catalase activity (mol/sec)	
	Plumule	Radicle
Flat surface	2.56^{a}	0.41^{a}
90^{o} turned	2.00^{a}	0.36^{ab}
1rpm	7.59^{c}	0.21^{ab}
2rpm	0.91^{b}	0.08^{a}
3rpm	2.65^{a}	0.08^{a}
F statistic	8.985	3.804
p-value	<0.001	0.018

Means on the same column or same row with similar alphabetic superscripts do not differ from one another (p>0.05)

Table 5. *Malondealdehyde responses of the shoots and roots of the test plant exposed to clinorotation.*

	Malondealdehyde (mol/sec)	
	Plumule	Radicle
Flat surface	2.38^{a}	1.47^{a}
90^{O} turned	6.05^{c}	4.79^{bc}
1rpm	4.79^{b}	6.44^{b}
2 rpm	2.81^{a}	4.19^{ab}
3rpm	6.13^{c}	3.71^{ab}
F statistic	28.764	2.004
p-value	<0.001	0.133

Means on the same column with similar alphabetic superscripts do not differ from one another (p>0.05)

Malondialdehyde responses of the shoot and root of the

test plants exposed to clinorotation presented in Table 5. Seedlings on flat surface had an MDA level of 2.38 mol/sec in the plumule and 1.47 mol/sec in the radicle. Comparatively, seedlings placed at 90 degrees had an MDA of 6.05 units in the plumule and 4.79 mol/sec in the radicle. At 3 rpm, plumule MDA level of 6.13 units was higher than in the radicle (3.71 mol/sec).

Bivariate correlation between, catalase and Malondealdehyde responses of the shoots and roots of the test plant exposed to clinorotation has been presented in Table 6 MDA in the plumule correlated significantly with CAT in the plumule, Similarly, there was significant correlation between CAT in the plumule and CAT in the radicle.

Table 6. *Bivariate correlation between, catalase and Malondealdehyde responses of the shoots and roots of the test plant exposed to clinorotation.*

	MDA (plumule)	MDA (radicle)	CAT (plumule)	CAT (radicle)
MDA in Plumule	1	0.026	-0.454*	0.132
MDA in radicle	0.026	1	-0.383	-0.306
CAT in Plumule	-0.454*	-0.383	1	0.486*
CAT in radicle	0.132	-0.306	0.486*	1

*. Correlation is significant at the 0.05 level (2-tailed).

Maize (Zea *Mays)* seedlings grown for four days on the horizontal 2-D Clinostat at 1rpm was 4.5 cm in length for the root while the shoot was 1.3 cm as against the normal surface data of 1.5 cm length on the root while 1.3 cm length on the shoot as indicated in Table 4, this is an indication that microgravity effect alters some activities in the developing state of seedlings. Changes in cell wall properties seem to be well correlated to the growth of each organ in maize seedlings. These results suggest that the growth responses to Micro-g conditions are regulated by both cell-wall mechanical properties and osmotic properties of stem cells, while supporting the hypothesis that under Micro-g in space the cell wall metabolism of seedlings is stimulated, reported in higher plants, leading to an increase in their extensibility [20-22, 59].

Accumulation of water at the tip of the root during clinorotation and also the elongation tendency which leads to tallness of the seedlings under Microgravity showed a high metabolic activity in this study compared to the one under normal surface (earth gravity).

Figures 1 & 2 show significant differences in their cell arrangement. Figure 1 has cells dispersed or scattered while Figure 2 had its cells packed and arrange in a concentric manner as a result of centrifugal force generated from the rotation of the clinostat. Furthermore, Figures 3 and 4 show significant differences. As the cells in Figure 4 were dispersed but not clustered, Figure 4 were dispersed and clustered. This is further evidence that main activities occured in the radicle of the plant. Results in root cap of *Z. mays* indicated patterns of differentiation and structure of all cells, as organelle specific, altered under Micro-g conditions. The distribution of amyloplasts (starch granules) happens in the statocytes of roots (columnella cells). Sedimentable amyloplasts are required for the graviperceptive function of columella cells. Differentiation of the putative graviperceptive cells of roots involves changes in relative volumes of amyloplasts [43, 45, 61]. Qualitative observations suggested that amyloplasts in columnella cells were characterized by a "probably low" starch grain (amyloplasts differentiate). Though details remain vague, gravitropism is initiated by sedimentation processes in shoot endoderm and root columnella cells, the sedimentation process itself representing the gravitropic signal. Investigations indicate that Micro-g altered the patterns and magnitude of changes characteristic of cellular differentiation evident in root caps of *Z. mays* [2, 5, 14-15].

Catalase activity was higher in the clinorotated plumule than in the radicle and in those not clinorotated (Table 4). When seedlings were rotated at 2 rpm for 6 hrs, CAT was 7.59 units compared to 2.56 units when placed on a flat surface. However, activities of CAT in the radicle were higher in the unrotated seedlings than when exposed to clinorotation. This increase of CAT in plumule against the radicle of clinorotated, could be as a result of the stress created by the rotation of the clinostat for the plant trying to survive in a very different environment compare to the one on normal surface. In chloroplasts of higher plants, for example, interaction of molecular oxygen with electrons carried by different transporters constantly produces ROS, whose levels may grow considerably in stress conditions [1, 3-4, 16].

Malondialdehyde responses of the shoot and root of the test plant exposed to clinorotation shows increase in activities. Seedlings on flat surface had an MDA level of 2.38 mole/sec in the plumule and 1.47 mole/sec in the radicle. Comparatively, seedlings placed at 90 degrees had an MDA of 6.05 mole/sec in the plumule and 4.79 mole/sec in the radicle. At 3 rpm, plumule MDA level was 6.13 mole/sec and was higher than in the radicle (3.71 mole/sec). The accumulated ROS can cause DNA damage, lipid peroxidation, and inactivation of certain enzymes. Plant cells respond to ROS by mobilizing the defense systems and enhancing the production of ROS scavenging proteins. In barley leaves grown on board the ISS, the transcription of genes encoding major antioxidant proteins was upregulated. It could also mean that this current study under microgravity could also be upregulated [10-12, 29]. The increase in antioxidant transcript levels was stronger than in plants subjected to salt stress, a well-known inducer of antioxidant biosynthesis. In spaceflight environment, barley leaves accumulated the transcripts of superoxide dismutase, ascorbate peroxidase, glutathione transferase, and catalase. It was previously shown that both ROS production and antioxidant enzyme activity are increased in artificial microgravity conditions in a clinostat [36-37, 71, 73-74]. The probability of oxidative

stress on board of spaceships has been repeatedly pointed out by many researchers. There was increased transcription of ROS eliminating genes in plant cells, in actual spaceflight environment, which means the increase in any of the part of the plant enzyme activities is as a result of the stress created.

4. Conclusion

This study has proven that in microgravity environment, certain characteristics are synonymous with plants in that environment, ranging from observation of water on the root tip, dispersed cells arrangement, relative increase in length and reduction in girth, all these leads to high rate of enzyme activities as was observed on the resulted presented compare to the plant observed under earth gravity. The high expression of the Cat and MDA in microgravity environment confirm that space travel pose different physiological challenges to living system as the system try to adapt to a new environment. The enzymes Cat and MDA are stress dependents which means plants could adjust to the current environment of microgravity with the high production of these stress managing biological molecules, this clearly shows that colonization of other planet using plants for life sustainability is very possible. This is an indication that for a successful colonization of other planets, more attention most be giving to plant biomolecules. There are numerous plants biomolecules that are directly involved in plant physiology, this study open more gateway to study in this respect.

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