American Journal of Life Sciences
2025, Vol. 13, No. 6, pp. 180–197
https://doi.org/10.11648/j.ajls.20251306.12

Research Article

Productivity Evaluation of Hormone-primed Maize Seedlings Exposed to Microgravity Environment Simulated with 2d Clinostat

Alexander Oseghale Orukpe[1, 2, *], Geoffrey Obinna Anoliefo[3], Beckley Ikhajiagbe[3], Prayer Eromosele Atumah[4]

[1]United Nations African Regional Centre for Space Science and Technology Education-English, Ile-Ife, Nigeria
[2]Space-Earth Environment Research Laboratory, University of Benin, Benin City, Nigeria
[3]Plant Biology and Biotechnology Department, University of Benin, Benin City, Nigeria
[4]Environmental Management and Sustainability Department, Anglia Ruskin University, Petersbrough, United Kingdom

Abstract

The biggest challenges in long space exploration has been sustainable supply of basic life support. Plants are reliable sources of sustenance and survival because of their ability to produce Oxygen, Water, and Food. This is much more important as it provides a more sustainable energy source during space travel. Unfortunately, microgravity which is a common phenomenon in space could be hindrance to plant growth and development. This study investigated response of plant growth promoters to enhance survival, yield and development of a *Zea mays* exposed to Microgravity simulated environment using 2D Clinostat. Plant growth stimulators that were used are Indole-3-Acetic Acid (IAA), Sodium Nitroprusside (SNP) and Thiourea (TU) at concentrations of 100 ppm and 500 ppm respectively. The *Zea mays* seeds were subjected to clinorotation at 0.5 rpm, and 2.5 rpm, and were observed for 120 hr. After 120hrs, the microgravity-exposed seedlings were acclimatized in experimental plants in a well-ventilated screen house for 5 days and thereafter transplanted on the field, where plant growth and yield responses were observed for 12 weeks. The study showed that addition of growth stimulators enhanced significant positive association between plant total dry weight and oxygen production efficiency. This study addresses Millennium Development Goal 1: Eradicate extreme poverty and hunger. This it does by providing information on the possible adoption of a combination of microgravity and growth stimulation as a measure for improvement of maize, as yield increase was more than 50% through the adoption of this technology. Conclusively, growth chemo-stimulation with IAA and SNP enhanced plant growth, development and high yield of maize exposed to simulated microgravity using 2D-Clinostat.

Keywords

Microgravity, 2D Clinostat, Simulation, Growth Stimulator

[*]Corresponding author: alexanorukpe@gmail.com (Alexander Oseghale Orukpe)

Received: 18 October 2025; **Accepted:** 31 October 2025; **Published:** 9 December 2025

1. Introduction

Space research is one of the most significant biotechnologies nowadays; the emphasis is on space research since there is a need to find alternate habitat for humans. Industrialization has put such a strain on the globe's resources that if they are not properly managed, the world may exceed it carrying capacity. One of the few alternatives remaining to cater for the world is to either lessen the intensity of man's intervention on earth, which may not be achievable because technology is progressing on a daily basis, or to explore other planets for man to relieve the pressure on the world. Astronauts that go into space normally carry supplies for energy and food, but these supplies are typically short-lived; once the supply is spent, space travel is terminated. The energy supply available to space travelers should be renewable. Plants are the only source of renewable resources that can provide both food and energy at the same time [25, 12, 34].

On October 4, 1957, the world's first artificial earth satellite called Sputnik 1 was launched by the Soviet Union (USSR) and the United States of America(USA). After that period, several other countries have made attempts at not only launching artificial satellites, but also putting the man on the moon. This feat actually is not basically for show of the country's level of technological advancement but probably to achieve a purpose that one of several purposes that is very important and that is the fact that the earth is gradually being overpopulated by humans and probably the need arises for man to seek other species, particularly in space [11].

Recent discovery has shown that apart from the Earth, perhaps there might be several other planets that have capacities for life sustenance. In recent times, the space journey to Mars has been trending, but the question is, why is space exploration very important? from the fact that it is a proof of the prowess of the world powers like the US, Canada, China, Germany Russia, it also, as earlier mentioned, provides the need for an alternative home [16, 17, 5, 19, 21].

Now the question would be why is this need for an alternative home? The pressure mounts from the overpopulation of the earth by humans and this concomitantly mounts pressure on the lean natural resources on Earth. Overpopulation eventually leads to gradual degradation of biodiversity and loss of habitat and if not taken care of, this eventually might return to cause a downward drop in human population which results from death, war and conflict. This actually eventually leads to humanitarian crisis, but it must be noted as stated in [36] that the impact of man has eventually increased the chances of a global catastrophe culminating in climate change [24, 29, 28, 3].

Haven noted that space travel probably presents an alternative for seeking for other planetary bodies where biological human life, or human sustenance can be found or can exist or subsist, it will therefore be important to enhance space travel research.

These resources are enormous and would eventually require huge sums of revenue, unfortunately these resources are also non-renewable and as such the space machine or the space vehicle would have to be well loaded with lots of these resources and that will contribute to increasing the weight of the space shuttle and also raising the amount of fuel that will be spent or burnt in the space travel.

However, the reliance on plants provides a better alternative since plants would provide these same requirements for life supports in space. Plants provide food, oxygen through photosynthesis, they are also a source of water vapor from photosynthesis and in most cases where plants have been shown to provide water through guttation.

Therefore, the reliance on plants will put forward oxygen, water, energy and nutrients become renewable resources rather than being termed un-renewable, suppose they were to be provided and packaged in the life support system. A number of studies on the relevance of plants in space and the relevance of plants as a support or as a source of life support for humans in space have been treated in literature [32, 45, 30].

The plant in space eventually amounts to exposing the plants themselves to a new kind of environmental condition different from what obtains on Earth, and this is termed microgravity. The effect of microgravity on plant morphology has been earlier discussed [23, 39, 45, 30]. The impact of microgravity on enzyme activities of plants have also been previously reported [1, 45, 30]. It must be noted that [27] reported the neurocognitive and psychological impact of space travel on man.

2. Justification of the Study

It is important to under study the effects of exposing crop plants to microgravity effects and assess productivity levels and capacity to provide close to what is required by humans in space and the adoption of Space based technology for possible crop improvement on earth. However, since microgravity effects on seed germination had earlier been determined to be suppressive [30] to what extent can the use of growth stimulants help alleviate this negative impact?

In this study, seeds of a test crop *Zea maize* L will be tested to evaluate germinability and germination potentials under microgravity conditions, and to see to what extent the use of stimulators can improve not only germinability but also seedling development and plant yield turnover. Because it had previously been reported that microgravity exposure impaired maize germination, there was a need to investigate whether these germination impairments could be corrected if the seeds were primed with plant growth-promoting substances such as Indole-3-acetic acid (IAA), Sodium Nitroprusside (SNP), and Thiourea (TU).

Plants begin with germination, which is why this study will focus on the initial few days of development, known as

the germination period. The ability of any plant to withstand stress has been shown to be influenced by particular chemical molecules known as growth stimulating chemicals, such as gibberellins, IAA, and so on.

Improved seed priming techniques can shorten the time between seed sowing and seedling emergence, resulting in rapid and uniform seedling emergence, high seed vigor, better and more uniform stand establishment, better allometric and yields in a variety of vegetable and flower species, as well as many field crops [12, 4]. Aside from crop establishment improvements, other advantages include improved drought tolerance, low temperatures, salinity tolerance, better weed competition, early flowering, and shorter time to maturity, and higher grain yield.

In this study, plant growth regulators are used as the primers. These substances, polyamines, and other organic sources have improved seed performance in many vegetable and field crops, including rice, by introducing them during priming and other pre-sowing treatments [9, 2, 47].

The aim of the study is to investigate the effects of exposure of maize seeds to germinant under microgravity condition on growth and yield responses of the maize plants, after having been chemo-primed with selected plant growth stimulators Indole-3-acetic acid (IAA), Sodium Nitroprusside (SNP), and Thiourea (TU).

The following are the objectives of the study: 1. Investigate the impact of the treatment regimen on the germination characteristics of the maize seed. 2. Determine possible influence of experimental treatments on the growth, morphology, and quantitative growth characteristics of the maize plant having been previously exposed to microgravity. 3.To conduct Phyto-assessment impact of clinorotation on quantitative growth and yield assessment of exposed maize seedlings.

3. Materials and Methods

3.1. Materials

3.1.1. Site Location

The experiment was conducted at the Space-Earth Environment Research Laboratory, University of Benin, operated by United Nation African Regional Centre for Space Science and Technology Education-English (UN-ARCSTEE) of the National Space Research and Development Agency (NASRDA). Part of the study was also conducted at the department of biochemistry, university of Benin, majority of this studies were the enzyme-based studies.

3.1.2. Materials Used for the Study Included

2D-Clinostat (United Nation Advanced Engineering Services Co Limited) use for Microgravity Simulation, Analytical Digital TX4202L (Shimadzu), Weighing Balance, Hot Plate (Emel), UV-VIS Spectrophotometer, Microscope with Camera, PCR

3.1.3. Reagents and Chemicals Materials

The Reagents and chemical materials that were utilized in this study included Agar Agar, distilled water, petri dishes, and other biochemical analysis reagents. edw

3.1.4. Maize Seeds

Maize variety (DK920 Hybrid) was obtained from Raymos Guanah Farms Ltd., Delta State. Viability of the seeds was ensured using the floatation viability seed testing described by [10].

3.1.5. Priming Material

The priming materials used for the experiment were solutions of sodium nitroprusside (SNP) and indole acetic acid (IAA). These were prepared into two (02) different concentrations; 100 and 500 mg/l respectively.

3.2. Methods

The previously microgravity exposed seeds were initially acclimatized and then transplanted to field for the field study. The laboratory study began with the preparation of agar.

The second stage, having had the petri dishes solidified, they were divided into two cycles. Whereas the first cycle involved inoculating the seeds on the petri dishes and then mounting on the clinostat. The clinostat was set to rotate at two different revolutions per minute; 0.5rpm and 2.5 rpm. The seeds were immersed in sodium nitroprusside (SNP) for one hour, they were also immersed in indole-3-acetic acid (IAA) and Thiourea (TU). Having been immersed for one hour, the seeds were removed and washed thoroughly before inoculation on the agar in the petri dish, which was thereafter mounted on the clinostat and rotated at two different speeds, 0.5 and 2.5 rpm.

Agar Preparation And Sowing of Maize Seeds

The preparation followed standard methods as used by [30]. The Agar was prepared by weighing 1.50 g in 100 mL of distilled water. The solution was then heated for 10 min with continuous stirring to achieve a homogenous solution. After a clear solution was observed, it was allowed to cool for another 10 min after pouring it on the Petri dishes labelled g and µg, sowing followed immediately.

The maize seeds were weighed using B3003T 300 g/0.001 g Portable Electronic Balance Laboratory Scale. The seeds (control or pretreated with either SNP, IAA or TU) were inserted into the Petri dishes containing the cooled agar, with the seeds standing upright. A total of 20 seeds were mounted on each Petri dish; and then sealed properly [30]. The Petri dishes containing the control experiment were left under the influence of gravity and placed on the laboratory bench, while the ones for microgravity were placed in turns on the 2D-Clinostat (2-D, One Dimension Clinostat, New York,

USA) for 120 h at 0.5 and 2.5 rpm.

Clinorotation

The different Petri dishes inoculated with the mounted maize seeds pretreated with the respective treatment concentration (100 mg/L IAA, 500 mg/L IAA; 100 mg/L SNP, 500 mg/L SNP, 100 mg/L TU and 500 mg/L TU respectively) were mounted on the Clinostat at 0.5 and 2.5rpm for 120 h.

Germination Parameters

Plant germination parameters measured were root length, shoot length, number of prominent roots, Wet weights of seedling, dry weight of seedling and those of seedlings were measured using the weighing balance after the day of termination of the experiment. Their respective dry weights were also measured after sun drying 72 hrs.

Germination Testing

Germination was assessed using various measurements and indices as earlier described [40, 13, 1, 18, 20, 35].

Germination Percentage: First day of germination (or Germinability) (FDG)

It is the time when the first germination was recorded

Last day of germination (LDG)

This is the last day when the seed germination was reported

Final germination percentage (FGP)

This is the germination percentage attained by the plant even beyond the time period

Peak period of germination (PPG) or Modal time of germination (MTG)

It is the time in which highest frequency of germinated seeds are observed and need not be unique.

Median germination time (MeGT), or Days required for 50% germination, T_{50}

Days required for 50% germination, T_{50}

T_{50} = Days required for 50% germination of the total number of seeds

Germination rate index (GRI)

$$S = \frac{N1}{T1} + \frac{N2}{T2} + \frac{N3}{T3} + \cdots + \frac{Nn}{Tn}$$

GRI =$[GP_1/1 + GP_2/2 + GP_3/3 + \ldots + GP_n/n]$

Where GP_1 is germination percentage at 1st day, GP_2 is germ percent at 2 days, GP_n is germ percent at n days. GRI reflects the percentage of germination on each day of the germination period.

Corrected germination rate index ($GRI_{corrected}$)

$S_{corrected}$ = GRI / FGP

The seedling vigor

SVI (I) = Seedling length x FGP

SVI (II) = (Root length + Shoot length) x FGP

SVI (III) = Seedling dry weight x FGP

Time spread of germination, or Germination distribution (TSG)

TSG = LDG - FDG

This is the time (in days) taken between the first and last germination events

Germination index, GI

$$GI = 10 \text{ x } (S_1+S_2+S_3+ \ldots +S_n) / (1*S_1 + 2*S_2 + 3*S_3 + \ldots + n * S_n)$$

WhereS_1, S_2, S_3, Sn are number of seeds that germinated per lot (or petri dish) at day 1, day 2, day 3 … day n

Mean daily germination, MDG

MDG = FGP/d, where d is the number of days it took to first arrive at the FGP

Daily germination speed, DGS

DGS = 1 /MDG. This is the reciprocal of MDG

Mean germination time, MGT

$$MGT = (1*S_1 + 2*S_2 + 3*S_3 + \ldots + n * S_n) / (S_1+S_2+S_3+ \ldots +S_n)$$

Or

$$\overline{T} = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni}$$

Where S_1, S_2, S_3, Sn are number of seeds that germinated per lot (or petri dish) at day 1, day 2, day 3 … day n.The lower the MGT, the faster a seed population has germinated. It is also called Length of Germination Time (LGT) or Germination Resistance (GR) or Sprouting Index (SI). It is the average length of time required for maximum germination of a seed lot.

Mean germination rate, MGR

MGR = 1 / MGT

Coefficient of velocity of germination, CVG

$CVG = [(G_1+G_2+G_3+ \ldots +G_n) / (1*G_1 + 2*G_2 + 3*G_3 + \ldots + n * G_n)] * 100$

Where G_1, G_2, G_3, Gn are germination percent per lot (or petri dish) at day 1, day 2, day 3 … day n. CVG gives an indication of the rapidity of germination.

Or, simply, CVG = MGR x 100

Germination capacity, GC

GC = FGP / N

Where N is number of seeds used in the bioassay

Growth Assessments

Growth parameters were constantly observed through the clinostat and after the required time for the exposure, which was five days, the seeds were dismounted from the clinostat and then growth and germination parameters were obtained, after which the seeds were transferred to the lab for further analysis.

In the second phase of the experiment, the seeds were not primed at all, in water were also inoculated on the petri dish. So therefore, invariably, the two sets of experiment in vitro were those mounted on the clinostat but had been priorly exposed to the growth stimulators, whereas the second phase of the experiment only included non-treated maize seeds that were mounted on the clinostat.

3.3. Morphological Growth Assessment

Morphological parameters determined in these studies included length of leaf, which was taken from the base of the leaf stalk up to the tip of the leaf, number of leaves, as well as the appearance of any physical anomaly. and normally. Stem girth, stem length, as well as shoot dry weight and root dry weight, including the average length of the longest root, were determined to ascertain the impact of treatment regimen on growth morphology of the maize plants. In order to assess productivity and net assimilation rate, it was important to determine if microgravity impacted on rate of assimilation of nutrients in the germinantes and also determine if such influences were significant. Results, I mean, leaf area index, relative growth rate, net assimilation rate, leaf area ratio, as well as leaf area duration, were all determined in the study according to [31].

3.4. Determination of Crude Protein Content

Nitrogen was determined using the micro-Kjeldahl method (A.O.A.C 2000) and crude protein content will be subsequently calculated by multiplying the nitrogen content by a factor of 6.25.

3.4.1. Principle

Proteins and other food components are digested with sulphuric acid in the presence of catalysts. Ammonium sulphate is produced from the total organic nitrogen in the food. Alkali is then used to neutralize the acid digest to produce ammonium which is steam distilled directly into a hydrochloric acid containing the indicator methylene red. Hydrochloric acid and ammonium react to produce ammonium chloride which is then titrated with sodium hydroxide. A blank determination is run to determine the nitrogen content of the reagents. The percentage nitrogen is calculated. The percent nitrogen multiplied by the conversion factor for that particular food produces the percent protein in a food.

3.4.2. Procedure

One millilitre of 4% $CuSO_4$, H_2SO_4 and 0.8g of K2SO4 were placed in the micro kjeldahl flask. Varying quantity of food samples depending on nitrogen content was added to the reagents in micro Kjeldahl flask. It was digested first at low temperature until frosting ceases, then at a high temperature, until the solution was clear, pale yellow or light blue. The flask was left to cool, and 4ml of distilled water was gradually added and content were distilled using a Kjeldahl distillation apparatus. 10 mL of 30% NaOH was used to liberate during distillation ammonium. Ammonium was collected in 0.01 M HCl (a drop of methylene red was added to the HCl.). The distillate was titrated with 0.01M NaOH. Nitrogen content of samples was calculated from the volume of HCl neutralized by NaOH.

3.4.3. Calculations

%nitrogen= titre value (blank) − titre value (distillate) x 0.14

Weight of sample

Protein = % nitrogen x 6.25

% protein = protein x 100

Initial weight

Determination of Total Sugar

Reagents required

R_3 (Reagent III) A

25g Ammoniummolydate was dissolved in 400ml distilled water and 21ml conc. H_2SO_4 was added.

R_3 (Reagent III) B

30g Sodium arsenate was dissolved in 25ml distilled water. A and B were mixed and made up to 500ml with distilled water.

R_2(Reagent II)

30g $CuSO_4$ was dissolved in 200ml of distilled H_2O and four drops of Conc. H_2SO_4 added.

R_1(Reagent I)

25g Sodium carbonate, 25g Potassium tartate, 20g Sodium hydrogen carbonate, 20g Sodium Sulphate were all dissolved in 500ml distilled H_2O and make up to I litre.

Reagent R_4 = 400ML (R1) + 16ml (R2)

3.5. Glucose Standard

0.1% glucose for standard calibration curve 100mg/ml (0.2 - 1.0µg/ml)

3.5.1. Preparation of Standard

200µg/ml was used to prepare the concentration of the standard curve (0.2 - 1.0µg/ml). Water was used to equilibrate tube 1 - 6. Tube 1 is blank. 1ml of R_4 was added to each tube and boiled for 20 minutes, then cooled. Iml of R_3 was added and shake vigorously to remove CO_2 and was allowed to stand for 10 minutes. 10ml of water was added and mixed before taking reading with spectrophotometer after blanking with tube 1. The absorbance values from the spectrophotometer were used to plot a calibration curve using the concentration of each tube.

3.5.2. Procedure for Test Samples

1.0ml of sample was pipetted into a test-tube. 1.0ml of R_4 was added and boiled for 20minutes. The tube was allowed to cool. 1ml R_3 was added and shaked vigorously to remove CO_2 and was allowed to stand for 10minutes. 10ml of distilled water was added to thetube and was shaked well before reading spectrophotometrically at 600nm. The absorbance value of the test sample was extrapolated from the standard curve to get the concentration (µg/ml) of sugar in the sample [10].

3.6. Data Analysis

Data obtained from the study were presented in means and standard errors of three replicates. Data were analyzed following two-way analysis of variance using GENSTAT (8th edition), where significant p-values were obtained. Differences between means were separated using Least Significant Differences.

4. Results

The effect of the microgravity and chemo-priming treatments on the maize seeds were significant, P less than 0.001. Elevated sugars were observed, particularly with growth enhancement using thiourea and seeds were hitherto exposed to microgravity. For example, whereas control seeds had a total sugar content of 0.5 rpm microgravity exposed seedling at 12 weeks. However, when the microgravity exposed seeds were treated with either sodium nitroprusside or thiourea, total sugars ranged from 1.69 9 to 2.24 milligram per meal (Figure 2).

Table 1 shows growth parameters of the maize plant after harvest of corn ears, particularly 12 weeks after the seeds were originally exposed to either microgravity or chemo-priming after microgravity. When seeds exposed to 0.5 rpm microgravity were sown (82.8 cm) as compared to plant height in the control (106.7 cm). Generally, there was significant impact of treatment regimen on plant height. Plant height in microgravity exposed seeds that were primed with 100 ppm IAA chemo-primed seeds.

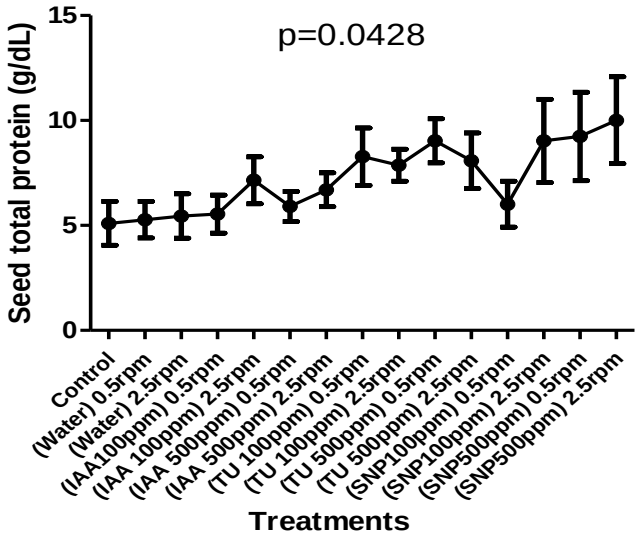

Figure 1. *Harvested Seed total protein at 12 weeks.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant difference.

Generally, leaf length was elongated due to microgravity; whereas leaf length in the control was 72.5 cm, in the microgravity exposed plants it ranged from 72.3 to 106.3 cm. When microgravity exposed plants were chemo-primed with sodium nitroprusside, leaf length ranged from 93.2 to 126.8 cm. Generally, the impact of treatments on number of leaves per plant of the test maize plant was significant.

It should be noted, however, that there were reductions in number of leaves for microgravity exposed plants that were chemo-primed when compared with the control. For example, whereas there were 16 leaves per plant in the control, exposing the plants to microgravity significantly raised the number of leaves to 21 seeds before they were eventually chemo-primed.

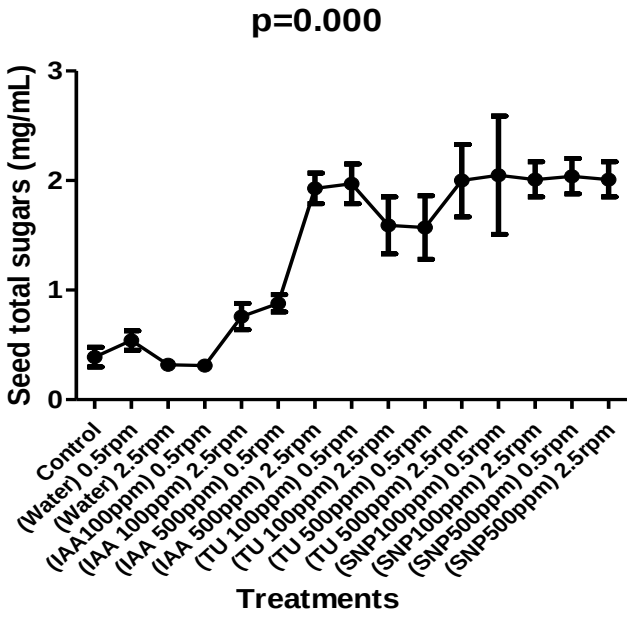

Figure 2. *Harvested Seed total sugars at 12 weeks.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant difference.

When microgravity exposed seeds were chemo-primed, number of leaves reduced to between 14 and 18 leaves per plant. Table 2 continues with plant growth parameters at 12 weeks after exposure to treatment regimen. In this case, results showed that there were no significant changes compared to the control in total dry weight. Whereas total dry weight in the control was 11.3 grams, in the microgravity exposed maize plant, it ranged from 9.2 to 13.4 grams. There was however significant delay in number of days taken for tasseling. Whereas it took 62 days for control plants to tassel, it however, for plants however, prime with IAA but we are not microgravity exposed to microgravity it took 56 days to tasseling.

For plants that were exposed to microgravity, the results showed that compared to the control there were no significant changesin the number of days to task them for micro-

gravity exposed plants, except when they were eventually chemo-primed. After this, there was significant delay for up to one week. evidence of foliar foraging was reported in Table 2. We refer to cases where leaves were eaten significantly by herbivores like insects, insect pests, and caterpillar. In this case, foliar foraging was higher in IAA-induced plants compared to other treatments.

Table 3 shows the quantitative growth assessment of maize plant after harvest, here the relative growth rate in the control was 0.0451gram per day. This the value however increased in sodium nitroprusside enhanced seed whether they were previously expose to microgravity or not (0.0478-0.0520gram per day), similarly increased in bio-mass duration in the sodium nitroprusside treated seed was reported (413.6 - 495.7 gram per day) compared to the control (459.6 gram per day). It was observed that the average growth rate in the IAA treatment where lesser (0.107 - 0.139) compared to the control (0.139 gram per day).

Table 1. *Growth parameters of maize plant after harvest of corn ears at 12 weeks after exposure to treatment.*

	Plant height (cm)	**Leaf length (cm)**	**Stem width (cm)**	**Average leaf area cm^2)**	****No. of leaves/ plant**
Control	106.7	72.5	4.1	195.8	16
(Water) 0.5rpm	82.8*	106.3*	5.2	255.2*	11*
(Water) 2.5rpm	132.8*	92.3*	6.3*	237.3*	21*
IAA 100 Ctr(G)	144.2*	87.2*	6.7*	232.1*	18
IAA 500 Ctr(G)	97.2	82.4	5.5	218.2	12*
(IAA100ppm) 0.5rpm	99.3	99*	5.6	213.4	13
(IAA 100ppm) 2.5rpm	80.8*	99.4*	4.8	238.6*	12*
(IAA 500ppm) 0.5rpm	83.7*	63.6	4.2	171.6	13
(IAA 500ppm) 2.5rpm	93.8	79.8	4.6	251.3*	12*
TU 100 Ctr(G)	100.3	90.2*	5.2	198.2	12*
TU 500 Ctr(G)	93.4	87.3*	4.7	192.3	15
(TU 100ppm) 0.5rpm	85.3*	78.5	4.5	176.5	12*
(TU 100ppm) 2.5rpm	89.6	79.2	3.5	170.6	10*
(TU 500ppm) 0.5rpm	90.4	82.3	4	207.2	13
(TU 500ppm) 2.5rpm	87.4*	72.1	4.1	172.9	12*
SNP 100 Ctr(G)	94.3	76.3	4.6	201.3	11*
SNP 500 Ctr(G)	92.4	89.2*	4.2	215.2	16
(SNP100ppm) 0.5rpm	118.2	121.4*	4.9	255.2*	18
(SNP100ppm) 2.5rpm	129*	114.2*	5.1	264.1*	19
(SNP500ppm) 0.5rpm	112.8	126.8*	4.6	213.4	16
(SNP500ppm) 2.5rpm	101.7	93.2*	4.2	189.1	14
LSD (0.05)	19.3	12.4	2.2	32.3	4
p-value	0.0394	<0.001	0.172	0.046	<0.001

*Means on similar column with asterisks different significantly from the control ($p<0.05$)

Table 2. *Growth parameters of maize plant after harvest of corn ears at 12 weeks after exposure to treatment.*

	Total dry wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)	No. of days to tasselling (days)	Foliar foraging (%)
Control	11.3	7.50	3.70	62	55.76
(Water) 0.5rpm	9.2	6.4	2.91	59	67.91
(Water) 2.5rpm	13.4	8.6	4.13	60	73.18
IAA 100 Ctr(G)	11.2	7.23	3.88	56*	81.25*
IAA 500 Ctr(G)	10.3	6.98	2.73	66	76.46
(IAA100ppm) 0.5rpm	10.2	7.8	2.60	71*	74.55
(IAA 100ppm) 2.5rpm	9.6	6.3	3.74	72*	78.44
(IAA 500ppm) 0.5rpm	9.2	6.8	3.20	68*	82.79*
(IAA 500ppm) 2.5rpm	8.9	6.7	3.20	69*	80.26*
TU 100 Ctr(G)	9.8	6.05	2.67	70*	76.54
TU 500 Ctr(G)	8.7	7.04	2.01*	68*	79.43
(TU 100ppm) 0.5rpm	8.6	6.8	2.27*	70*	65.34
(TU 100ppm) 2.5rpm	8.8	6.7	2.50*	68*	79.52
(TU 500ppm) 0.5rpm	9.6	6.9	2.90	65	68.66
(TU 500ppm) 2.5rpm	8.9	6.82	2.90	70*	70.03
SNP 100 Ctr(G)	9.3	6.88	2.93	70*	75.34
SNP 500 Ctr(G)	10.3	7.04	2.16	71*	82.35*
(SNP100ppm) 0.5rpm	11.6	6.9	3.90	69*	72.86
(SNP100ppm) 2.5rpm	12.3	7.5	3.70	72*	68.92
(SNP500ppm) 0.5rpm	11.5	7.58	3.20	67	71.23
(SNP500ppm) 2.5rpm	10.5	7.02	3.10	68	78.55
LSD (0.05)	2.9	1.6	1.2	6	24.41
p-value	0.713	0.384	0.227	0.068	0.452

*Means on similar column with asterisks different significantly from the control ($p<0.05$)

Figure 3, however shows net assimilation rate of the maize plant calculated after harvest of the corn, here net assimilation rate in the control was 0.018gram per cm^2 per day. Apart from exposure to microgravity at 2.5 rpm where net assimilation rise to 0.0019gram per cm^3 per day, there were generally reduction in net assimilation compared to the control.

Net assimilation rate in this study, describes the increase in dry matter area in the plants. It is very useful quantitative plant index that is used to indicate the assimilatory capacity of the maize plant. Biomass duration is the product of the biomass and the time period in which biomass is maintained. This is even important because it indicates how persistent biomass presents and it is also useful for calculation of maintenance respiration overlong period of time.

Figure 4, shows leave area duration of the plant and in this case, there were general increase in leave duration when compare to the control. Whereas leaf area duration in the control was 8059cm^2 per day, the maximum value of above 14000cm^2per day was obtained in the treated plant.

The leaf area duration is the product of the leaf time area and the period which leaf area is maintained, it is also critically important because it evaluates the period which plants appear green. Haven't put these three indices together, the current study try to estimate the capacity for oxygen production efficiency based on Nitrogen, Net assimilation rate, Leaf area duration and Biomass duration. In this case oxygen production efficiency calculated as percentage day was 3.42 percent day, however it was elevated in the microgravity exposed seeds without chemo priming (3.62-3.69percent day) similar increase were obtained in sodium nitroprusside chemoprimed seedling irrespective of exposure to microgravity (3.41-3.58 percent day).

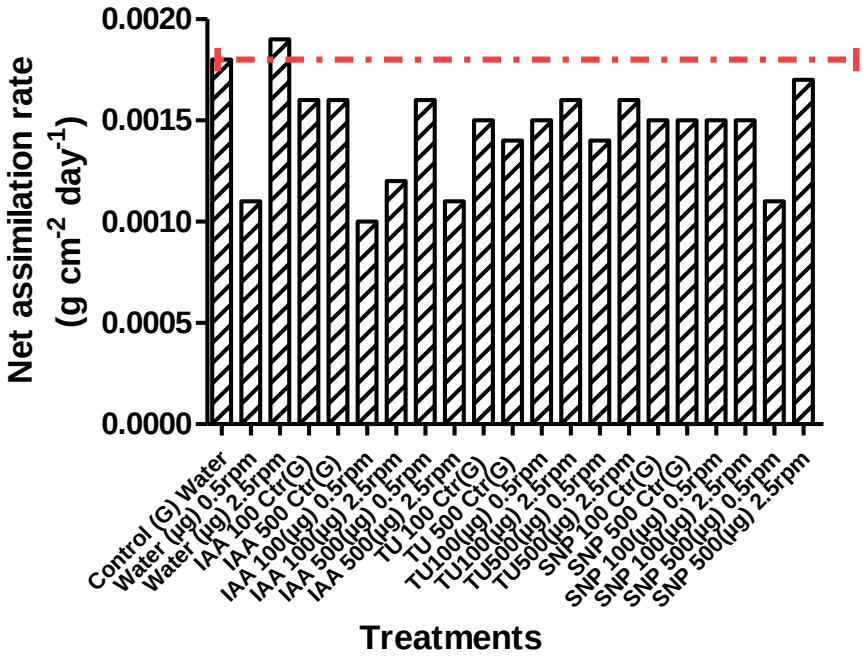

Figure 3. *Net assimilation rate of maize plant calculated after harvest of corn ears at 12 weeks after exposure to treatment.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant difference.

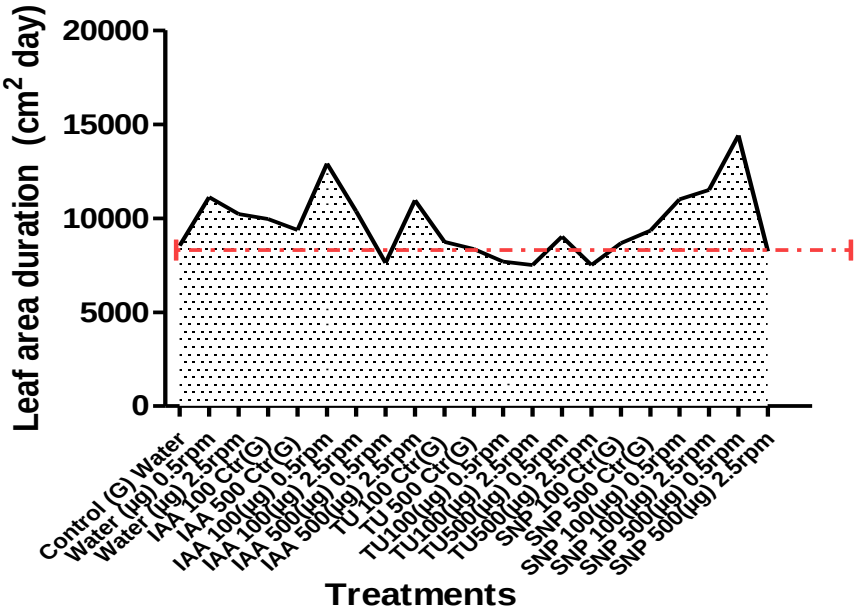

Figure 4. *Leaf area duration of maize plant calculated after harvest of corn ears at 12 weeks after exposure to treatment.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant diff.

Table 3. *Quantitative growth assessment of maize plant after harvest of corn ears at 12 weeks after exposure to treatment.*

	RGR (g g-1 day-1)	BMD (g day)	AGR (g day-1)
Control (G) Water	0.0451	459.6	0.139
Water (µg) 0.5rpm	0.0348	386.7	0.109
Water (µg) 2.5rpm	0.0515	538.4	0.167
IAA 100 Ctr(G)	0.0510	450.3	0.139
IAA 500 Ctr(G)	0.0466	417.1	0.127
IAA 100(µg) 0.5rpm	0.0372	424.2	0.122
IAA 100(µg) 2.5rpm	0.0331	406.9	0.113
IAA 500(µg) 0.5rpm	0.0456	373.3	0.113
IAA 500(µg) 2.5rpm	0.0378	369.9	0.107
TU 100 Ctr(G)	0.0486	395.4	0.121
TU 500 Ctr(G)	0.0444	353.9	0.107
TU100(µg) 0.5rpm	0.0438	349.2	0.105
TU100(µg) 2.5rpm	0.0467	356.3	0.109
TU500(µg) 0.5rpm	0.0462	389.1	0.118
TU500(µg) 2.5rpm	0.0452	362.0	0.110
SNP 100 Ctr(G)	0.0514	373.7	0.116
SNP 500 Ctr(G)	0.0520	413.6	0.128
SNP 100(µg) 0.5rpm	0.0467	469.7	0.143
SNP 100(µg) 2.5rpm	0.0493	495.7	0.153
SNP 500(µg) 0.5rpm	0.0496	465.0	0.143
SNP 500(µg) 2.5rpm	0.0478	424.2	0.130

Key: Relative Growth Rate (RGR); Biomass duration (BMD); NAR Net assimilation rate; AGR Average growth rate; LAD leaf area duration; Shoot-root ratio (SRR). rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant diff.

Figure 7, shows the root shoot ratio of maize plants calculated after harvest of corned years at 12 weeks after exposure to treatment. Results generally shows that in the control shoot to root ratio was 1.97. however, there was slight increase in the shoot to root ratio in the microgravity exposed seeds. When microgravity exposed seeds were treated with IAA, TU and SNP there were significant increase to shoot to root ratio from 2.16 to 3.68.

The dicksons quality index as presented in Figure 8, is a necessary index that helps to show the capacity of seedlings to present better morphology qualities when transferred to the field. In many cases they are also regarded as an index that underscores the plant general vigor. Whereas the Dickson quality index 001 was 0.40 in the control, in the microgravity exposed seeds there were significant increase from 0.51-0.58. Significant increase was also observed in the microgravity exposed seeds chemo-primed IAA and those chemo-primed with SNP. However, reduced DQI was obtained in all the seeds that were chemo-primed with thiourea TU. Irrespective of the microgravity status or concentration of the thiourea utilized in this experiment.

Figure 6A, 6B and 6C various stages of maize development: Figure 6A maize seedlings been acclimatized after clinorotation; Figure 6B maize plants during sinking; figure 6C maize stands after harvest.

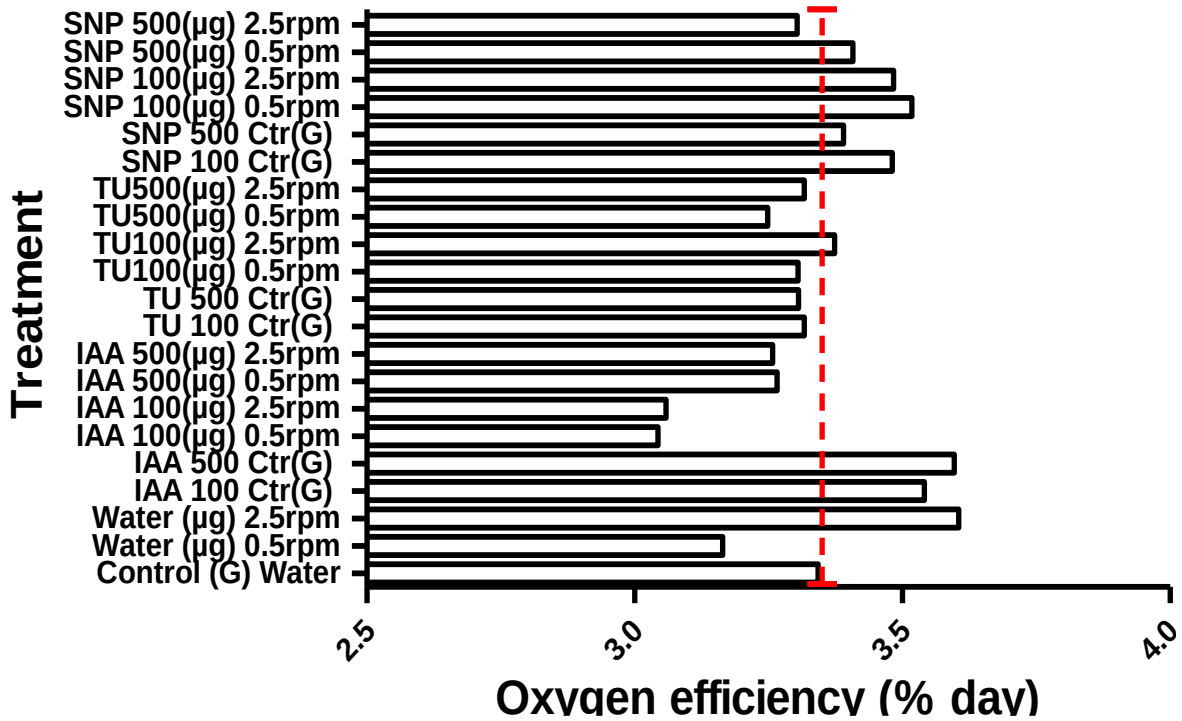

Figure 5. *Oxygen production efficiency.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant diff.

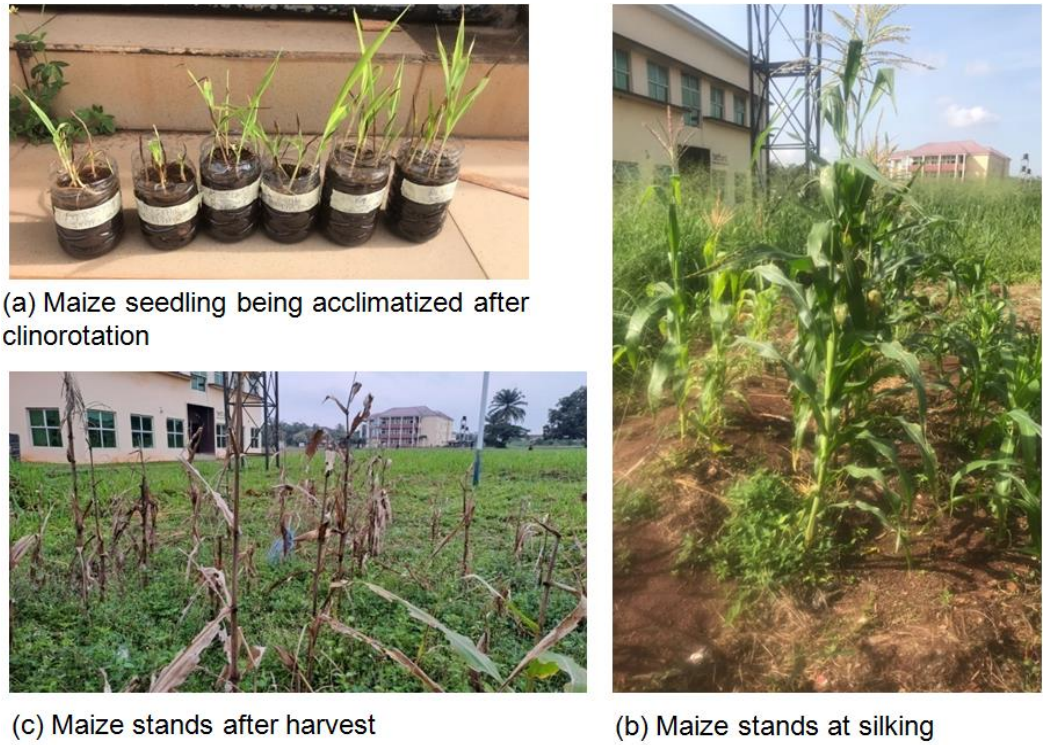

Figure 6. *a, b c.: Maize plants at different stages of growth during the experiment.*

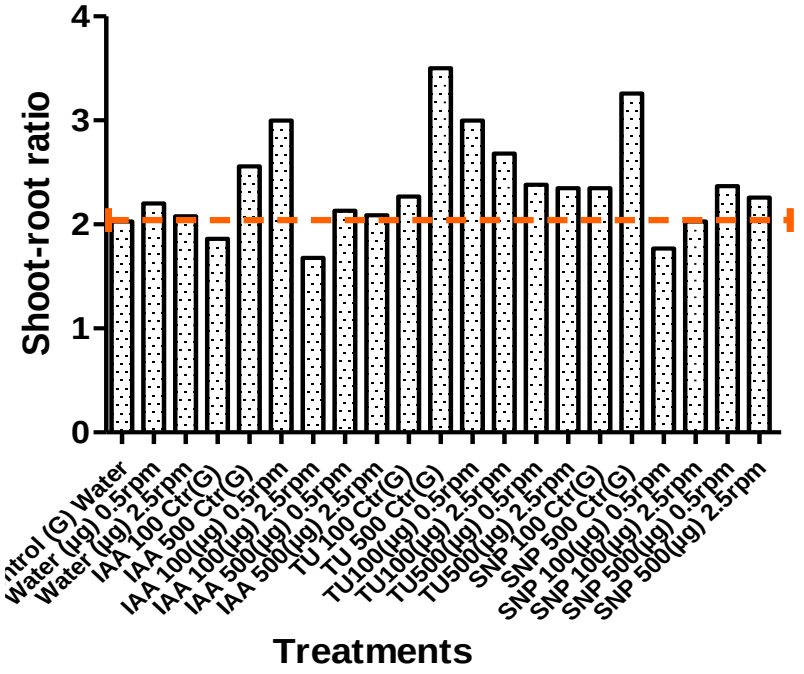

Figure 7. *Shoot-root ratio of maize plant calculated after harvest of corn ears at 12 weeks after exposure to treatment.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant diff.

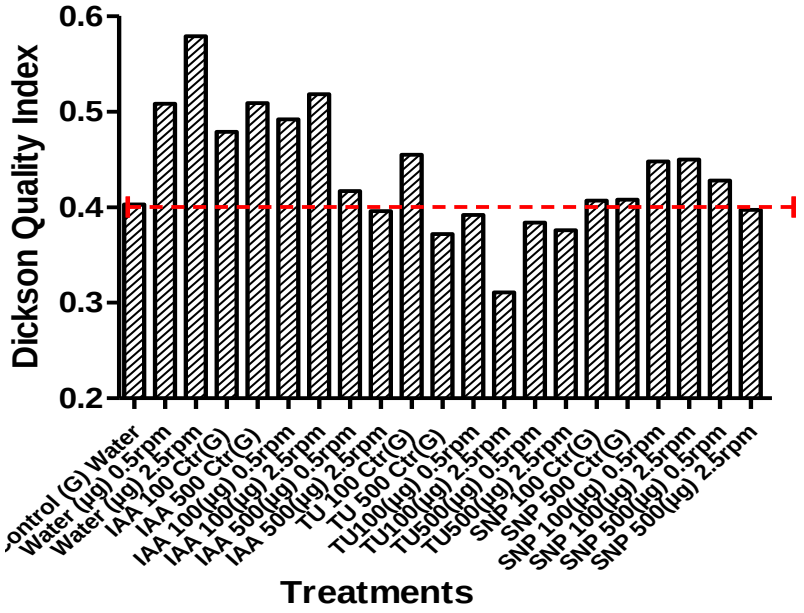

Figure 8. *Dickson Quality Index of maize plant calculated after harvest of corn ears at 12 weeks after exposure to treatment.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole-3- acetic acid; G under normal gravity; LSD Least significant diff.

Table 4 shows each parameter of the maize plants expose to microgravity and chemo-priming treatments. Results generally shows that for ear weight there were no significant differences even after plant has been exposed previously to microgravity. However, for seeds that were exposed to IAA without microgravity, significant increase in ear weight were obtained (107.4 gram per dry weight as compared to 92.5 per dry weight in the control). It was generally observed that ear weight, cub weight, number of seeds per cub, shelling percent as well as seed weight per cub were generally observed in the microgravity exposed seeds that were chemo-primed with either 100micrograms and 500 micrograms.

KEY:

CTRL	Control (G) Water	F	IAA 100(µg) 2.5rpm	L	SNP 500(µg) 2.5rpm	R	TU100(µg) 2.5rpm
A	IAA 100(µg) 0.5rpm	G	IAA 500(µg) 2.5rpm	M	IAA 500(µg) 0.5rpm	S	TU500(µg) 0.5rpm
B	Water (µg) 0.5rpm	H	SNP 100(µg) 2.5rpm	N	IAA 100 Ctr(G)	T	SNP 500 Ctr(G)
C	SNP 100(µg) 0.5rpm	I	SNP 500(µg) 0.5rpm	O	TU100(µg) 0.5rpm		
D	Water (µg) 2.5rpm	J	TU 100 Ctr(G)	P	TU500(µg) 2.5rpm		
E	IAA 500 Ctr(G)	K	TU 500 Ctr(G)	Q	SNP 100 Ctr(G)		

Figure 9. *Maize ears harvested from test plants.*

Table 4. *Yield parameters of maize plant exposed to microgravity.*

	Ear weight (g DW)	Cob weight (g DW)	No. of seeds per cob	Shelling (%)	Seed wt. per cob (g DW)
Control (G) Water	92.5	73.5	104	79.4	59.4
Water (µg) 0.5rpm	77.2	68.6	112	88.9	49.7
Water (µg) 2.5rpm	95.2	74.2	137	77.9	57.7
IAA 100 Ctr(G)	95.3	76.3	189*	80.1	54.9
IAA 500 Ctr(G)	107.4*	78.6	204*	73.2	53.9
IAA 100(µg) 0.5rpm	115.1*	81.6	124	70.9	58.5
IAA 100(µg) 2.5rpm	91.6	77.5	194*	84.6	54.8
IAA 500(µg) 0.5rpm	99.8	89.3*	160*	89.5	42.3*
IAA 500(µg) 2.5rpm	63.5*	46.7*	150*	73.5	38.9*
TU 100 Ctr(G)	89.3	62.3	355*	69.8	42.1
TU 500 Ctr(G)	68.1*	58.9*	192*	86.5	31.8*
TU100(µg) 0.5rpm	83.1	60.3*	112	72.6	38.2*
TU100(µg) 2.5rpm	83.6	77.4	141	92.6	45.8
TU500(µg) 0.5rpm	70.9*	48.5*	102	68.4	28.6*
TU500(µg) 2.5rpm	66.9*	47.4*	124	70.9	25.8*
SNP 100 Ctr(G)	82.9	68.7	95	82.9	43.8
SNP 500 Ctr(G)	61.8*	46.3*	118	74.4	37.5*

	Ear weight (g DW)	Cob weight (g DW)	No. of seeds per cob	Shelling (%)	Seed wt. per cob (g DW)
SNP 100(µg) 0.5rpm	113.8*	90.7	102	79.7	61.4
SNP 100(µg) 2.5rpm	147.3*	123.5*	302*	83.8	98.5*
SNP 500(µg) 0.5rpm	140.7*	118.3*	324*	84.1	85.7*
SNP 500(µg) 2.5rpm	134.6*	105.2*	284*	78.2	86.7*
LSD (0.05)	19.4	13.2	41	15.25	18.4
p-value	<0.001	0.027	0.003	0.263	0.015

*Means on similar column with asterisks different significantly from the control ($p<0.05$); rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant difference.

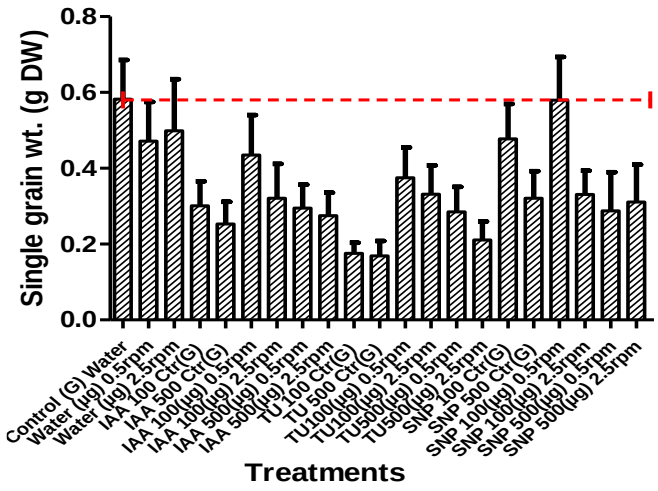

Figure 10. *Single grain weight.*

Key: rpm - revolutions per minute in the 2D clinostat; TU Thiourea, SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant diff.

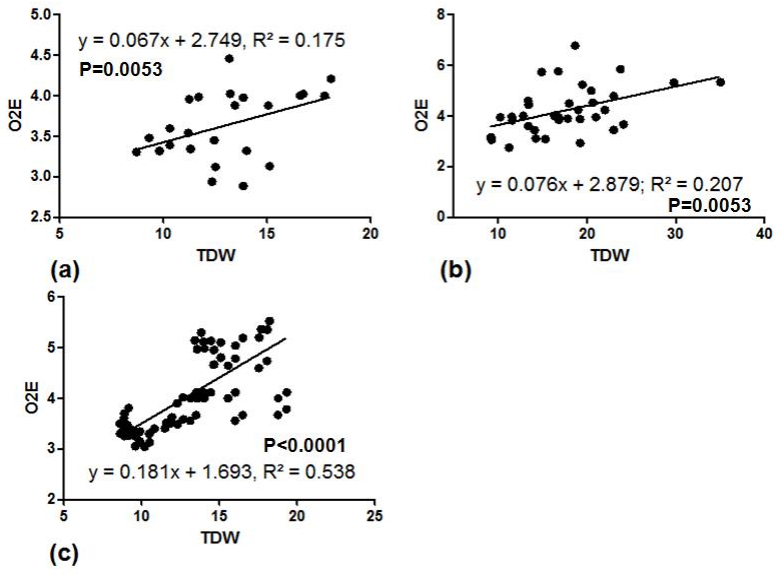

Figure 11. *a, b, c Linear regression showing association between total dry weight of maize plant (TDW) and oxygen production efficiency (O2E) under (a) gravity (b) microgravity without growth stimulation (c) microgravity with growth stimulation.*

5. Discussion

The result of the above research had shown that plant has the capacity to survive during long space exploration and also provide the necessary life sustaining parameters like Oxygen, Food and environmental cleaning by removing carbon dioxide through the process called photosynthesis.

On the survival level of plant as it concerns the microgravity environment, the research had shown that plant can overcome the stress posed by microgravity environment.

One of the key physiological and general body biochemical needs of Astronauts and Cosmonauts as the case maybe is oxygen, this research had shown that plants subjected to microgravity had the tendency to produce oxygen based on the data presented. This goes to show that the photosynthetic ability of plants on board a space craft during long space exploration will not pose a threat to scientists on board, as the plant will have the capacity to provide the needed oxygen [22, 36].

As it has been reported on plants grown onboard a space craft that there was delay in flowering compare to the control grown on earth, same was observed during the research where in the plants under earth gravity flowers earlier than the one grown on microgravity. It was observed in this research that there was at least two weeks difference of the flowering between plant subjected to simulated microgravity with treatment and the control which was under earth gravity [33, 26].

The combination of microgravity simulated environment using clinostat instrument created by [43], has shown high productivity rate as this research provided a proof of crop productivity level when the seeds were harvested after 12 weeks in the field this will help to increase food security on earth [27, 38, 37, 7].

Net Assimilation Rate (NAR) is the Rate of increase in dry matter per unit leaf area. It indicates the assimilatory capacity of plant. Biomass Duration is the Product of biomass and the time period in which biomass is maintained. It indicates the biomass persistence and useful for calculation of maintenance respiration over times Leaf Area Duration (LAD) is the Product of leaf area and the time period which leaf area is maintained. It determines the duration of greenness of crop. Therefore, a mean of these the association among NAR, LAD, BMD estimated the oxygen production efficiency [40, 41, 15].

Generally, the research presented growth suppression due to microgravity exposure, the addition of growth stimulators, particular Sodium Nitroprusside (SNP), enhanced general plant morphological growth, development and antioxidant enzyme response. The study showed that addition of growth stimulators enhanced significant positive association between plant total dry weight and oxygen production efficiency. This study addresses Millennium Development Goal 1: eradicate extreme poverty and hunger. This it does by providing information on the possible adoption of a combination of microgravity and growth stimulation as a measure for improvement of maize, as yield increase was more than 50% through the adoption of this technology. Conclusively, growth chemo-stimulation with IAA and SNP enhanced plant growth, development and high yield of maize exposed to simulated microgravity using 2D-Clinostat [14, 42, 29, 44, 47].

Plants are reliable sources of sustenance and survival because of their ability to produce Oxygen, Water, and Food. This is more important as it provides a more sustainable energy source during space travel. Unfortunately, microgravity which is a common phenomenon in space, hamper plant development. This study investigated the ability for plant growth promoters to enhance survival, yield and development of a Zea mays [6, 8, 32].

After 120hrs, the microgravity-exposed seedlings were acclimatized in experimental plants in a well-ventilated screen house for 5 days and thereafter transplanted on the field, where plant growth and yield responses were observed for 12 weeks.

Although there was growth suppression due to microgravity exposure, the addition of growth stimulators, particular Sodium Nitroprusside (SNP), enhanced general plant morphological growth, development and antioxidant enzyme response [46]. The study showed that addition of growth stimulators enhanced significant positive association between plant total dry weight and oxygen production efficiency. This study addresses Millennium Development Goal 1: eradicate extreme poverty and hunger. This it does by providing information on the possible adoption of a combination of microgravity and growth stimulation as a measure for improvement of maize, as yield increase was more than 50% through the adoption of this technology. Conclusively, growth chemo-stimulation with IAA and SNP enhanced plant growth, development and high yield of maize exposed to simulated microgravity using 2D-Clinostat.

6. Conclusion

This research had shown that maize can survive in microgravity environment and also serve as source of food, oxygen and water during long space exploration and mars colonization. However, the effect of microgravity on maize could have some effects on its growth and development but the use of growth stimulators like Indole-3-acetic acid (IAA), Sodium Nitroprusside (SNP) will significantly reduce the effects of microgravity on maize during long space exploration and food security and environmental sustainability on earth.

7. Recommendations

The Nigeria government and government of other developing countries should dedicate more funds into space re-

search so they will not be left out from alternative home building.

More research should be done on the combination of clinostat and chemo-priming for seeds productivity for food security on earth.

UNOOSA (United Nation office for Outer Space Affairs) should provide more clinostat for researchers to do more studies especially 3D-Clinostat for further research in three dimensional forms.

All should be encouraged to meet up with the condition set by UNOOSA in order to be awarded clinostat.

Awareness should be created on this technology by research and educational institutions.

Abbreviations

BMI	Body Mass Index
UNOOSA	United Nation Office for Outer Space Affairs
USSR	Union of Soviet Socialist Republic
ASEB	Aeronautics and Space Engineering Board

Acknowledgments

Special appreciation to the Space-Earth Environment Research Laboratory, University of Benin, Edo State, Nigeria, for giving the permission to use their facilities. This study was privately funded.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Aeronautics and Space Engineering Board (ASEB). (1997). *Advanced Technology for Human Support in Space*. National Research Council, National Academy Press, Washington, D.C., U.S.A. 34 p.

[2] Ashraf, M. and Foolad, M. R. (2005). Pre-sowing seed treatment - a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy*88:223-271.

[3] Bai, B., Sikron, S., Gendler, T., Kazachkova, Y., Barak, S., Grafi, G., Khozin-Goldberg, I. and Fait, A. (2011). Ecotypic variability in the metabolic response of seeds to diurnal hydration dehydration cycles and its relationship to seed vigor. *Plant and Cell Physiology*53(1):38-52.

[4] Bajehbaj, A. A. (2010). The effects of NaCl priming on salt tolerance in sunflower germination and seedling grown under salinity conditions. *African Journal of Biotechnology*9:1764-1770.

[5] Berkovich, J. A., Krivobok, N. M., Siniak, J. E., Zaitsev, E. R., Monakhov, B. N., Protasov, N. N., Abramov, L. Kh., Samsonov, N. M. and Farafonov, N. S. (2000).*Perspectives of Developing Space Greenhouse for the International Space Station," 6th European Symposium on Space Environmental Control Systems*. Noordwijk, Netherlands. 22p.

[6] Bewley, J. D., Bradford, K., Hilhorst, H. and Nonogaki, H. (2013). Seeds: Physiology of Development, Germination and Dormancy. 3rd Edition. New York: Springer.

[7] Bhuiyan, M. S. H., Malek, M. A. and Hassan, A. B. A. (2019). Mutation determination of rice by using RAPD primers. *International Journal of Agricultural Research* and Innovation *Technology*. 9(1): 1-7.

[8] Campbell, W. F., Salisbury, F. B., Bugbee, B., Klassen, S., Naegle, E., Strickland, D. T., Bingham, G. E., Levinskikh, M., Iljina, G. M., Veselova, T. D., Sytchev, V. N., Podolsky, G., McManus, W. R., Bubenheim, D. L., Stieber, J. and Jahns G. (2001). Comparative floral development of mars-grown and ethylene-treated, earth-grown Super Dwarf wheat. *Journal Plant Physiology*, 158: 1051-1060.

[9] Caprong I, Corbineau, F., Dacher, F., Job, C., Côme, D. and Job, D. (2000). Sugar beet seed priming: effects of priming conditions on germination, solubilization of 11-S globulin and accumulation of LEA proteins. *Seed Science Research*10:243-254.

[10] Cohen, G., Dembiec, C. and Marens, J. (1970) Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34, 30-38.

[11] Daneshvar, A., Tigabu, M. and Karimidoost, A. (2017). Flotation techniques to improve viability of *Juniperus polycarpos* seed lots. *Journal of Forestry Research* 28: 231-239.

[12] Dauzart, A. J. C., Vandenbrink, J. P. and Kiss, J. Z. (2016). The effects of Clinorotation on the host plant, *Medicago truncatula*, and its microbial symbionts. *Frontiers in Astronomy and Space Sciences* 3(3):1-10.

[13] Farooq, M., Basra, S. M. A., Wahid, A., Khaliq, A. and Kobayashi, N. (2009). Rice seed invigoration: a review. In: Lichtfouse E (ed) Organic farming, pest control and remediation of soil pollutants: sustainable agricultural reviews. Springer Science, Amsterdam.

[14] Gordon, A. G. (1971). The germination resistance test - A new test for measuring germination quality ofcereals. *Canadian Journal of Plant Science* 51: 181-183.

[15] Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R. and Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*14: 9643-9684.

[16] Hensel, W. and Sievers, A. (1980). Effects of prolonged omnilateral gravistimulation on the ultrastructure of statocytes and on the graviresponse of roots. *Planta* 150: 338-346.

[17] Ihase, L. O, Horn, R., Anoliefo, G. O., Eke, C. R., Okwuagwu, C. O. and Asemota, O. (2014). Assessment of an oil palm population from Nigeria Institute for Oil palm Research (NIFOR) for simple sequence repeat (SSR) marker application. *African Journal of Biotechnology*, 13 (14):1529 - 1540.

[18] Ikhajiagbe, B., Igiebor, F.A. and Ogwu, C. (2021). Growth and yield performances of rice (*Oryza sativa* var. nerica) after exposure to biosynthesized nanoparticles. *Bulletin of the National Research Centre*45(62): 1-13.

[19] ISTA. (1999). International Rules for Seed Testing. International Seed Testing Association (ISTA), Seed Science and Technology, 287, supplement.

[20] Jules, K., McPherson, K., Hrovat, K., Kelly, E. and Reckart, T. (2004). A status report on the characterization of the microgravity environment of the International Space Station. *Acta Astronautica*55:335-364.

[21] Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceedings of the Royal Society of New South Wales* 138: 65-75.

[22] Kiss, J., Wolverton, C., Wyatt, S., Hasenstein,K.H., Van Loon J. (2019). Comparison of microgravity analogs to spaceflight in studies of plant growth and development. *Frontiers of Plant Science* 3:12- 22.

[23] Kittang Jost, A.-I., T. Hoson, and T.-H. Iversen. (2015). "The Utilization of Plant Facilities on the ISS the composition, growth, and development of plant cell walls under microgravity conditions." *Plants* 5: 44-62.

[24] Klymchuk, D. O., Vorobyova, T. V., Chapman, D. K. and Brown, C. S. (2003). Changes in vacuolation in the root apex cells of soybean seedlings in microgravity. *Advances in Space Research*, 31:2283 - 2288.

[25] Kumari, N and Thakur, S.K (2014). Randomly Amplified Polymorphic DNA - A brief review. *American Journal of Animal and Veterinary Sciences* 9(1): 6 - 13.

[26] Monje, O., G. Stutte, and D. Chapman. (2005). "Microgravity does not alter plant stand gas exchange of wheat at moderate light levels and saturating CO_2 concentration." *Planta* 222 (2): 336-345.

[27] Musgrave, M. E., A. Kuang, L. K. Tuominen, L. H. Levine, and R. C. Morrow. (2005). "Seed storage reserves and glucosinolates in Brassica rapa L. grown on the International Space Station." *Journal of the American Society for Horticultural Science* 130 (6): 848-856.

[28] Nelson, M., Allen, J., Alling, A., Dempster, W. F. and Silverstone, S. (2003). Earth applications of closed ecological systems: relevance to the development of sustainability in our global biosphere. *Advance Space Resources*, 31(7):1649 - 1656.

[29] Nyman, J. and Haszeldine, R. N. (1959). Oxidation of polyhalogeno-compounds. Part III. Thermal oxidation of some chlorofluoroethanes. *Journal of The Chemical Society* 75: 420-422.

[30] Odeh, R., and Guy, C. (2017). Gardening for therapeutic people-plant interactions during long duration space missions. *Open Agriculture*, 2:1-13.

[31] Orukpe, A. O., Anoliefo, G. O. and Ikhajiagbe, B. (2021). Effects of Clinorotation on the Enzyme activities and morphology of *Zea mays* Seedlings. *American Journal of Life Sciences* 9 (1): 11-18.

[32] Özalkan, C., Hasan, T., Sepetolu, H. T., Daur, I. and Sen, O. F. (2010). Relationship between some plant growth parameters and grain yield of Chickpea (*Cicer arietinum* L.) during different growth stages. *Turkish Journal of Field Crops*, 15(1): 79-83.

[33] Penley, N. J., Schafer, C. P. and Bartoe, J. D. F. (2002). The International Space Station as microgravity research platform. *Acta Astronautica*50:691-696.

[34] Porterfield, D. M. (2002). The biophysical limitations in physiological transport and exchange in plants grown in microgravity. *Journal of Plant Growth Regulation*, 21:177 - 190.

[35] Poulet, L., Fontaine, J. P. and Dussap, C. G. (2016). Plant's response to space environment: a comprehensive review including mechanistic modelling for future space gardeners. *Botany Letters*, 163:337 - 347.

[36] Ranal, M. A. and Santana, D. G. (2006). How and why to measure the germination process? *Brazilian Journal of Botany* 29: 1-11.

[37] Raymond, M. W. (2017). *The Role of Plants in Space Exploration*. National Aeronautics and Space Administration Exploration Research and Technology, Kennedy Space Centre, Florida, USA. 24 p.

[38] Sadia, T., Zahida, H. P. and Rabbani, M. A. (2012). Molecular characterization of traditional and improved rice cultivars based on random amplified polymorphic DNAs (RAPDs) markers. *African Journal of Biotechnology*, 11(45):10297 - 10304.

[39] Silje, A. W., Liz H. C., Irene K. and Jost, A. K. (2014). Effect of the extraterrestrial environment on plants: recommendations for future space experiments for the MELiSSA higher plant compartment. *Life Sciences in Space Research*,4:189 - 204.

[40] Stutte, G. W., Newsham, G., Morrow, R. C. and Wheeler, R. M. (2011). *Concept for sustained plant production on ISS using VEGGIE capillary mat rooting system*. 41st International Conference on Environmental Systems, Portland, Oregon. Pp23.

[41] Timson, J. (1965). New Method of Recording Germination Data. *Nature* 207: 216.

[42] Tryggvason, B. V., Duval, W. M. B., Smith, R. W., Rezkallah, K. S., Varma, S., Redden, R. F. and Herring, R. A. (2001). The vibration environment on the International Space Station: its significance to fluid-based experiments. *Acta Astronautica*48: 59-70.

[43] Tuominen, L. K., L. H. Levine, and M. E. Musgrave. (2009). "Plant secondary metabolism in altered gravity." *Methods in Molecular Biology* 547: 373-386.

[44] United Nations Office for Outer Space Affairs (2013). *Teacher's Guide to Plant Experiments in Micro-g. Programme on Space Applications*. UNOOSA, New York. 45p p.

[45] Yang, Y., Rao, Y., Xu, J., Shao, G., Leng, Y., Huang, L., Wang, L., Dai, L., Zhang, G., Hu, J., Zhu, L., Li, C., Gao, Z., Guo, L., Qian, Q. and Zeng, D. (2014). Genetic analysis of sugar-related traits in rice grain. *South African Journal of Botany*, 93: 137 - 141.

[46] Zabel, P., Bamsey, M., Schubert, D. and Tajmar, M. (2016). Review and analysis of over 40 years of space plant growth systems. *Life Sciences in Space Research,* 10: 1-16.

[47] Zhao, T., Deng, X., Xiao, Q., Han, Y., Zhu, S. and Chen, J. (2020). IAA priming improves the germination and seedling growth in cotton (*Gossypium hirsutum* L.) via regulating the endogenous phytohormones and enhancing the sucrose metabolism. *Industrial Crops and Products*155: 112788.