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Effects of Selected Plant Growth Stimulators on Enhancing Germinability and Germination Parameters of *Zea mays* L. under Microgravity Conditions Simulated by a Two-Dimensional Clinostat

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ABSTRACT

The Earth has become increasingly overcrowded as a result of rapid urbanization and population growth, with strong predictions that its carrying capacity could be overstretched soon. As a result, it is important to test the possibilities of growing plants under space exploration conditions, especially gravitational balance. Since microgravity impedes plant development, it is important to evaluate the extents by which plant growth stimulators can reverse or enhance this trend. A total of 12 maize seeds were weighed and placed sideways in petri dishes and inoculated with plant growth stimulators, indole acetic acid (IAA), gibberellic acid (GA), and ascorbate (AA). They were clinorotated at different rates (0.5, 1.0, and 2.0 rpm), while the control seeds were just placed on a balanced table. Results of this research showed that under microgravity, the maize seeds had a decreased level of germination percentage with increasing clinorotation rates at 72 hrs, compared to the control group. But when stimulated with IAA, GA and AA, they improved in germination percentage, compared to the control, even under microgravity conditions. The seedling dry weight, germination time and other germination parameters also showed similar improvements. Comparatively, the three growth stimulators showed no major variations in their ability to improve germination percentage under micro-gravitational impact. However, IAA caused more improvements in seedling vigor, compared to the other growth regulators, while GA had more effects on the rate of germination. This research confirmed the possibilities of improving germinability in maize seeds under space exploration conditions.

Introduction

As the planet becomes increasingly overcrowded because of accelerated urbanization and global population growth, which are coupled with technological, economic developments, recent efforts have been aimed at finding alternative

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places for humans to live (Vandenbrink et al., 2014). The concern is that the Earth's carrying capacity would reach its maximum potential, and the planet will be unable to meet the world's ever-increasing population needs for food, nutrients, oxygen, and other resources. Scientists have started looking for alternative habitats in order to avoid a potential humanitarian catastrophe. This has aided research into efforts that can help humans approach alternative planets to see what possibilities there are in the event of a humanitarian crisis (Braun et al., 2018). However, it is also worth noting that in the field of space science, space exploration can be difficult to accomplish if the resources are not available in sufficient quantities (Raymond, 2017).

According to Soga et al. (2002), humans who go on board any spacecraft will need metabolic energies for their bodies in addition to the energy required to power the spacecraft. Traveling through space could take years, and since humans are heterotrophs, resources such as oxygen for metabolic energy production would be needed in the form of food. Furthermore, these two forms of human sustenance-based criteria for space travel are mostly plant-based. Plants, for example, have long been thought to be the best oxygen generators, considering the use of synthetic methods (Stutte et al., 2002). Plants consume CO_2 which is a by-product of human metabolism, and then give off oxygen, which is needed for human survival. Photosynthesis is also very important, especially in driving the process for generating ATP, which humans require for metabolism (Papaseit et al., 2000). Secondly, the primary producers in food chains are plants (Kering and Zheng, 2015), so much so that plants cannot be taken away from the human mechanism of survival.

During space travel, one of the greatest effects on plants is the influence of gravity (Levine, 2010; Vandenbrink et al., 2014; Braun et al., 2018; Kiss et al., 2019; Orukpe et al., 2021). This ubiquitous force usually influences plant development, productivity, and morphology at all levels, from the molecular level to the whole plant (Vandenbrink et al., 2014). Previous studies have shown that changes in gravitational forces usually affect growth and development in plants. Other physical phenomena governed by gravity include buoyancy, convection, and sedimentation, all of which have an effect on a number of physical and chemical processes, as well as on plant growth and development. Buoyancy, for example, affects gaseous exchange, cellular respiration, and photosynthesis, but it is also a characteristic of densities (Braun et al., 2018).

Previous studies have also shown that prolonged exposure to micro-gravitational forces by astronauts usually impair plant metabolism (Morrow et al, 2005; Ikhajiagbe and Musa, 2020). Furthermore, changes in gravity have reportedly affected protein metabolism and, of course, protein synthesis (Koryum and Chapman, 2017). Since proteins are needed for the regulation of cellular activities and for the control of metabolic processes, anything that affects protein production, hinders or improves such processes, can eventually affect metabolic processes and metabolic regulation (Monje et al., 2000). However, some plant hormones such as indole acetic acid have reportedly improved plant metabolic processes and cell division, thereby enhancing growth properties in plants (Ikhajiagbe and Musa, 2020).

Indole Acetic Acid (IAA), Gibberellic Acid (GA) and Ascorbic Acid (AA) are plant growth stimulators that can manipulate a range of growth and developmental phenomena in a variety of crops (Koryum and Chapman, 2017). Plant height, number of leaves per plant, and fruit size have all been found to increase in response to IAA, resulting in larger seed yields (Kapgate et al., 1989; Lee, 1990). Gibberellic Acid (GA) influences many aspects of plant growth and development, including growth, flowering, and ion transport, while Ascorbic Acid (AA) plays a regulatory role in promoting productivity in many plants (Shah, 2004). Ascorbic acid controls phytohormone-mediated signaling, as well as a variety of physiological processes in plants for stress tolerance. It regulates these processes by acting as a cofactor for a variety of enzymes (Barth and Mario, 2006, Smirnorf and Wheeler, 2000, Farooq et al., 2013). These growth stimulators have also been documented in previous research to help plants survive in stressful situations (Smirnorf and Wheeler, 2000, Farooq et al., 2013). For example, Soga (2002) reported the possibility for growth stimulation of plants under microgravity conditions in space. Furthermore, a previous report by Orukpe et al. (2021) showed the effect of micro-gravitational forces on maize seeds under 0.5 and 1.0 clinorotation. A significant decrease in seed growth capacities and germinability were observed. Accordingly, the current research aims to evaluate the possibilities of growing maize plants under a model gravitational force, occasioned by microgravity and stimulated with phytohormones (IAA, GA and AA).

Materials and Methods

The experiment was conducted at the Space-Earth Environment Research Laboratory,

University of Benin, under the Centre for Atmospheric Research of the National Space Research and Development Agency (NASRDA). The maize seeds were obtained from the New Benin Market, Benin City, Edo State, Nigeria. Agar was prepared, following standard methods, according to Orukpe et al. (2021).

Sowing the maize seeds

The maize seeds were weighed individually using a digital weighing balance, Model No. NBT-A200. The seeds were inserted into the petri dishes containing the cooled agar, and were aligned at the two sides of a clinostat. A total of 12 seeds were sown on each petri dish, based on the carrying capacity of the petri dishes, which were then sealed properly. The petri dish containing the control was left under the influence of gravity on a balanced table, while the seeds meant for micro-gravitational force were placed on the clinostat for 72 hrs under different rotation levels (0.5 rpm, 1.0 rpm and 2.0 rpm).

Exposure of seeds to growth stimulators

The maize seeds were primed in the selected growth stimulators before they were eventually exposed to clinorotation for 72 hrs. The seeds were pre-treated with 150 ppm GA, 150ppm IAA and 150ppm AA, respectively. The seeds were soaked in the above treatment for 90 mins before sowing, according to a relevant method (Musa and Ikhajiagbe, 2021).

Clinorotation

The experiment was prepared in two batches. The first batch was placed in clinorotation at 0.5, 1.0 and 2.0 rpm and was studied for 72 hours, while the second batch was first exposed to growth stimulation in indole acetic acid, gibberellic acid and ascorbate, respectively, before subjecting them to clinorotation at 0.5, 1.0 and 2.0 rpm in the 2-D Clinostat (Dimension clinostat, UN, New York, USA) (Fig. 1). In both batches, the control seeds were placed on the laboratory bench for 72 hours.

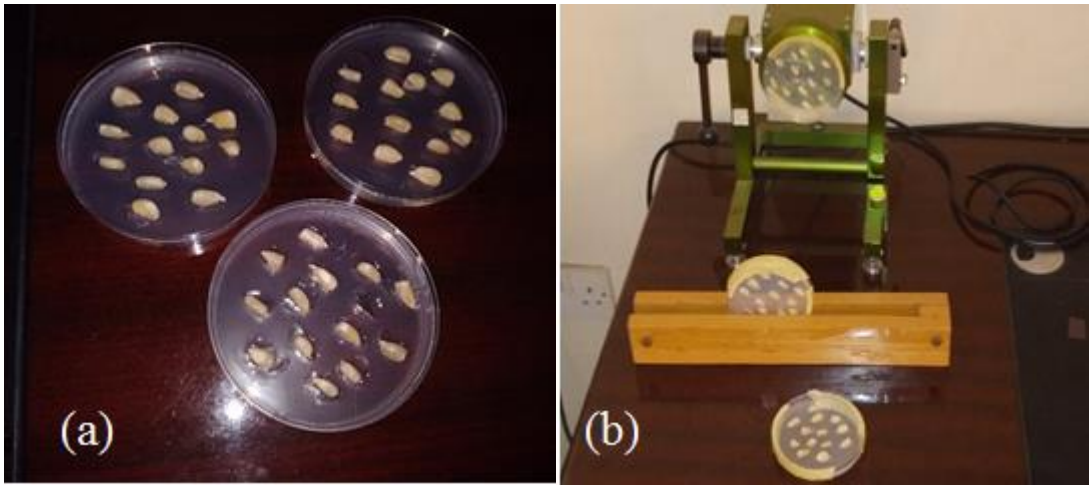

Fig. 1. (a) The arrangement of seeds in petri dishes before (b) placement into the clinostat

Germination properties

Several plant germination parameters were measured, including the amount of time to the first appearance of germination, root length, shoot length, number of prominent roots, fresh weights of seedling, and dry weight of seedling. The measurements were conducted a day after the termination of the experiment. Their respective dry weights were also measured after sun-drying for 72 hrs. All seed germination parameters were calculated according to a relevant protocol (AOSA, 1983).

Statistical analysis

Assuming the existence of homogeneity in the experimental set-up, a completely randomized design was adopted. Mean values of 3 replicates were presented and separated by Duncan's Multiple Range Test. Germination indices were computed according to previous methods by Edwards (1932), Al-Mudaris (1998), Farooq et al. (2005), Ranal and Santana (2006), ISTA (2015), Aravind et al. (2020). These included assessments of final germination percentage, median germination time, and seedling vigor.

Results

Micro-gravitational impact before exposure to plant growth stimulants

The effects of gravity on the final germination percentage of *Zea mays* and seedling dry weight (g) before exposure to plant growth stimulators is

depicted in Figure 2. The final germination percentage at 72 hrs was 60% at 2.0 rpm in the clinostat, 68% at 1.0 rpm and 80% at 0.5 rpm, compared to 94% in the control. A similar result was observed in the seedling dry weight. These results showed a significant decrease in germination percentage and seedling dry weight, parallel to an increase in the clinostat rate.

The amount of time required by seeds to germinate, after their exposure to microgravity, was recorded on a gradual basis (Fig. 3). The results showed that when seeds were exposed to microgravity, germination was significantly delayed in all clinostat rates (0.5 rpm = 45 hrs, 1.0 rpm = 54 hrs and 2.0 rpm = 55 hrs). However, in the control petri dishes where seeds were not subjected to micro-gravitational influence, it took only 40 hrs for the seeds to germinate. This showed that germination was delayed by 15 hrs under the micro-gravitational impact at 2.0 rpm.

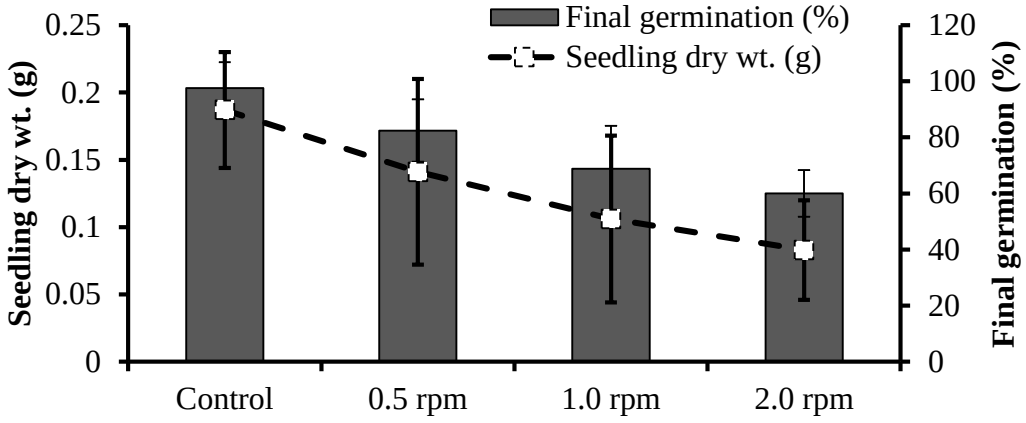

Fig. 2. Effects of microgravity on the final germination percentage and seedling dry weight of *Zea mays* at 72 hrs after the initiation of germination

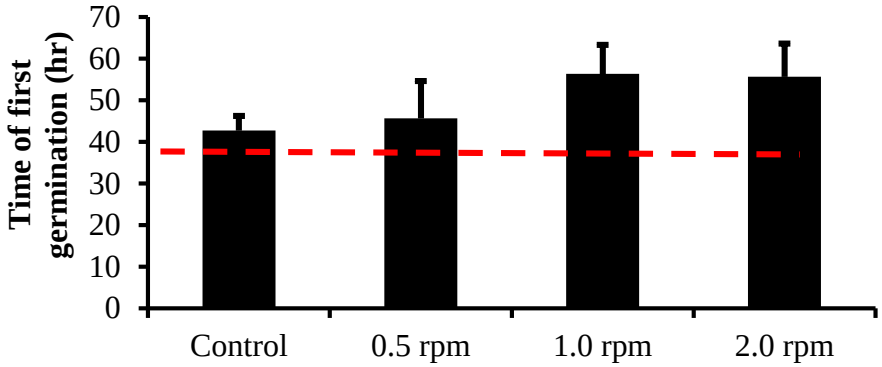

Fig. 3. The amount of time required by seeds to show the first instance of germination, following exposure of maize seeds to micro-gravitational forces

Micro-gravitational impact after exposure to plant growth stimulants

GA was added to the maize seeds before exposure to microgravity to test its effectiveness in improving maize seed germinability and germination parameters (Table 1). At first, the GA improved germination parameters in the presence of gravity, compared to the control (H_2O). The germination percentage of GA-induced microgravity-exposed seeds at 72 hrs showed significant variations compared to the control (92.98-97.45%, $p<0.05$). Even under microgravity, the GA-induced seeds germinated earlier than those of the control (H_2O). However, no significant change was observed in the germination time with microgravity exposure at different intensities. Median germination time (T50) was observed to be shorter (24.05 hrs) in the case of GA-induced seeds under gravity, while an increase in T50 was observed with higher rates of clinostat. Daily germination speed improved with the application of GA, although it led to no significant decrease in the speed (in response to microgravity-induction). Generally, the results showed stronger effects because of GA induction, followed by a slow decrease in germination,

parallel to an increase in clinostat levels.

When maize seeds were stimulated by IAA (Table 2), it improved the germination percentage even under microgravity. The first germination appeared at 35.97 hrs in IAA-stimulated seeds under 0.5 rpm clinostat, whereas the least germination percentage was observed in the control (H_2O) (Fig. 4). The peak germination period was 45.96-53.95 hrs ($p \leq 0.05$) according to Table 2. The germination rate index, which is also known as the germination speed, ranged from 41.06 to 66.54 hrs ($p \leq 0.05$). Microgravity-exposed seeds, which were previously exposed to indole acetic acid, had a significant increase in the fresh weight of seedlings (0.69 - 0.81g), compared to 0.5g of the control. However, when the seeds were exposed to indole acetic acid (0.15g-0.41g), there were no major variations in the dry weight in relation to the degree of simulated microgravity ($p \leq 0.05$).

Table 1. Effects of gibberellic acid on the germinability and germination parameters of *Zea mays* after exposure to microgravity

Parameters	Control (H_2O)	Control (GA)	Simulated microgravity			p-value
			0.5 rpm	1.0 rpm	2.0 rpm	
Germination Percent (%) at 72hrs	92.98[a]	97.45[b]	96.90[b]	96.23[b]	95.52[b]	0.783
Germination Time (hrs)	58.34[a]	49.92[b]	50.95[b]	52.92[b]	56.94[b]	***0.038***
Median Germination Time (T50) (hrs)	32.94[a]	24.05[b]	27.95[a]	27.94[b]	29.96[a]	***0.042***
Final Germ. Percent (%)	98.98[a]	99.02[a]	98.90[a]	98.23[a]	97.52[b]	0.623
Mean Daily Germination	33.27	53.27	51.27	50.27	48.27	NA
Germination Index	2.8[a]	3.6[b]	3.4[b]	2.8[a]	2.8[a]	0.264
Time for the First Germination (T0)	64.96[a]	44.07[b]	47.96[b]	47.96[b]	49.97[a]	0.413
Time for the last Germination (Tg)	97.92[a]	76.11[b]	79.95[b]	83.92[b]	87.94[b]	0.004
Time Spread of Germination (Tg-To)	36.96[a]	51.03[b]	49.99[b]	46.96[b]	41.98[b]	0.041
Peak Period of Germination	56.95[a]	73.01[a]	69.95[b]	63.95[b]	62.96[b]	0.092
Germination Rate Index or Speed (GRI)	48.06[a]	66.6[a]	63.25[b]	61.06[b]	63.24[b]	0.293
Rate of Germination (hrs/ seed)	5.29[a]	7.04[b]	6.72[b]	6.29[b]	6.96[b]	***0.022***
Seedling Vigor	819.21[a]	970.21[b]	949.18[b]	919.21[b]	929.58[b]	***0.007***
Daily Germination Speed	0.03	0.05	0.05	0.04	0.04	NA
Length of Roots(cm)	5.1[a]	7.7[b]	7.5[b]	7.1[b]	7.0[b]	***<0.001***
Length of Stem(cm)	3.1[b]	5.5[a]	4[b]	4.1[ab]	4.3[b]	0.053
*No of Roots	6[ab]	8[b]	8[b]	8[b]	8[b]	0.772
Wet Seedling Weight(g)	0.53[b]	0.79[a]	0.75[a]	0.73[a]	0.62[a]	0.127
Dry Seedling Weight(g)	0.15[b]	0.32[a]	0.26[a]	0.25[a]	0.20[a]	0.089
Original Seed Dry Weight(g)	0.31[b]	0.37[a]	0.36[a]	0.38[a]	0.37[a]	0.152

*Mean values are rounded off to the nearest integer. Results with similar letters are not significantly different ($p \leq 0.05$)

As previously mentioned, and similar to the case of GA and IAA, the effects of AS were apparent on the germination percentage in microgravity-exposed seeds (Table 3). The final germination percentage ranged from 94.16 to 98.98 percent ($p \leq 0.05$). While the results in Figure 1 showed that exposing seeds to microgravity up to 2.0 rpm significantly delayed germination, the results in Table 3 showed that soaking the seeds in ascorbic acid and then exposing them to microgravity up to 2.0 rpm did not significantly delay germination, compared to the control. In this case, the time taken for the first signs of germination to appear ranged between 31.97-42.98 hrs ($p \leq 0.05$). The average time required for germination was 36.96 hrs in the control, 18.77 hrs when the seeds were first exposed to vitamin C (but not to microgravity), and 11.99-19.98 hrs when the seeds were first soaked in ascorbic acid and were then exposed to simulated microgravity. There were no major variations in seedling dry weight, regardless of the degree of simulated microgravity, as the seedling dry weight ranged from 0.11g (in response to 2.0 rpm) to 0.25g (in response to 1.0 rpm) ($p \leq 0.05$) (Tables 1 and 2). Asymptotic values of significance resulted from comparing the effects of these growth stimulators on the germination of maize seeds (Table 4). By comparing the capacity of the three growth stimulators to ameliorate the influence of microgravity on seed germination, no major variations were found in the ability of the three growth stimulators to influence the germination percentage or the time taken for the first and last occurrence of germination under the micro-gravitational impact. According to the results, the

absence of variation was caused by clinorotation at 0.5 rpm. However, indole acetic acid improved seedling vigor more than the effects of GA and AA under the influence of microgravity at 0.5 rpm.

Table 2. Effects of indole acetic acid on the germinability and germination percentage of *Zea mays* after exposure to microgravity

Parameters	Control (H_2O)	Control (IAA)	Simulated microgravity			p-value
			0.5 rpm	1.0 rpm	2.0 rpm	
Germination Percent (%) at 72hrs	92.98^{a}	98.96^{a}	98.84^{a}	98.56^{a}	98.51^{a}	0.382
Germination Time (hrs)	79.92^{a}	60.84^{ab}	51.95^{b}	61.94^{ab}	61.94^{ab}	0.053
Median Germination Time (T50) (hrs)	62.94^{a}	53.12^{ab}	43.96^{b}	53.95^{ab}	51.95^{ab}	0.084
Final Germ. Percent (%)	98.98^{a}	95.96^{a}	99.24^{a}	95.23^{a}	98.21^{a}	0.382
Mean Daily Germination	33.27	33.27	33.27	33.27	33.27	NA
Germination Index	2.8^{a}	3.7^{a}	3.9^{a}	3.7^{a}	3.7^{a}	0.127
Time for the First Germination (T0)	42.96^{a}	40.09^{a}	35.97^{a}	39.96^{a}	41.96^{a}	0.521
Time for the Last Germination (Tg)	79.92^{a}	50.78^{b}	51.95^{b}	61.94^{ab}	61.94^{ab}	0.047
Time Spread of Germination (Tg-To)	36.96^{a}	21.11^{b}	13.98^{b}	21.98^{ab}	19.98^{b}	***0.023***
Peak Period of Germination	53.95^{a}	48.08^{a}	45.96^{a}	49.95^{a}	49.95^{a}	0.068
Germination Rate Index or Speed (GRI)	41.06^{a}	53.3^{a}	66.54^{a}	53.25^{a}	53.25^{a}	0.204
Rate of Germination (hrs/ seed)	5.29^{a}	4.26^{ab}	3.42^{b}	4.14^{ab}	4.11^{ab}	***0.003***
Seedling Vigor	819.21^{b}	970.09^{a}	1228.8^{a}	789.24^{b}	909.12^{a}	***<0.001***
Daily Germination Speed	0.03	0.03	0.03	0.03	0.03	NA
Length of Roots(cm)	5.14^{b}	5.61^{b}	9.49^{a}	5.69^{b}	5.59^{b}	***0.015***
Length of Stem(cm)	3.1^{a}	3.6^{a}	2.8^{a}	2.2^{a}	3.5^{a}	0.083
*No of Roots	6^{a}	6^{a}	6^{a}	4^{b}	6^{a}	***0.035***
Wet Seedling Weight(g)	0.53^{b}	0.83^{a}	0.81^{a}	0.69^{b}	0.73^{ab}	***0.021***
Dry Seedling Weight(g)	0.15^{a}	0.41^{a}	0.31^{a}	0.17^{a}	0.21^{a}	0.053
Original Seed Dry Weight(g)	0.38^{a}	0.31^{a}	0.28^{a}	0.32^{a}	0.31^{a}	0.127

* Mean values are rounded off to the nearest integer. Results with similar letters are not significantly different ($p \leq 0.05$)

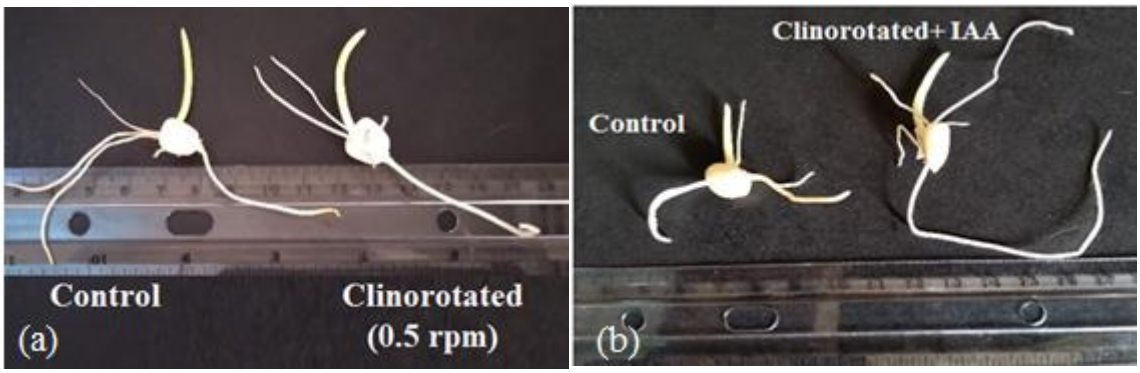

Fig. 2. (a) Clinorotated seedling and (b) IAA-primed clinorotated seedling, compared with the control at 50 hrs after the initiation of germination

Similarly, by the micro-gravitational impact at 0.5 rpm, indole acetic acid had a stronger influence on root length in germinated maize seeds. Compared to IAA or AA, GA most likely influenced germination time at 1.0 rpm. Also, GA was more likely to affect the peak time and rate of germination, compared to the effects of the other two growth stimulants. Table 4 showed that indole acetic acid and ascorbic acid improved seedling vigor more than the effect of GA when microgravity reached 2.0 rpm in the clinostat. Compared with the control seeds under normal gravity, Table 5 indicates the percentage of differences in the amount of time required for the first signs of germination to appear and, also, reveals the final seedling dry weight at 72 hrs. The results showed that under simulated gravity, there was a substantial decrease in the amount of time (44.9%) required for the first germination, due to the influence of GA, IAA, and AA, implying that the growth stimulators had an accelerated influence on germination time. The results showed that the seedling dry weight increased significantly when microgravity-exposed seeds

were treated with GA, IAA, and AA for 72 hrs (Table 5). The seedling dry weight increased by 198.1% under the influence of GA at 1.0 rpm, and by 201.2% under the influence of IAA at 2.0 rpm (Table 5). The implication is that exposing germinating seeds to microgravity could better boost their growth, if they were primed earlier with GA, IAA, and AA.

Table 3. Effects ascorbic acid on the germinability and germination parameters of *Zea mays* after exposure to microgravity

Parameters	Control (H_2O)	Control (Vit. C)	Simulated microgravity			p-value
			0.5 rpm	1.0 rpm	2.0 rpm	
Germination Percent (%) at 72hrs	98.98^{a}	94.16^{a}	96.87^{a}	98.32^{a}	97.46^{a}	0.468
Germination Time (hrs)	79.92^{a}	63.42^{ab}	49.95^{b}	51.95^{b}	54.95^{b}	***<0.001***
Median Germination Time (T50) (hrs)	62.94^{a}	51.25^{ab}	43.96^{b}	39.96^{b}	41.96^{b}	***0.005***
Final Germ. Percent (%)	98.98^{a}	94.16^{a}	96.87^{a}	98.32^{a}	97.46^{a}	0.468
Mean Daily Germination	33.27	33.27	33.27	33.27	33.27	NA
Germination Index	2.8^{a}	3.7^{a}	3.8^{a}	3.9^{a}	3.9^{a}	0.137
Time for the First Germination (T0)	42.96^{a}	42.98^{a}	37.96^{a}	31.97^{a}	35.97^{a}	0.329
Time for the last Germination (Tg)	79.92^{a}	63.22^{ab}	49.95^{b}	51.95^{b}	54.95^{b}	***<0.001***
Time Spread of Germination (Tg-To)	36.96^{a}	18.77^{ab}	11.99^{b}	19.98^{ab}	18.98^{b}	0.058
Peak Period of Germination	53.95^{a}	50.92^{a}	37.96^{b}	34.97^{b}	37.96^{b}	0.038
Germination Rate Index or Speed (GRI)	41.06^{b}	43.3^{b}	63.24^{ab}	73.23^{a}	73.23^{a}	***0.004***
Rate of Germination (hrs/ seed)	5.29^{a}	4.01^{ab}	3.33^{b}	3.52^{b}	3.78^{b}	***<0.001***
Seedling Vigor	819.21^{a}	790.02^{ab}	999.03^{a}	539.48^{b}	999.03^{a}	***0.027***
Daily Germination Speed	0.03	0.03	0.03	0.03	0.03	NA
Length of Roots(cm)	5.12^{b}	4.81^{bc}	8.99^{a}	3.53^{c}	7.19^{a}	***<0.001***
Length of Stem(cm)	3.1^{a}	3.1^{a}	1.1^{b}	1.9^{b}	2.8^{ab}	***0.033***
*No of Roots	6^{ab}	5^{b}	5^{b}	5^{b}	8^{a}	***0.014***
Wet Seedling Weight(g)	0.53^{a}	0.64^{a}	0.71^{a}	0.78^{a}	0.76^{a}	0.283
Dry Seedling Weight(g)	0.15^{a}	0.12^{a}	0.22^{a}	0.25^{a}	0.11^{a}	0.138
Original Seed Dry Weight(g)	0.38^{a}	0.29^{a}	0.32^{a}	0.29^{a}	0.32^{a}	0.562

* Mean values are rounded off to the nearest integer. Results with similar letters are not significantly different ($p \leq 0.05$)

Discussion

Before maize seeds were stimulated with plant growth promoters, a reduced level of germination was observed when the seeds were exposed to microgravity. This indicated perturbations of many biological phenomena due to the effect of altered gravity (De Micco et al., 2013). This is consistent with previous research by Orukpe et al. (2021) about the role of microgravity as a major impediment to maize seed germination.

In order to ameliorate this effect, researchers discovered that the exposure of seeds to growth stimulants or seed pre-treatments improved seed germination rate, seedling vigor, and growth statistics, thereby reducing the effect of microgravity on maize seed germinability ($p \leq 0.05$). Despite the fact that the responses differed with different growth stimulators, the three growth stimulators in the current research (i.e. GA, IAA, and AA) had different effects on maize seed germinability. GA improved germination by extending the peak time and speed of germination, while IAA improved root length and seedling vigor. Ascorbic acid yielded the most notable result by improving seedling vigor. In the clinostat, IAA and AA increased seedling vigor more than GA did, when the microgravity reached 2.0 rpm.

The three distinct phases of seed germination are imbibition, lag phase, radicle emergence, and seedling growth (Bradford, 1995). Zabel et al. (2016) demonstrated that the first seeds which germinate have a higher chance of survival. Seed pre-treatment significantly increased the final germination percentage, median germination period, peak germination length, germination time, and seedling vigor, even under the influence of simulated microgravity. Accordingly, the results of the current research are consistent with previous findings by Musa and Ikhajiagbe (2021) and Mshelbula et al. (2015). Priming the seeds with growth regulators boosted seed and seedling vigor through metabolic and biochemical processes that occur during regulated hydration, followed by dehydration (Becerra-Vázquez et al., 2020).

The current study showed that the growth regulators had no significant effects on the number of roots, thereby confirming a previous work by Hoson et al. (1992) about the effects of

clinorotation on cress (*Lepidium sativum*), pea (*Pisum sativum*) and azuki beans (*Vigna angularis*). The increase in seed germination, as a result of seed priming in the current research, was consistent with earlier findings by Murungu et al. (2004) and Basra et al (2006). GA had a greater effect on the germination rate in response to all concentrations as compared to other priming agents. This was likely because GA contributed to the regulation of many plant growth processes and developmental stages (Hedden and Phillips 2000; Sevik and Guney (2013); Zhang et al (2007). GA-treated seeds rapidly synthesized various amino acids and amides (Gupta and Mukherjee, 1982), thereby justifying the fast occurrence of germination.

Table 4. Asymptotic values of significance arising from a comparison of the effects of gibberellic acid (GA), indole acetic acid (IAA) and ascorbic acid on the germination parameters of *Zea mays*, as influenced by each level of simulated microgravity

Parameters	Microgravity at 0.5 rpm		Microgravity at 1.0 rpm		Microgravity at 2.0 rpm	
	p-value	Remark	p-value	Remark	p-value	Remark
Germination Percent (%) at 72hrs	0.705	NS	0.111	NS	0.461	NS
Germination Time (hrs)	0.744	NS	0.028	* (GA)	0.383	NS
Median Germination Time (T50) (hrs)	0.068	NS	0.074	NS	0.089	NS
Final Germ. Percent (%)	0.561	NS	0.31	NS	0.137	NS
Germination Index	0.238	NS	0.084	NS	0.11	NS
Time for the first Germination (T0)	0.372	NS	0.188	NS	0.288	NS
Time for the last Germination (Tg)	0.004	NS	0.471	NS	0.09	NS
Time Spread of Germination (Tg-To)	0.037	NS	0.1	NS	0.051	NS
Peak Period of Germination	0.083	NS	0.03	** (GA)	0.056	NS
Germination Rate Index or Speed (GRI)	0.264	NS	0.189	NS	0.07	NS
Rate of Germination (hrs/ seed)	0.02	NS	0.019	* (GA)	0.112	NS
Seedling Vigor	0.006	* (IAA)	0.192	NS	0.011	* (IAA, V)
Length of Roots(cm)	0.023	* (IAA)	0.088	NS	0.061	NS
Length of Stem(cm)	0.048	NS	0.069	NS	0.089	NS
Number of Roots	0.695	NS	0.119	NS	0.07	NS
Wet Seedling Weight(g)	0.114	NS	0.112	NS	0.197	NS
Dry Seedling Weight(g)	0.08	NS	0.075	NS	0.1	NS
Original Seed Dry Weight(g)	0.137	NS	0.091	NS	0.117	NS

NS not significant; * significant (*p<0.05, **p<0.01); growth stimulator in parenthesis is the one that most significantly enhanced germinability

Table 5. Changes in the percentage of the required amount of time for the first occurrence of germination and final seedling dry weight at 72 hrs, compared to the control seeds under gravity

Plant growth stimulator	Simulated microgravity		
	0.5 rpm	1.0 rpm	2.0 rpm
Δ% Time to first germination			
150 ppm GA	-1.3	-25.4	-37.1
150 ppm IAA	-23.2	-30.7	-26.3
150 ppm Vit. C	-16.7	-44.9	-37.1
Δ% Seedling dry wt.			
150 ppm GA	128.1	198.1	165.1
150 ppm IAA	31.6	63.5	201.2
150 ppm Vit. C	-12.3	101.9	32.5

Δ% percentage change. Values are percentage of changes in the required time for the first occurrence of germination and final seedling dry weight at 72 hrs, compared to the control seeds under gravity. Whereas negative values indicate a decrease in the percentage, positive values indicate an increase in the measured parameter, compared to the control seeds under gravity

Heavier dry weights of seeds in the control, compared to the seeds of the microgravity treatment, may have resulted because the microgravity perturbation disrupted the water

use efficiency of the seeds even under the influence of growth stimulators. Since the clinostat motion improved seed drying, which affected seed weight (Bradford, 2006), the microgravity condition acted as a stress factor on the plants. In general, microgravity has the potential to disrupt plant growth processes, but the effects of this stress can be minimized by the application of growth stimulators (Cowles et al., 2008), thereby actualizing the possibilities of seed germination even during space exploration (Ikhajiagbe et al., 2007).

Conclusion

Since the findings of this research showed the potential of using plant growth stimulators (i.e. IAA, GA and AA) to improve maize germination even under the influence of microgravity, the novelty of this research gave positive hopes of administering the germination of maize seeds under gravitational forces. Therefore, this can partly assist researchers in further understanding the extent to which the academic sector can prepare for future space explorations. Further research is encouraged to explore the effects of other plant growth stimulators and to test their effectiveness on other important cereals.

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Conflict of Interest

The authors declare no competing interests.

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