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Changes in Macro Elements Content in Plant Tissues Subjected to Clinostat

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Abstract

Plants regulate metabolism under the effect of gravity, which affects growth direction, morphogenesis and biochemistry by changing the metabolic pathways. In present study, the effect of microgravity stimulation on biogenic elements and growth profiles of rice, wheat, barley, and pea during early growth stages was investigated. Clinostat was used for gravity stimulation, which was applied at embryonic stage. Seeds were grown under laboratory conditions at 24 ± 2°C and 3 days-old seedlings were exposed to the microgravity generated by clinostat rotation at 40 rpm for 96 h and 90 rpm for 70 h. The biogenic elements and growth parameters were measured. The seedling Fresh weight (Fwt) and Dry weight (Dwt) recorded to be higher under gravity stimulation at 40 rpm for 96 h clinostat rotation. The micro- and macro-nutrient were also enhanced under same set up. The seedlings exposed at clinostat rotation for 70 h at 90 rpm did not affect both minerals and growth parameters, which revealed that microgravity has variable and stimulatory effect on plant characteristics under specific conditions. Results revealed that gravity stimulation applied through clinostat rotation at low rotation has positive effect on biogenic elements as well as growth parameters in plant, which could be used practically to enhance growth.

Keywords: Microgravity; Clinostat; Micro-element; Macro-element; Plant growth

Introduction

Microgravity affects the biochemistry of cellular organelles and the formation of cell cultures in real and simulated microgravity (r- and s- μg) attracted the attention of scientific community since microgravity has significant effect on plant characteristics. In r-and s-μg various cell types were found to form 3D structures. Microgravity generated through clinostat are in use in different forms i.e., one axis and two axis or can be applied horizontally or vertically [1]. Clinostats provide opportunities to study the effects of simulated microgravity under controlled-environment using controls. Random Positioning Machines (RPM), also called 3D clinostats is recognized well to offer a good simulation of microgravity for higher plants [2].

The plant growth and morphogenesis is controlled by internal mechanism, however, the internal mechanism may change under the effect external factors i.e., light, gravity, temperature, and water and resultantly, change in growth patterns since growth regulating parameters are changed. Gravity is one of the major external factors, which has significant effect plant growth [3]. Researchers evaluated the plant growth under free fall or parabolic flight condition and gravity obtained by these methods was insignificant since the microgravity was too low, which did not affected the plant growth and morphogenesis considerably. Therefore, for accelerated growth and changes during morphogenesis high microgravity is required. The clinostat has been used for microgravity generation and effect on plant growth characteristics was found to be significant. A horizontal clinostat is convenient since compensates for the unilateral influence of gravity [4]. To date, three-dimensional clinostat are in practice due to side effects of horizontal clinostat like flow of air or solution in a constant direction and the chronic stimulation of the lateral sides of the materials equipped with two rotation axis placed at right angles [5]. Using three-dimensional clinostat, plant materials can be rotated in three dimensions. Various reports are documented regarding effect of clinostat on plant characteristics and it has been reported that effect on growth and other parameters was insignificant under short term rotation; however, long term rotation furnished positive response on plant growth and growth regulating parameters [3,6-10].

In view of positive effect of clinostat rotation on plant growth and growth regulating mechanisms, it was hypothesized that microgravity generated by clinostat rotation can alter the growth and biogenic elements. Therefore, the principle objective of present study was to appraise the effect of microgravity (generated by clinostat rotation) on rice, wheat, barley germination and biogenic elements. The seedlings were rotated using clinostat under two different condition and effects were measured based on germination, growth as well as micro and macro nutrients in rice, wheat and barley plants.

Materials and Methods

Germination of local plant (Rice, Wheat, Barley and Pea)

The authentic seeds of rice, wheat, barley and pea were purchased from local market, Baghdad, Iraq. Before exposure to clinostat rotation, seeds were sterilized with 1.75% NaOCl for 10 min. A 10-15 seeds were soaked in 2 mL of the sterilization solution in a 2 mL Eppendorf tube with intermittent shaking.

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After 10 min, seeds were rinsed twice with 95% ethanol, and then seeds were spread on the surface of the tube under a laminar flow hood until complete evaporation of ethanol. After drying, seeds were placed in Petri dish containing 10 mL agar medium (15 mg agar/L) and the culture media was maintained at 24 ± 2°C in growth chamber at dark condition for 3 days. Later, the petri plates contains germinating seeds were divided into sets and subjected to clinostat rotation for microgravity exposure. A half seed of each cultivar was exposed to clinostat rotation 40 rpm for 96 h and other half to clinostat rotation of 90 rpm for 70 h at 90°, as shown in Table 1. The speed of clinostat rotation is important parameter in generating the microgravity. Therefore, slow-rotating clinostat 40 rpm and fast-rotating 90 rpm were used.

Response measurements

The effect of clinostat rotation was measured on the basis of fresh and dry weights assimilation as well as minerals i.e., K, Ca, Mg, Na, Zn, Mn, Cu, and Fe. For mineral analysis, a 1 g seedling from each replicates was dried in an oven for 24 h at 105°C (until a constant weight), dried samples were grinded and mineral contents like K, Ca, Na, Mg, Mn, Zn, Cu and Fe were determined by the standard method (aqua regain digestion method) [11] using atomic absorption spectroscopy (A Analyst 30, Perkin Elmer). For fresh and dry weights measurement, three seedlings of each cultivar were selected randomly and fresh weight was estimated using analytical balance. For dry weight measurement, the seedlings used for fresh weight were dried in an oven for 24 h at 105°C (until a constant weight) and weighed; data was averaged and analyzed using the SAS 2000 software. The means were separated using the standard deviation method [12].

Type of Plant	Time for Gravity (Hour)	Rotational speed (rpm)	Rotational Axis Angle	Rotation Direction
Rice	96	40	90°	Clockwise
	70	90		
Wheat	96	40	90°	Clockwise
	70	90		
Barley	96	40	90°	Clockwise
	70	90		
	70	90		
Pea	96	40	90°	Clockwise
	70	90		

Table 1: Clinostat construction (40, 90 rpm) used in germination plant for duration of 96 and 70 h.

Results and Discussion

Two types of experiments were conducted i.e., rotation at 40 rpm for 96 h and 90 rpm for 70 h for microgravity stimulation. The seedling growth exposed to 90 rpm rotation for 70 h and 40 rpm rotations for 96 h have been shown in Figures 1 and 2, respectively. The distribution of marco and micro elements in tissues of Barely, Wheat, Rice, and Pea changed considerably under clinostat rotation of 40 rpm for 96 h (Table 2). Higher fresh and dry weight reached 0.632 mg and 0.436 mg respectively for Pea seedling in control conditions. For Macro-elements, higher accumulation was found as followed: higher accumulates in P 0.6% for Pea seedling in control treatment while 3.7%, 1.4% of K and Na in Rice (Anber) at control treatment and Clinostat for 96 h and 40 rpm clinostat rotation respectively. Wheat (Mixibac) and Rice (Jasmine) recorded higher accumulation for Ca, Mg (4.9%, 6.5%) in control and clinostat microgravity respectively. In related to Micro-elements Rice (Anber) recorded higher accumulation reached 4.24 mg/kg in Clinostat condition.

Concerning to effect of microgravity at 90 rpm and 70 h clinostat rotation, data in Table 3 revealed that higher fresh and dry weight reached 0.9318 mg and 0.9318 mg respectively in pea seedling at 90 rpm and 70 h clinostat rotation. For Macro-elements, higher accumulation was found as followed: P 7.9% for Wheat (Abu-ghrab) seedling under Clinostat gravity condition. Whereas 5.5% accumulation of K in Pea seedling under Clinostat gravity condition. For Na Pea seedling accumulated 0.91% in control treatment while Pea and Wheat (Abu-ghrab) accumulated 4.1% of Ca for each whereas Wheat (Mixibac) accumulated 6.4% of Mg in control conditions. In related to Microelements, Pea recorded higher accumulation reached 6.45 (mg/kg) in Clinostat condition.

Wheat as compared to barley, pea and rice under the effect of microgravity stimulation, generated by clinostat, however, these contents were higher than control. The mineral contents

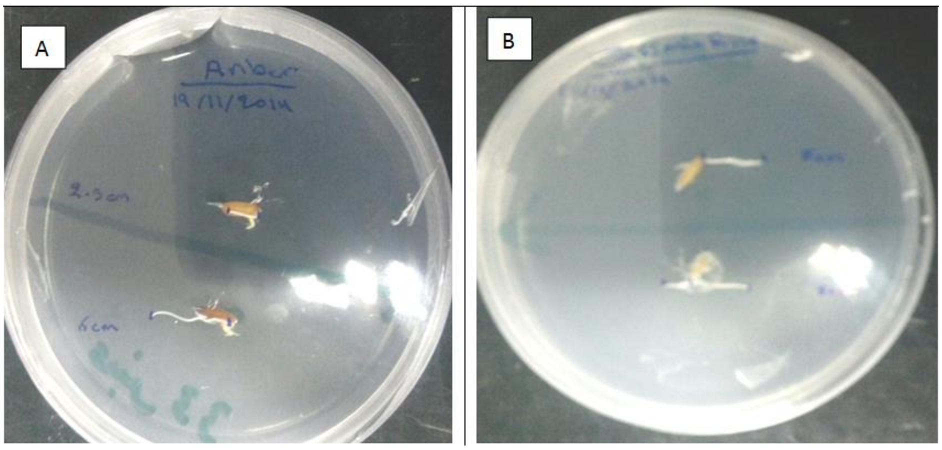

Figure 1: Seed germination after 70 hour under gravity conditions (A) and control (B) in 15 m/L Agar medium.

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recorded in plant tissue revealed that microgravity enhanced and accumulated element in plant tissues and P, K, Na, Ca and Mg concentrations in crass plant were recorded to be 0.4%, 2.1%, 0.3%, 1.8% and 2.8% in control plants, whereas these values were 0.7%, 3.4%, 0.1%, 2.1% and 10% under the effect of clinostat rotation 40 rpm rotation for 96 h. In barely plant, the minerals accumulation was also recorded to higher versus control. A 0.5%, 3.1%, 0.8%, 3.0%, 2.3% and 2.35 mg/kg of P, K, Na, Ca, Mg and Zn contents were recorded, respectively in control, whereas these values were 0.7%, 3.4%, 0.1%, 2.1%, 10.0% and 3.94 mg/kg, respectively in barely seedlings under the effect of microgravity at clinostat rotation 40 rpm rotation for 96 h (Table 3). Wheat seedlings also accumulated higher contents of macro-element and microelement under microgravity stimulation effect and up to 0.08%, 0.025%, 0.4%, 2.0%, 0.7%, 3.1%, 2.5% and 1.49 mg/kg P, K, Na, Ca, Mg and Zn were recorded, whereas these values were 0.07%, 0.021%, 0.2%, 1.6%, 0.8%, 4.9%, 3.0% and 0.53 mg/kg) in control wheat plants, respectively. In rice

Figure 2: Seed germination after 96 hour under gravity conditions (A) and control (B) in 15 mg/L Agar medium.

Species	Statement	Fwt (mg)	Dwt (mg)	Macro-elements (%)					Microelements (mg/kg)
				P	K	Na	Ca	Mg	Zn
Barley	Control	0.2 ± 0.012	0.02 ± 0.0014	0.5 ± 0.026	3.1 ± 0.14	0.8 ± 0.035	3.0 ± 017	2.3 ± 0.17	2.35 ± 0.19
	Clinostat	0.11 ± 0.010	0.025 ± 0.0012	0.5 ± 0.023	3.4 ± 0.25	0.4 ± 0.023	2.0 ± 0.12	2.1 ± 0.19	3.48 ± 0.21
Wheat (Mixibac)	Control	0.07 ± 0.0031	0.021 ± 0.0013	0.2 ± 0.011	1.6 ± 0.74	0.8 ± 0.039	4.9 ± 0.29	3.0 ± 0.19	0.53 ± 0.31
	Clinostat	0.08 ± 0.0042	0.025 ± 0.0013	0.4 ± 0.024	2.0 ± 0.1	0.7 ± 0.034	3.1 ± 0.14	2.5 ± 0.17	1.49 ± 0.68
Wheat (Abu-ghrab)	Control	0.07 ± 0.0031	0.02 ± 0.0014	0.2 ± 0.012	2.6 ± 0.12	0.9 ± 0.041	4.2 ± 0.21	1.2 ± 0.58	1.56 ± 0.79
	Clinostat	0.08 ± 0.0032	0.021 ± 0.001	0.3 ± 0.014	2.3 ± 0.13	0.6 ± 0.03	3.0 ± 0.21	2.2 ± 0.12	0.88 ± 0035
Rice (Jasmine)	Control	0.033 ± 0.0014	0.017 ± 0.007	0.1 ± 0.009	2.0 ± 0.1	1.0 ± 0.45	2.0 ± 0.12	3.0 ± 0.14	3.58 ± 0.23
	Clinostat	0.032 ± 0.0026	0.035 ± 0.0026	0.2 ± 0.01	1.8 ± 0.92	5.0 ± 0.28	3.5 ± 0.19	6.5 ± 0.29	2.79 ± 0.19
Rice (Anber)	Control	0.033 ± 0.0012	0.017 ± 0.006	0.2 ± 0.01	3.7 ± 0.17	0.5 ± 0.03	0.7 ± 0.025	1.9 ± 0.78	3.92 ± 0.21
	Clinostat	0.034 ± 0.0024	0.019 ± 0.008	0.3 ± 0.012	3.0 ± 0.13	1.4 ± 0.72	1.4 ± 0.68	1.9 ± 0.79	4.24 ± 0.24
Pea	Control	0.632 ± 0.031	0.436 ± 0.0023	0.6 ± 0.026	1.3 ± 0.56	0.5 ± 0.27	1.2 ± 0.59	1.6 ± 0.74	1.42 ± 0.69
	Clinostat	0.366 ± 0.021	0.093 ± 0.0041	0.5 ± 0.021	1.8 ± 0.84	2.1 ± 0.16	0.9 ± 0.05	1.3 ± 0.67	1.49 ± 0.71

Table 2: The mineral contents and growth (fresh and dry weight) in plants seedling under microgravity effect at 96 h and 40 rpm clinostat

Species	Statement	Fwt (mg)	Dwt (mg)	Macro-elements (%)					Microelements (mg/kg)
				P	K	Na	Ca	Mg	Zn
Barley	Control	0.2142 ± 0.014	0.10682 ± 0.052	3.1 ± 0.108	2.4 ± 0.88	0.89 ± 0.04	2.9 ± 0.16	1.4 ± 0.65	1.1 ± 0.10
	Clinostat	0.1517 ± 0.063	0.1754 ± 0.061	2.4 ± 0.106	3.4 ± 1.02	0.65 ± 0.03	2.8 ± 1.35	1.27 ± 0.54	3.1 ± 0.22
Wheat (Mixibac)	Control	0.1795 ± 0.056	0.05072 ± 0.0025	2.0 ± 0.080	1.9 ± 0.08	0.24 ± 0.01	2.9 ± 0.14	6.4 ± 0.35	0.6 ± 0.05
	Clinostat	0.169 ± 0.088	0.08458 ± 0.0041	2.1 ± 0.081	1.7 ± 0.44	0.61 ± 0.03	1.6 ± 0.07	1.7 ± 0.62	0.78 ± 0.04
Wheat (Abu-ghrab)	Control	0.127 ± 0.061	0.03674 ± 0.0021	4.5 ± 0.21	0.2 ± 0.01	0.2 ± 0.001	2.7 ± 0.13	3.9 ± 0.26	0.69 ± 0.03
	Clinostat	0.1348 ± 0.069	0.06186 ± 0.0031	7.9 ± 0.42	0.7 ± 0.34	0.64 ± 0.03	4.1 ± 0.20	4.1 ± 0.25	1.1 ± 0.10
Rice (Jasmine)	Control	0.0969 ± 0.0052	0.0191 ± 0.0029	1.8 ± 0.035	0.16 ± 0.07	0.51 ± 0.02	1.9 ± 0.05	2.4 ± 0.15	1.2 ± 0.01
	Clinostat	0.1075 ± 0.051	0.02388 ± 0.0018	4.1 ± 0.258	0.21 ± 0.08	0.47 ± 0.02	1.5 ± 0.08	2.7 ± 0.16	1.1 ± 0.01
Rice (Anber)	Control	0.0896 ± 0.0047	0.029525 ± 0.001	2.1 ± 0.100	0.9 ± 0.05	0.3 ± 0.01	2.4 ± 0.11	3.7 ± 0.21	1.2 ± 0.01
	Clinostat	0.1104 ± 0.052	0.03368 ± 0.0022	2.7 ± 0.134	1.0 ± 0.05	0.25 ± 0.01	0.9 ± 0.04	4.6 ± 0.26	1.3 ± 0.01
Pea	Control	0.51325 ± 0.026	0.3245 ± 0.0021	2.9 ± 0.19	4.9 ± 2.98	0.91 ± 0.04	4.1 ± 0.20	1.87 ± 0.88	1.9 ± 0.87
	Clinostat	0.9318 ± 0.044	0.58408 ± 0.023	2.1 ± 0.67	5.5 ± 1.56	0.61 ± 0.20	3.52 ± 0.27	1.4 ± 0.063	6.45 ± 0.34

Table 3: The mineral contents and growth (fresh and dry weight) in plants seedling under microgravity effect at 90 rpm and 70 h clinostat rotation.

seedlings, the P, K, Na, Ca, Mg and Zn contents were recorded to be 0.034%, 0.019%, 0.3%, 3.0%, 1.4%, 1.4%, 1.9% and 4.24 mg/kg under microgravity effect and control showed the P, K, Na, Ca, Mg and Zn contents of 0.033%, 0.017%, 0.2%, 3.7%, 0.5%, 0.7%, 1.9% and 3.92 mg/kg, respectively. The pea seedlings under microgravity showed similar behavior regarding mineral accumulation under the effect of microgravity stimulation versus control at 40 rpm for 96 h clinostat rotation.

The clinostat rotation at 90 rpm for 70 h effect on growth and mineral contents was evaluated and it was observed the effect of 90 rpm for 70 h clinostat rotation on both mineral contents as well as growth parameters was insignificant, which was due zero gravity because the clinostat rotation at 90 rpm unable to induce gravity. As it can be seen from data presented in Table 3, the control seedling (no clinostat rotation) showed higher mineral contents as well as fresh and dry weights. It is reported that the clinostat centrifugal force is important in generating microgravity, which is in proportion to the distance between the sample and the axis of rotation and the rotational speed (revolutions per minute). In current experiments, the rotational speed was kept high, and centrifugal force moved the statoliths towards the cell wall. The forced of 5.37 g^{-2} in the centrifugal acceleration acting on objects rotated in a one-axis. Clinostat rotation at 90 rpm for 70 h decreased the fresh and dry weights and minerals as compared to control in all plant species. The rotation speed may disrupt elements in plant tissue. Results revealed that at 90 rpm clinostat rotation for 70 h affected the plant characteristics negatively. Under clinostat rotation at 90 rpm for 70 (zero gravity), the response of other plant species were also recorded to be similar regarding growth and minerals accumulation (Table 3). These findings are in line with previous studies Wolff et al. [10] and Kitaya et al. [13] studied the photosynthetic rate in different plant species and it was observed that photosynthetic rate reduced when gravity levels reduced 1.0 to 0.01. However, Jiao [8] reported that the chemical and mechanical properties of the cell modified under altered gravity conditions. The growth, auxin transport, circumnutation, and starch metabolism was also affected positively under the effect of microgravity stimulation [6]. The polysaccharides per unit length of hypocotyl in radish (*Raphanus sativus L.*) and *Arabidopsis thaliana* were also enhanced under gravity stimulation [14].

Present investigation and reported studies revealed that microgravity may enhanced plant characteristics positively i.e. growth, minerals and physiological attributes. The artificial microgravity stimulation by clinostat rotation has the ability to bring these changes and biologists employed successfully to change the microgravity environment for plants and promising results have been reported [15]. It is well known that the rate of plant cell growth is regulated by the osmotic potential and the mechanical extensibility of the cell wall. Since clinostat rotation has direct effect on cell sap and as a result of rotation, the differential growth is likely to occur under clinostat rotation due microgravity generation. Under the effect of clinostat rotation, differences in the metabolic turnover of the cell wall constituents have been reported. The microtubule orientation may also involve in differentiating the plant organs. The accelerated growth in response of favorable curvature might be the reason of accumulation of biogenic elements, which involved in metabolic pathways and resultantly, plant shows positive response to microgravity stimulation by clinostat. The changes in cell wall properties and modifications in growth pattern were also correlated with microgravity stimulation [3]. The results of present study of minerals accumulation and growth of barely, wheat, rice, and pea revealed that microgravity stimulation, generated by clinostat assimilated more minerals and faster seedling were achieved since under zero gravity both minerals and growth were recorded to be low versus control. Therefore, clinostat rotation at 40 rpm (slow) for 96 h enhanced the plant characteristics versus 90 rpm (fast) for 70 h rotation. Present investigation showed the dependence of growth intensity and accumulation of minerals in plants under gravity stimulation and these finding are in line with previous studies that gravity stimulation has potential to affect biochemical in plants tissue, which can change the response of respective tissue/organ [4,6,16-21]. Based on results and importance of plants as a source of food and bioactive compounds [22-24], the use of this technique is suggested to enhance the plant characteristics and growth which will be helpful in enhancing the yield.

Conclusion

The effect of microgravity stimulation on biogenic elements and growth of rice, wheat, barley and pea during early growth stages was investigated. The effect of microgravity stimulation was compared with zero gravity. The rice, wheat, barley and pea species showed higher growth and accumulation of more minerals under the effect of microgravity stimulation. The responses measured for both clinostat conditions were significantly different, which revealed that microgravity has variable and stimulatory effect on plant characteristics. Based on results it was concluded that slow rotation of clinostat has promising efficiency in enhancing the plant biological characteristics versus fast rotation of clinostat. So, clinostat rotation at 40 rpm for 96 h furnished better results as compared to 90 rpm for 70 clinostat rotation. Future studies should be focused on growth, biochemical, and physiological parameters at lateral stages of development whether microgravity stimulation has long term effect on plant characteristics or short term.

Conflict of Interest

No author has any financial or other conflict of interest to declare.

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