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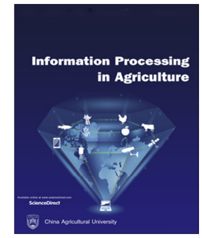

Effect of gravistimulation on amino acid profile of pea, rice, corn, wheat during early growth stages

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The amino acids are one of the major cellular components of plants, which are involved in different metabolic pathways. In present study, effect of artificial gravistimulation on amino acid profiles of pea, rice, corn, wheat during early growth stages was investigated. One-axis clinostat was used for gravistimulation application, which was applied at embryonic stage. Amino acid profile was measured in 10-days old seedlings of pea, rice, corn and wheat cultivars. The effect of clinostat rotation was also evaluated under salt stress and MS medium supplement. Germinated pea, rice, corn and wheat seedlings were grown under the gravity condition for specific time interval. Corn and wheat seeds showed slow germination as compared to pea and rice cultivars. The rate of amino acid formation under gravity condition was significantly higher than control (un-treated seedling). The variation in amino acid profile of pea, rice, corn and wheat cultivars vary deferentially. Results revealed that gravistimulation applied through clinostat has positive effect on amino acid profile in plant tissue and future studies should be focused on growth, biochemical, physiological at lateral stages of growth.

1. Introduction

Scientists demonstrated that the clinostat has ability to simulate the growth of plants. A clinostat continually rotates plant through 360° to eliminate a set direction for gravity, which also prevents the hormone, auxin, from accumulating on one side of the stem or roots. As a result, the clinostat may induce unusual growth in seedlings. Roots may grow toward stems, and stems may grow horizontally, rather than upwards under gravity effect [1].

The microgravity conditions can be produced by free fall or parabolic flight on earth, however, the effect of microgravity generation is small and resultantly, changes in plant growth and morphogenesis may change negligibly. A clinostat with

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a horizontal axis, produce unilateral gravity, which influence the plant growth and morphogenesis significantly. Microgravity influence the cellular functions and cellular components under constant microgravity [2]. This technique is highly interesting and attracted the attention of researchers, which opens new horizon in understanding the effect of microgravity plants morphogenesis, growth and metabolism regulation [3]. However, it needs to carry out research on plant genetic, which will be ultimately upgraded to cereals and vegetables for the production of high quality food and other valuable bioactive compounds [4–10].

To date, few researchers have been studied the effects of gravity on plant developmental processes i.e., the effect of microgravity on cell proliferation within the root meristem [11,12] and using parabolic flights, the studies of Hausmann et al. [13] and Aubry-Hivet et al. [14] showed that changes in gene and protein expression in *Arabidopsis thaliana* cells were significant and fundamental changes in metabolic pathways were also revealed by these researchers. It is reported by Hoson et al. [2,15] that microgravity supports that microtubules played important role in mediating gravity resistance in *Arabidopsis*. Furthermore, Nakashima et al. [16] evidenced that actin might also be an important modulator of root growth in space, whereas Scherer and Pietrzyk [17] reported the root coiling in *Arabidopsis* under the effect of microgravity. Galland [18] and Göttig and Galland [19] used sporangiophore of *Phycomyces blakesleeanus* to investigate graviperception and graviresponses. Analyses of spores revealed that gravity affected expression of a PM-type Ca^{2+} ATPase [20]. Scherer and Quader [21] revealed a transient increase of endocytosis in tobacco pollen tubes under the effect of microgravity. Kordyum [22] highlighted a short overview of real and simulated microgravity on cell components, including statolith positioning, mitochondria, tubulin and the endoplasmic reticulum. Although significant progress has been made in identifying stimulus-responsive elements, the nature of the sensors remains elusive and Iida et al. [23] summarized mechanosensitive channels in Arabidopsis, named MCA1 and MCA2, and their putative role in gravity sensing in Arabidopsis. Tatsumi et al. [24] described recent progress in mechanosensitive channels controlled by the actin cytoskeleton. The involvement of the actin cytoskeleton in gravity perception is further investigated in plant cells using imaging tools i.e., Grolig et al. [25] reported the role of actin in organelle movement in the sporangiophore of the *zygomycete P. blakesleeanus*. Auxin transport is an essential component of the signaling pathway of roots and shoots under gravitropism. The report of Ueda et al. [26] complemented this view by examining the relationship between polar auxin transport and graviresponse in the context of microgravity. A comprehensive analysis of gene expression of floral buds revealed that hypergravity substantially changes expression of genes involved in the biosynthesis of phytohormones such as abscisic acid and auxin [27]. Nasir et al. [28] demonstrated the changes in expression of stress related genes under microgravity in *Euglena gracilis*.

From above discussion, it was hypothesized that microgravity can alter the morphogenesis and growth during germination. Therefore, present study was aimed to appraise the effect of microgravity (generated by clinostat) on pea, rice, corn and wheat cultivars. The principle objective was to investigate the effect on microgravity on amino acid profile under under normal, salt stress and Murashige and Skoog medium supplementation.

2. Materials and methods

2.1. *Microgravity stimulator*

UNOOSA is launching the zero-gravity Instrument Distribution Project and in UN/Malaysia Expert Meeting on Human and Space Technology in November 2011, gravity instruments were distributed to selected institutions (Universities, and research laboratories worldwide). The main objective of the project was to raise awareness and launch this research in education and research institutes, particularly in developing countries. One-axis clinostat (UNOOSA, USA) was used for microgravity generation and experimental set up is shown in Fig. 1 (The clinostat has a horizontal rotational axis perpendicular to the gravity vector on the ground). Growing seeds were exposed around the axis by rotation. The clinostat provided a simulated microgravity condition in equalizing the gravity vector. The direction of the rotational axis can be varied from 0° (parallel to the ground) to 90° (perpendicular to the ground). The rotation speed can be freely selected from 0 to 90 rpm with a 0.5 rpm increment from 0 to 20 rpm and a 5 rpm increment from 20 to 90 rpm. A one-axis clinostat has one limitation if a sample placed on the end of the rotational axis, it remained away from the axis and sample can not be exposed equally. The two-dimensional clinostat rotation cannot effectively compensate for the gravity exerted on all parts of the sample body [29]. This limitation was avoided by selecting small samples size of seeds.

There are several physical factors which affect the performance of one-axis clinostat. The first factor is the angle between the rotational axis and the true horizontal plane [30]. If the angle is one degree, the axial residual acceleration is 0.02*g*. It is important to set the rotational axis within 0.5° from the true horizontal plane of 10–2*g*. The second factor is the centrifugal force if a sample is placed away from the rotational axis [31]. A simple calculation shows that if a sample is placed one centimeter away from the rotational axis

Fig. 1 – Experimental setup of one-axis clinostat.

and the rotation speed is 10 rpm, the amount of the centrifugal force exerted on the sample was in the order of 10–3*g*.

2.2. Plant material

Local cultivar of Pea, Rice, Corn, and Wheat were planted in the plant agar medium in the Petri dish. The germinated seeds of Pea, Corn, Rice, and Wheat were grown under the gravity. The clinostat conditions used for seedling exposure to microgravity are shown in Table 1, the clinostat rotated in random direction and speed was increased from 1 to 90 rotations/min. The sample stage and illumination are settled on the two opposite sides of the inner frame. The sample and control were grown under similar condition. The experiments were conducted in an air-conditioned room with ambient air and the temperature of 23 ± 1 °C. The light dark cycle was 12–12 h, and light intensity was 200 L mol m^{-2} S^{-1}. The control plants grew in the same culture conditions as the plants but without clinorotation. After stipulated time period (Table 1), plant seedlings were harvested and subjected to amino acid analysis.

2.3. Salt stress

The Pea, Rice, Corn, and Wheat cultivars were grown hydroponically under four level of saline conditions (tap water (0.5 dS/M), well water 1 (4 dS/M), well water 2 (8 dS/M) and well water 3 (12 dS/M)) (well water-fresh water collected from different wells). Before seed germination, the water samples were autoclaved at 1.04 kg cm^2, 121 °C, for 20 min and seeds were placed in Petri dish containing 10 mL agar medium (agar medium was prepared by dissolving agar (15 g/L) in double distilled H_2O and mixture was kept till solidified. The culture medium was maintained at 24 ± 2 °C in growth chamber in dark for 3 day. The half germinated seed were subjected to gravity stimulation (Table 1) and reaming germinated seeds were used as control.

2.4. Murashige and Skoog medium

Seeds were placed in Petri dish containing 10 mL double distilled H_2O contains solidified MS medium (8 g/L) (Table 2) and culture medium were maintained at 24 ± 2 °C in growth chamber in dark for 3 day. The half germinated seed were subjected to gravity stimulation (Table 1) and reaming germinated seeds were used as control.

Table 2 – MS medium composition supplied along with microgravity application.

Components	Amount (mg/L)
MS salts	4300
Thiamine	10
Nicotinic acid	1.0
Pyridoxine	1.0
Inosital	100
Sucrose	3000
Vio-agar	7000

2.5. Amino acid analysis

Amino acids in seedlings of Pea, Rice, Corn, and Wheat were estimated in triplicate following reported method elsewhere [32]. Briefly, 3 mL of HCl (6 N) was added in 0.1 g of sample in a test tube. The test tube placed in the oven at 110 °C for 1 h. Then, volume was increased to 50 mL by adding distilled water and mixture was filtered. The samples were dried in rotary evaporator at 40 °C and solid samples were melted in 2 mL of HCl (0.01 N) and transferred to tightly closed test tube and stored at −4 °C until analysis. Amino acids analyzer (Shimadzu 20AT LC System (Shimadzu, Tokyo, Japan) was used for amino acid analysis.

3. Results and discussion

3.1. Amino acid

Various local crops were selected for evaluation of microgravity effect on amino acid profiles. The corn varieties (39 and 5) amino acids contents are shown in Fig. 2AB, respectively. Under the effect of clinostat microgravity, the amino acid concentration found to be different in corn cultivars and corn 39 cultivar showed higher amino acid contents as compared to corn 5 cultivar and control (plant grown without microgravity application) also showed lower amino acids contents versus both corn cultivars. Results revealed higher proportion of glutamic acid, isoleucine and glutamine amino acids under the effect of microgravity as compared to other amino acids. The amino acids such as arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, thre-

Table 1 – Clinostat conditions used for microgravity stimulation.

Type of plant	Time for gravity (h)	Rotational speed (rpm)	Rotational axis angle (°)	Rotation direction
Corn parents (5)	9	90	90	Clockwise
Corn parents (39)	9	90	90	Clockwise
Wheat	5	90	90	Clockwise
Pea	5	90	90	Clockwise
Rice amber 33	6	90	90	Clockwise
Rice jasmine	6	90	90	Clockwise

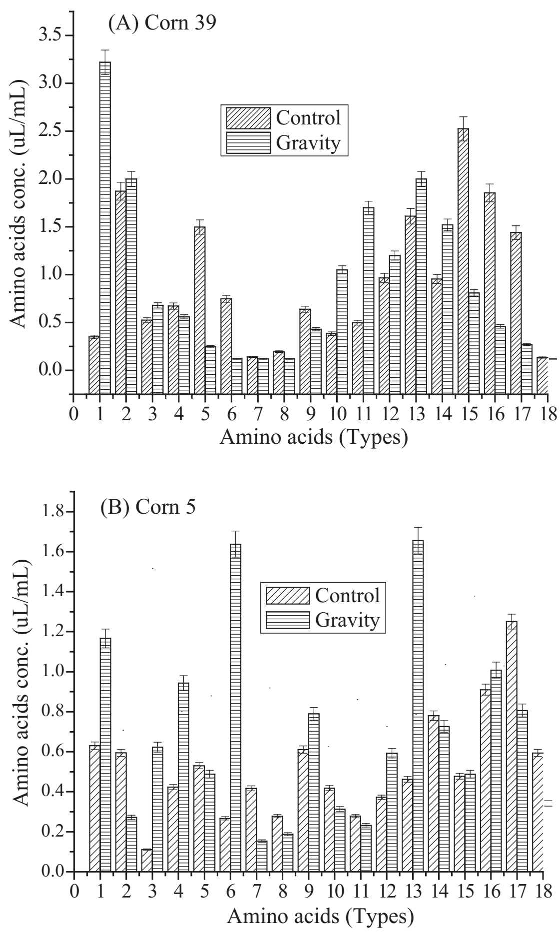

Fig. 2 – Effects of clinostat rotation on the amino acids concentration of corn; (A) corn variety 39 and (B) corn variety 5.

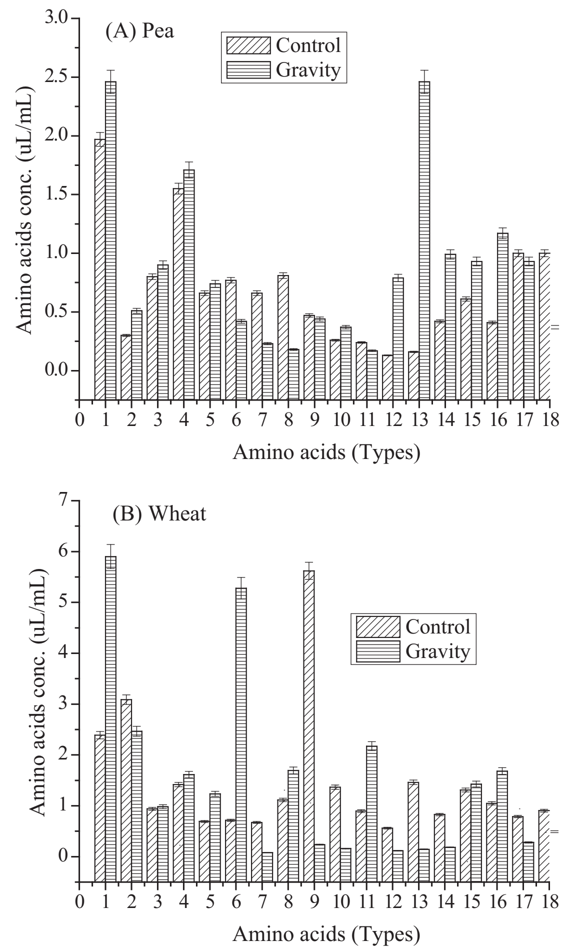

Fig. 3 – Effects of clinostat rotation on the amino acids concentration; (A) pea cultivar and (B) wheat cultivar.

onine, tryptophan, tyrosine and valine contents were recorded to be 3.22, 2.00, 0.68, 0.56, 0.25, 0.12, 0.12, 0.12, 0.43, 1.05, 1.70, 1.20, 2.00, 1.52, 0.81, 0.46, 0.27 and 0.12 (μL/mL), respectively in corn 39 cultivar and similar trend was observed for corn cultivar 5 with negligible variation among individual amino acids. Similarly, pea also showed variable amino acid contents under the effect of microgravity stimulation, however, higher than control. The arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine contents were 2.46, 0.51, 0.90, 1.71, 0.74, 0.42, 0.23, 0.18, 0.44, 0.37, 0.17, 0.79, 2.46, 0.99, 0.93, 1.17, 0.93 and 0.37 (μL/mL) in pea cultivar (Fig. 3A). The amino acid contents of wheat also enhanced under the effect of microgravity and 5.903, 2.467, 0.982, 1.614, 1.235, 5.279, 0.081, 1.695, 0.237, 0.160, 2.173, 0.117, 0.145, 0.183, 1.428, 1.684, 0.279 and 0.491 (μL/mL) arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine were recorded in wheat cultivar (Fig. 3B). In comparison to control both pea and wheat showed considerably higher amino acid contents under the effect of microgravity. The seeds grown in water from different sources (well water 1, 2 and 3) and subjected to gravity stimulation showed similar response regarding amino acids in plant tissues.

3.2. *Amino acids concentration under salt stress and microgravity stimulation*

It is well know that plants metabolism also depends upon external factors and salinity is one of them, which adversely affect the plant growth and yield. Salinity is the buildup of soluble salts by which saline soils are formed, which adversely effects plant growth salt ions create the critical conditions for plants survival by intercepting different plant mechanisms such as physiological disorders, osmotic potential, water intake by plants is restricted [33]. Therefore, the effect of microgravity on amino acids concentration under salt stress is important to study and response thus obtained is shown in Fig. 4AB for jasmine and amber (rice cultivars), respectively. Amino acids increased considerably in the plants grown under clinostat rotation and under salt stress. Values of 2.49, 2.05, 0.83, 1.68, 0.65, 1.06, 0.72, 0.50, 2.54, 2.21, 2.75, 1.49, 2.92, 1.51, 0.57, 1.49, 1.16 and 0.30 (μL/mL) of arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and

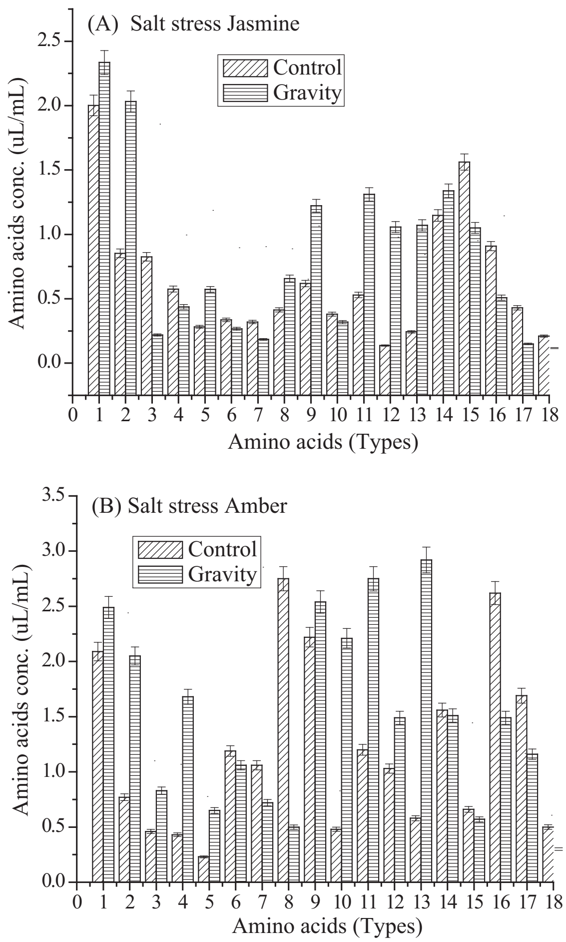

Fig. 4 – Effects of clinostat rotation on the amino acids concentration under salt stress; (A) rice variety jasmine and (B) rice variety Amber.

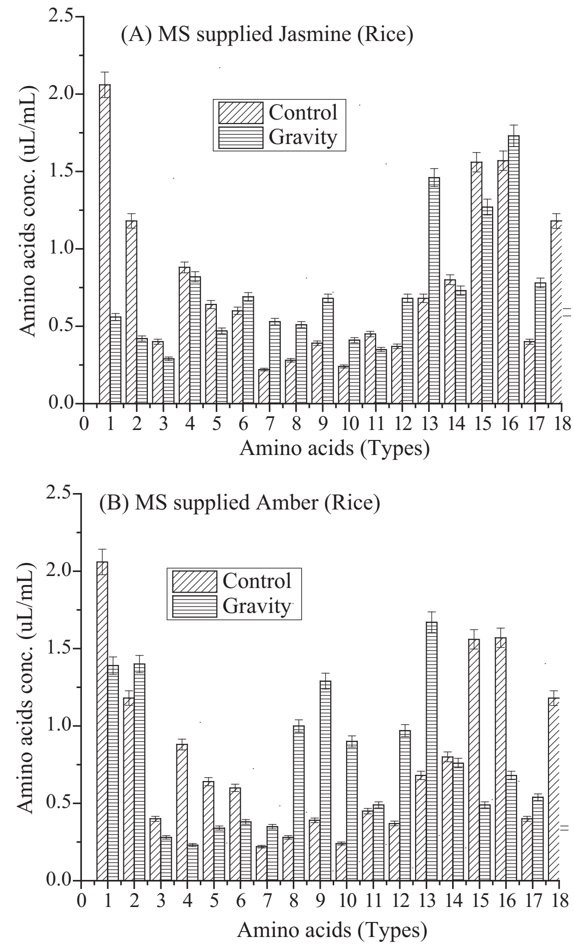

Fig. 5 – Effects of clinostat rotation on the amino acids concentration under MS medium supplementation; (A) rice variety jasmine and (B) rice variety amber.

valine were observed in amber rice variety, whereas these values were 64.76, 86.02, 18.51, 70.50, 19.68, 12.40, 7.30, 6.64, 43.34, 45.24, 22.19, 26.79, 98.25, 41.55, 55.87, 27.69, 22.46 and 17.65 (μL/mL) were recorded in jasmine rice variety under salt stress, which indicate that salt stress was unable to effect the amino acids under the effect of microgravity stimulation. However, the amino acids concentration found to be variable among both rice cultivar and this difference was due to different rice varieties. Jasmine rice variety showed higher amino acids concentration versus Amber variety. Overall, the amino acids contents enhanced in both rice varieties, grown under clinostat rotation + under salt stress versus only salt stress (control).

3.3. Amino acids profile under MS medium supplementation

Plants grown under clinostat rotation showed that clinostat rotation affected the concentration of amino acids positively versus control under MS supplementation; the microgravity effect was also different in both species (Fig. 5AB). Amino acids concentrations of 2.056, 1.176, 0.401, 0.881, 0.643, 0.599, 0.217, 0.277, 0.386, 0.240, 0.448, 0.375, 0.679, 0.801, 1.560, 1.569, 0.402 and 1.180 (μL/mL) of arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine were observed in Jasmine rice variety, whereas these values were 0.559, 0.423, 0.286, 0.818, 0.468, 0.688, 0.528, 0.505, 0.684, 0.412, 0.350, 0.676, 1.456, 0.733, 1.269, 1.725, 0.776 and 0.586 (μL/mL) for arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine were observed in Amber rice variety, respectively. In comparison to plants types, it was observed that under similar MS medium supplementation, the amino acid profile vary among plants, which clearly indicates the effect of clinostat rotation since clinostat rotation enhanced the amino acid profile. Both amber rice and jasmine varieties showed different amino acid profile, which were recorded to be higher in Jasmine rice variety versus Amber rice variety and both showed higher amino acid concentration in MS supplementation + under microgravity effect versus control (only MS supplementation).

Previous studies also highlighted the effect of gravity on plant growth and positive growth behaviors under the influence of clinostat have been reported i.e., lentil (*Lens culinnrias* L. cv. Verte du Puy) seedlings grown in space (Spacelab D1 Mission), with seedlings grown on a slowly rotating clinostat were compared. Seeds were germinated and grown under microgravity and 1*g*-centrifuge in space. The root lengths, root orientation, distribution of amyloplasts, location of the nucleus in statocytes were studied. Root length and orientation were similar for roots grown under microgravity. The amyloplasts were identically distributed in statocytes in roots exposed horizontally clinostat rotation. However, the location of the nucleus was similar in vertically rotated roots and under microgravity. Author concluded that that horizontal clinostat rotation simulates microgravity better than vertical clinostat rotation [34]. In another study, the growth was studied under microgravity conditions. It was observed that growth was stimulated and lateral expansion suppressed in shoot and roots under microgravity condition. The changes in cell wall properties and modifications in growth pattern were correlated with the effect of microgravity [2]. The results of present study of the essential amino acids and amino acids under salt stress and MS medium of rice, corn, pea, crass were also enhanced under microgravity as compared to control. The level of amino acids in tissues was many folds higher than control. It is well known that tissues accumulate free amino acids under the conditions of stress such as salt, water deficiency, because they belong to the most hydrophilic and somatically active compounds [35]. In another study, tobacco pollen tubes having an elaborate cytoskeleton was study under microgravity effect and it was observed that endocytosis is increased transiently in microgravity within 3 min. This inhibited by the calcium blocker verapamil suggests that calcium was lower in the tip, which is known to increase endocytosis in the pollen tube [21]. Similarly, [16] studied how microgravity impacts root growth in *A. thaliana*. Studies showed that the actin cytoskeleton negatively regulates root gravity responses, leading the assumption that actin might also be an important modulator of root growth in space. From results, it was proved that root responses under gravity on Earth, endogenous directional growth patterns of roots under microgravity are suppressed by the actin cytoskeleton. Modulation of root growth in space by actin could be facilitated in part through its impact on cell wall architecture. The photosynthesis process was also influenced under altered gravity [4]. Author conducted that the ultrastructure and state of the photosynthetic apparatus in *A. thaliana* leaf mesophyll cells at the different stages of plant development under clinostat rotation were affected and [36] also studied the structural and functional characteristics of the photosynthetic apparatus in 15-day old *Brassica rapa* plants under space shuttle in Columbia (STS-87). An average volume of one mesophyll palisade cell increased approximately twice and the chloroplast number per cell by 69.8%. The stromal thylakoids, starch grains and plastoglobuli also increased by 19.4, 20.6 and 2 times, respectively. Hoson et al. [15] compared the growth of inflorescence stem under microgravity conditions with ground and on-orbit 1*g* conditions. The stems were 10–45% longer and their growth rate was 15–55% higher under microgravity conditions than those under 1*g* condition. Seedlings germinated and grew for 6 days under the altered gravity conditions of horizontal clinostat rotation and centrifugation decreased growth relative to the control (vertically rotated). Starch concentration in the cotyledons was lower under clinostat rotation and was higher under the effect of centrifugation as compared to the control. The ADP glucose pyrophosphorylase was affected by the gravity treatments; being lower in the cotyledons of the horizontally rotated plants and higher in the cotyledons of the centrifuged plants relative to the control [29]. In another study, plant roots response was studied under altered gravity, which induces asymmetric cell growth leading to root bending. Results revealed that common and mutation-related genes differentially expressed in response to transient microgravity phases. Gene ontology analysis of common genes revealed lipid metabolism, response to stress factors and light categories as primarily involved in response to transient microgravity phases, which suggests that fundamental re-organization of metabolic pathways functions upstream of a further signal mediating hormonal network is involved. Moreover, repetitive exposure to microgravity/hypergravity and gravity/hypergravity flight phases induced an up-regulation of auxin responsive genes in wild type plant roots. Researchers reported that accumulation of amino acids in over ground organs is part of active reaction of plant organs on insufficient water supply. Present investigation showed that dependence of growth intensity and accumulation of free amino acids in plants under gravity stimulation applied affected the amino acid concentration positively 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 [11,12,16,19,22,29,37–39]. Based on results and importance plants [10,40–46], the use of this technique might be helpful in enhancing the plant characteristics and ultimately growth.

4. Conclusions

In present investigation, effects of gravistimulation on amino acid profiles of pea, rice, corn, wheat was investigated. The gravistimulation produced through clinostat and it was observed that all cultivars i.e., pea, rice, corn, wheat showed enhanced amino acids concentration versus control. The clinostat rotation effect was also studied under salt stress and MS medium supply and responses thus obtained revealed that under salt stress and MS medium supply, the amino acids concentrations were also higher in rice varieties, which indicates that microgravity can mitigate the effect of salt stress on plant tissues. Based on results, it can be concluded that clinostat rotation can affect the biochemical in plant tissue positively and this technique can be used to enhance the plant germination, growth and ultimately, productivity in agriculture sector.

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