



Comparative Studies on Effects of Simulated Microgravity on Growth and Photosynthetic Parameters in Rice and Mungbean

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Abstract

Microgravity or low gravity environment of space can be simulated on Earth by using clinorotation with appropriate speed of rotation. Previous reports show that microgravity environment (real or simulated) affects growth, chlorophyll content and photosynthetic performance in different plants. However, most of the reports showed variation in results. Earlier studies carried out on simulated microgravity (SMG) using clinorotation in our laboratory showed increase in growth, chlorophyll content and various yield attributes in case of laboratory and field grown rice. Subsequent studies were carried out on mungbean seedlings. In the present work, comparative aspects of these studies are reported. Results of the present study showed increase in growth, chlorophyll content and chlorophyll fluorescence parameters in SMG samples as compared to control samples in rice and mungbean seedlings. Interestingly, percentage of enhancement in growth, chlorophyll content and chlorophyll fluorescence parameters of SMG samples is significantly higher in rice (monocotyledonous) seedlings as compared to mungbean (dicotyledonous).



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Introduction


On Earth, all living beings experience gravitational acceleration of 9.8 m/s^2 , termed as one Earth's gravity or simply 1 g . If this value is reduced to 10^{-3} to 10^{-6} times 1 g then it is called as microgravity or low gravity or simply μg . To achieve the condition of microgravity one has to either travel into deep space (real microgravity) or it can be simulated on Earth by

using different platforms such as parabolic flights, sounding rockets, drop towers or more commonly used instruments by plant biologists, clinostat (simulated microgravity or SMG). Recent studies revealed that gravity has had a profound impact on the form, structure and function of plants (Gilles Clement, 2005). The instrument called clinostat developed by Von Sachs (1879) has been widely

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used to simulate microgravity condition on ground (Jack Van Loon, 2007). Previous reports show that microgravity (real or simulated) affects growth, chlorophyll content and photosynthetic performance in different plants. However, variation in results was obtained in studies carried out by using clinostat.

For instance, increase in shoot length in rice seeds (*Sasanishiki*) under clinorotation was also observed by Takakura *et al.* (1996). The enhancement in growth as measured by root, shoot length and weight are consistent with similar observations on soybean (Hilaire *et al.*, 1996), azuki bean (Hoson *et al.*, 1992) as well as pea and maize (Shimazu *et al.*, 2001) using 3-D clinostat. Jagtap *et al.* showed similar increase in root shoot length and weight as well as chlorophyll content in rice seedlings when rotated at 2 rpm in 1-D clinostat (Jagtap *et al.*, 2010). The photosynthesis process might also be affected as observed in 4 - 10 days old garden cress seedlings when exposed to 3-D clinostat rotated at lower rpm (Yamada *et al.*, 1993). The proton permeability in pea chloroplasts grown under slow clinorotation (2 rpm) was increased (Mikhaylenko *et al.*, 2001). The rate of CO₂ assimilation was found to be greater in *Pisum sativum* grown on a horizontal axis clinostat as compared to control (Brown *et al.*, 1996).

Though the enhancement in growth, chlorophyll content and photosynthetic performance was observed in different plants under microgravity, some of the previous reports showed no change or decrease in plant growth and development (Briarty *et al.*, 1995, Kiss *et al.*, 1998, Levine *et al.*, 2001), chlorophyll content (Laurinavichius *et al.*, 1986, Moleshko *et al.*, 1991, Mikhaylenko *et al.*, 2001) and photosynthetic performance (Tripathy *et al.*, 1996, Chen *et al.*, 2013) in space and clinorotation.

Thus, these results show variation and do not show consistency. The discrepancies in the results may be due to the difference in methodologies, different environmental conditions and use of different species (Aarouf *et al.*, 1999). These inconsistent results could also be due to the duration of exposure to clinorotation (Jagtap *et al.*, 2011). Besides these factors, it would also be interesting to study whether clinorotation has similar effects on monocotyledonous and dicotyledonous plants. Therefore, in the present work, the attempt has been made to study and compare the seed germination, growth and

especially the chlorophyll a fluorescence (O-J-I-P) transient and other important photosynthetic parameters in rice and mungbean seedlings grown under slow rotating (2 rpm) clinostat under similar environmental conditions. The response against simulated microgravity was compared in two plants viz., monocotyledonous rice and dicotyledonous mungbean.

Materials and Methods

Seed Selection

Healthy seeds of monocotyledonous rice (*Oryza sativa* 'PRH-10') and dicotyledonous mungbean [*Vigna radiata* (L.) Wilczek] were selected for the experiment. Seeds were surface sterilized by using 0.5 % fungicide (Uthane M-45) and thoroughly washed with distilled water (D/W) to remove the traces of fungicide. Seeds were then soaked in D/W for 24 hours at room temperature. After 24 hours, these seeds were mounted on 0.8 % agar gel in Perspex beakers of volume 150 ml. Total 8 seeds per beaker were mounted on a periphery of a circle of radius 1.5 cm.

Simulated Microgravity (Clinorotation) Treatment

Beakers were covered with polythene bags containing five holes at upper side for ventilation and placed on a clinostat with one rotation axis developed in-house. Seeds were rotated continuously for 5 days at a constant speed of 2 rpm (simulated microgravity value approx. 7×10^{-5} g). Most of the previous studies on various plant seeds and seedlings were carried out at 2 rpm.

Measurements

Percentage seed Germination and Growth

Percentage seed germination and growth parameters were measured on 5th day from sowing. Orientations of root and shoot were continuously monitored.

Chlorophyll Extraction

Chlorophyll was extracted by using DMF (N, N-dimethyl formamide) as a solvent (Porra *et al.*, 1989). Shoots from 5 days old seedlings were immersed in DMF solution and stored at 8 – 10 °C for overnight. On the next day, the solution was filtered using Whatman® filter paper. Filtered chlorophyll solution was used for spectroscopic measurement. Absorption spectra of chlorophyll were recorded for both control and clinorotated samples by using UV-Visible spectrometer (Perkin Elmer Lambda 950). Chlorophyll - a, chlorophyll - b and total chlorophyll

content were calculated from absorption spectra by using Arnon formula (Arnon, 1949).

Chlorophyll Fluorescence Measurements

The fifth day expanded leaf was used to measure chlorophyll fluorescence with portable Handy PEA (Hansatech Instruments, Kings Lynn, UK). After dark adaptation of leaves for ten minutes, a single strong 1 s-light pulse ($3500 \mu\text{mol}/\text{m}^2/\text{s}$) was applied on them with the help of three light-emitting diodes (650 nm). The fast fluorescence kinetics (F_0 to F_m) of control and simulated microgravity treated seedlings was recorded during $10 \mu\text{s}$ to 1 s. The chlorophyll fluorescence parameters were analyzed on the basis of data extracted from the O-J-I-P transient (Strasser *et al.*, 2000).

Results

Growth

The results of the present study showed no significant difference in the percentage seed germination in rice and mungbean. Figure 1 shows photographs of control and clinorotated rice seedlings recorded on 2nd day from sowing. As seen from the figure, control seedlings clearly follows normal gravitropism behaviour i.e. shoots were grown upwards and roots grown downwards (towards the direction of gravity). However, in clinorotated seedlings, direction of shoots and roots was random i.e. shoots were oriented outward (towards the open end of the beaker). Similar trend for control and clinorotated samples was observed in case of mungbean seedlings.

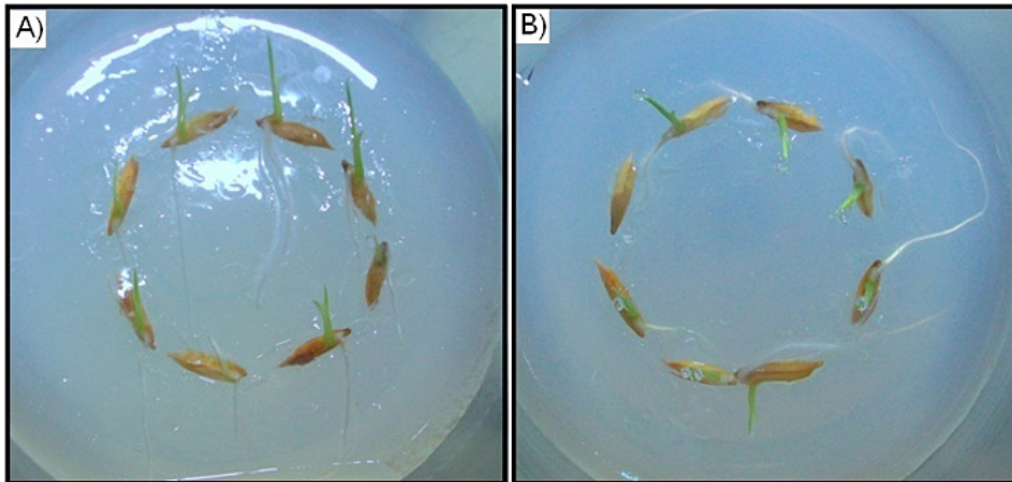


Fig. 1: Representative photograph showing behaviour of 2 days old rice seedlings for A) control and B) clinorotated samples.

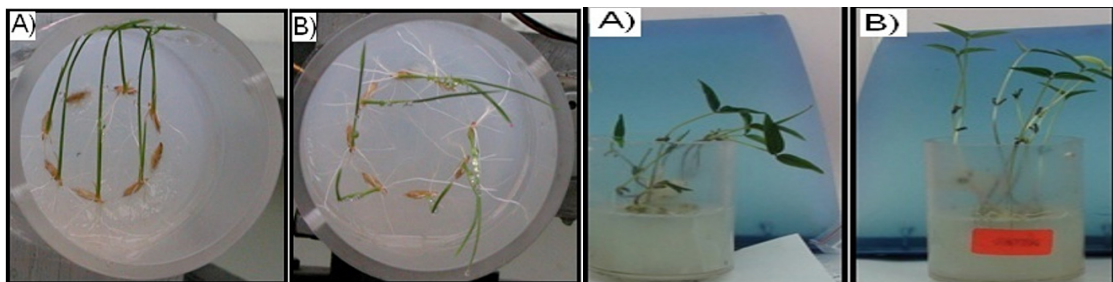


Fig. 2: Representative photograph showing growth of 5 days old rice and mungbean seedlings for A) control and B) clinorotated samples.

Figure 2 shows representative photograph of growth of rice and mungbean seedlings on 5th day from sowing. A comparison between root lengths and shoot lengths in rice and mungbean seedlings is given in figure 3. Figure 4 represents a comparison between

leaf area in rice and mungbean seedlings. As shown in figure 3, enhancement in root and shoot length was observed in rice and mungbean in clinorotated samples than control samples.

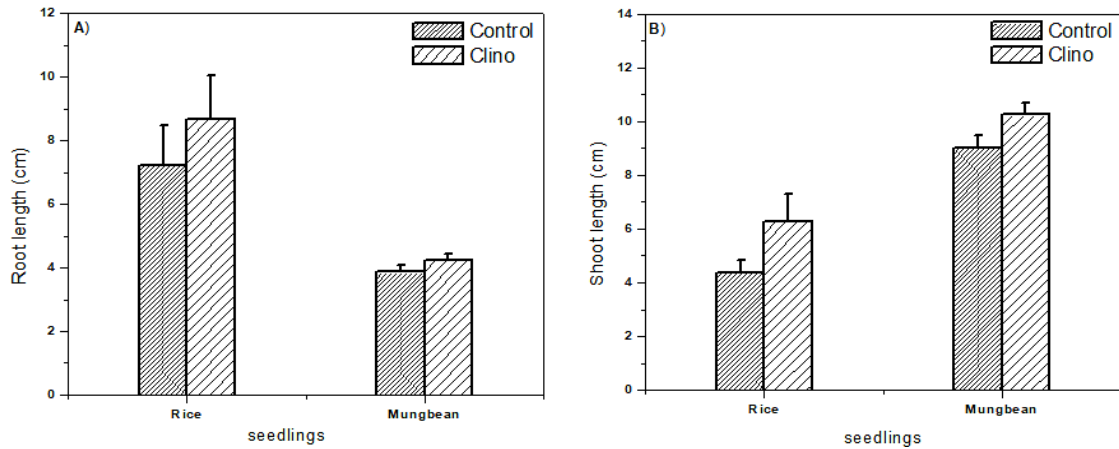


Fig. 3: Changes in growth parameter A) root length, B) shoot length in rice and mungbean after five days sowing. Each value represents \pm SE of (n = 24) seeds in three repeated experiments.

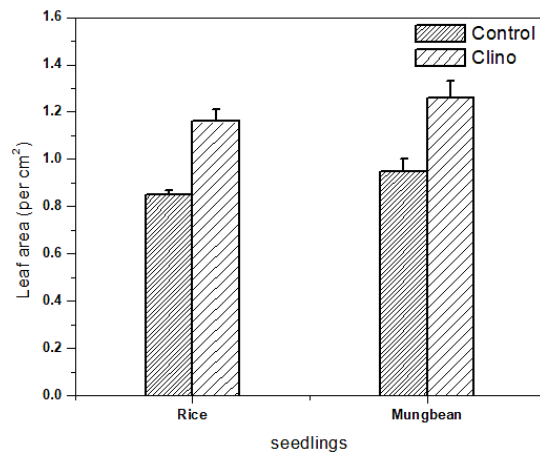


Fig. 4: Effect of clinorotation on leaf area in rice and mungbean seedlings. Each value represents \pm SE of (n = 24) seeds in three repeated experiments.

Interestingly, the percentage increase in root length in mungbean was found to be less (9 %) as compared with that in rice (17 %). Similarly, percentage increase in shoot length was also less in mungbean (12 %) as compared with that in rice

(30 %). It is seen from figure 4 that the leaf area was found to be increased in clinorotated samples than control samples in rice and mungbean. The percentage increase in leaf area was also marginally less in mungbean (25 %) as compared with that of rice (27%).

Chlorophyll Content

Results of chlorophyll content showed significant increase in chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) in clinorotated samples than control samples in rice as well as mungbean. Increase in chlorophyll content in clinorotated samples was also observed by Abilov (1986), Aliyev (1987) and Jagtap (2011). However, chlorophyll a/b ratio was found to be decreased in clinorotated samples than control samples in all three seedlings. Table 1 shows the percentage of increase or decrease in chlorophyll a, chlorophyll b and total chlorophyll contents and chlorophyll a/b ratio in clinorotated samples with respect to the controls. As shown in table 1, percentage increase in chlorophyll contents in clinorotated samples in rice was more (Chl a-52 %; Chl b-59 % and total chl-53

%) as compared with that in mungbean (Chl a-19 %; Chl b-35 % and total chl-22 %). However, percentage decrease in chlorophyll a/b ratio in rice is less (14 %) as compared with mungbean (21 %).

Table 1: Percentage increase or decrease (indicated by - sign) in chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio in clinorotated samples with respect to those of the controls. This table is a representative of 3 experiments.

	Percentage increase or decrease	
	Rice	Mungbean
Chlorophyll-a	51.56 %	18.89 %
Chlorophyll-b	58.60 %	34.68 %
Total chlorophyll	52.91 %	21.82 %
Chlorophyll a/b	-14.36 %	-21.09 %

Chlorophyll Fluorescence Parameters

Chlorophyll a fluorescence measurement is useful non invasive probe for monitoring the PS II behavior in plants under different environmental conditions (Strasser BJ and Strasser RJ, 1995, Strasser *et al.*, 2000). The fluorescence transient curve during illumination is labeled as O-J-I-P (Strasser BJ and Strasser RJ, 1995; Strauss *et al.*, 2003). The analysis of fluorescence parameters based on theory of fluxes indicates the changes of absorbed, dissipative, trapping and electron fluxes named

JIP test. The fluorescence curve begins with initial fluorescence Fo (O) and achieve maximum fluorescence Fm (P) via two intermediate steps J and I with respect to time. The entire curve is divided into three stages O-J, J-I and I-P. The OJIP test can be used to study the behavior of PSII when plants are exposed to different environmental conditions. The (Fv/Fm) is more sensitive to the reaction centre side. The ratio of Fv/Fm in healthy leaves is about 0.78 - 0.83 regardless of plant species (Abbaspoor and Streibig, 2007).

Table 2: A - Effects of Clinorotation on chlorophyll fluorescence parameters in rice and mungbean on fifth day. Values are means ± SE (n=10). * and ** indicates the significant differences after treatments (P< 0.05 and P<0.01 respectively). Values indicated in parenthesis are percentage increase in clinorotated samples with respect to the control.

Chlorophyll fluorescence Parameters	Rice		Mungbean	
	Control	Clino	Control	Clino
Fv/Fm	0.76 ± 0.02 (6%)	0.81* ± 0.007	0.81 ± 0.005 (1.80%)	0.83* ± 0.003
Fm/Fo	4.31 ± 0.28 (18.59%)	5.30* ± 0.12	5.53± 0.46 (9.87%)	5.92± 0.32
Fv/Fo	3.34± 0.28 (22%)	4.29* ± 0.12	4.45± 0.16 (9.37%)	4.91* ± 0.11
PI (abs)	0.55 ± 0.03 (27%)	0.77**± 0.02	2.53 ± 0.01 (12.91%)	2.91* ± 0.16
PI total	0.76± 0.05 (24%)	1.05**± 0.03	1.90 ± 0.02 (23%)	2.48* ± 0.26

Table 2: B - Effects of Clinorotation on chlorophyll fluorescence parameters in rice and mungbean on fifth day. Values are means \pm SE (n=10). * indicate the significant differences after treatments ($P < 0.05$). Values indicated in parenthesis are percentage decrease in clinorotated samples with respect to the control.

Chlorophyll fluorescence Parameters	Rice		Mungbean	
	Control	Clino	Control	Clino
δRo	1.44 \pm 0.19 (Negligible or no change)	1.37 \pm 0.63	0.43 \pm 0.002 (6.65%)	0.46 \pm 0.02
ϕRo	0.41 \pm 0.02 (50%)	0.84 \pm 0.26	0.21 \pm 0.01 (7.55%)	0.23 \pm 0.001
ρRo	0.54 \pm 0.04 (45%)	0.95 \pm 0.37	0.25 \pm 0.02 (5.69%)	0.27 \pm 0.01
ϕDo	0.23 \pm 0.01 (-19.23%)	0.19* \pm 0.04	0.19 \pm 0.005 (-9.84%)	0.17* \pm 0.03
Dlo/RC	0.87 \pm 0.008 (-38.54%)	0.54* \pm 0.14	0.48 \pm 0.06 (-12.33%)	0.42* \pm 0.04

Table 2: C - Effects of Clinorotation on chlorophyll fluorescence parameters in rice and mungbean on fifth day. Values are means \pm SE (n=10).

Chlorophyll fluorescence Parameters	Rice		Mungbean	
	Control	Clino	Control	Clino
Vj	0.62 \pm 0.02	0.59 \pm 0.008	0.40 \pm 0.04	0.40 \pm 0.02
Vi	0.44 \pm 0.03	0.45 \pm 0.003	0.74 \pm 0.05	0.73 \pm 0.05
ϕEo	0.29 \pm 0.03	0.33 \pm 0.10	0.48 \pm 0.01	0.49 \pm 0.02
ΨEo	0.39 \pm 0.05	0.41 \pm 0.01	0.60 \pm 0.02	0.60 \pm 0.004
ABS/RC	3.75 \pm 0.34	3.72 \pm 0.08	2.54 \pm 0.13	2.44 \pm 0.18
TRo/RC	2.87 \pm 0.31	3.01 \pm 0.06	2.04 \pm 0.10	2.01 \pm 0.15

An increase was observed in Fv/Fm, Fm/Fo, Fv/Fo ratios. Performance index (PI abs) and PI total were also significantly increased (Table 2 A). There was also increase in photosynthetic yields or flux ratios such as δRo , ϕRo , and ρRo (Table 2 B). Density of reaction centers was also increased. However, no significant change (Table 2 C) was observed in relative variable fluorescence at Vj and Vi ϕEo , ΨEo . ABS/RC, TRo/RC. There was decrease (Table 2 B) in dissipation energy yield like Dlo/Rc and ϕDo under clinorotation as compared to the control. This may have happened, due to the reoxidation of QA which was increased by the electron transfer from PSII to PSI.

Discussion

In SMG seedlings, direction of shoots and roots was found to be random in rice as well as mungbean seedlings. Such random growth behavior of root and shoots in clinorotated samples was also observed by (Shimazu *et al.*, 2001) in etiolated pea (*Pisum sativum* L. cv. Alaska) and maize (*Zea mays* L. cv. Golden Cross Bantam) seedlings grown under 3-dimensional clinostat.

The enhancement in growth is consistent with those obtained by Aarouf where, *Brassica napus* seedlings grown for 5 days on the horizontal clinostat exhibited a faster development than in the controls.

The biomass (fresh weight) of the root system, and also that of the shoot, was greater on the horizontal clinostat than in the controls (Aaroufet *et al.*, 1999). Similar results were obtained by Levine (Levine *et al.*, 1990) and Jagtap (Jagtap *et al.*, 2011) on rice seedlings.

As far as chlorophyll fluorescence parameters are concerned, the increase in F_v/F_m ratio could be associated with decrease in energy dissipation ϕDo and Dio/Rc in the antennae of PSII. However, PSII activity was unchanged under random positioning machine (RPM) condition as evident from chlorophyll fluorescence and thermoluminescence studies (Chen *et al.*, 2013) as compared to 1 g control. Aluminium toxicity decreased F_v/F_m indicated that photoinhibition occurs in leaves exposed to Al (Jiang *et al.*, 2008). Our findings lead to the hypothesis that, no photoinhibition occurs under clinorotation. No change in ϕEo in rice and mungbean plants also supports this hypothesis.

F_v/F_o represents the maximum primary yield of PSII. It also indicates the activity of water splitting complex at the donor side of PSII (Jafarina and Shariati, 2012). An increase was observed in F_v/F_o under clinorotation in mungbean and rice. Our results are consistent with that of strawberry and carnation (Zhao *et al.*, 2002). In rice and mungbean, the ratio F_v/F_o increased by 22 % and 9.37% ($p < 0.05$), respectively during clinorotation as compared to the control (Table 2 A). It indicates that clinorotation plays significant role in increasing the activity of water splitting complex. The decrease in the ratio F_m/F_o indicates the damage to the photochemical apparatus (Lacuesta *et al.*, 1992). In the present study, the ratio of F_m/F_o in mungbean increased by 10% under clinorotation with respect to the control. These findings suggest that clinorotation does not cause damage to the photosynthetic apparatus. Fluorescence rise at I step was observed when treated with different concentration of aluminium (Al) in Citrus leaves, indicating that Aluminium (Al) inhibits on the acceptor side of PSII (Jiang *et al.*, 2008). An increase of V_j and V_i were used as probe for the inhibition of the electron transport at the acceptor side of the PSII (Lu 1999). On the basis of obtained results no change in V_j and V_i suggests that no photoinhibition occurred under clinorotation. Thus it can be concluded that clinorotation does not affect

the acceptor side of PSII. The parameter performance index (PI abs) is used to determine the plant vitality with respect to different stress (Strasser *et al.*, 2004, Zheng *et al.*, 2009) An increase in performance index (PI abs) in response to clinorotation was mainly due to increase in the photochemical efficiency and photosynthetic electron transport. It is also accompanied by decreased ϕDo - quantum yield for energy dissipation. Increase in performance index (PI) in rice is about 27 % ($p < 0.05$) higher in clinorotated samples than control. It is also accompanied by decreased ϕDo - quantum yield (19.23 %; $p < 0.05$) for energy dissipation, Dio/RC - dissipation energy (38.54 %; $p < 0.05$) per reaction centre. Increase in performance index (PI) in mungbean is about 12.91% ($p < 0.05$) higher in clinorotated samples than control. It is also accompanied by decreased ϕDo - quantum yield (9.84 %; $p < 0.05$) for energy dissipation, Dio/RC - dissipation energy (12.33%; $p < 0.05$) per reaction centre.

Conclusion

The study revealed that simulated microgravity (SMG) led to increased growth, chlorophyll content, and chlorophyll fluorescence in both monocotyledonous rice and dicotyledonous mungbean seedlings, compared to control samples. However, the chlorophyll a/b ratio decreased significantly in SMG samples. Monocotyledonous rice showed a more pronounced improvement in growth and chlorophyll content compared to dicotyledonous mungbean. This pattern of response aligns with observations in other stress conditions. Monocotyledonous rice displayed greater adaptability to SMG than dicotyledonous mungbean. Further research at the cellular and molecular levels is needed to understand these adaptive mechanisms and potentially identify marker proteins or growth hormones associated with environmental stresses, including gravity.

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Conflict of Interest

The Authors declare no Competing Financial or Non-Financial Interests.

Data Availability Statement

Not applicable

Ethics Approval Statement

Not Applicable

Authors' Contribution

Sagar S. Jagtap: Design, optimization and conduction of experiments, result analysis, manuscript writing and editing. S M Kamble: Conduction of experiments, result analysis, Jyotsana Dixit: Result analysis, manuscript writing and editing, Pandit Vidyasagar: Experimental planning, manuscript editing and revision, supervisor.

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