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RESEARCH ARTICLE

Effects of simulated microgravity on rice (MR 219) growth and yield

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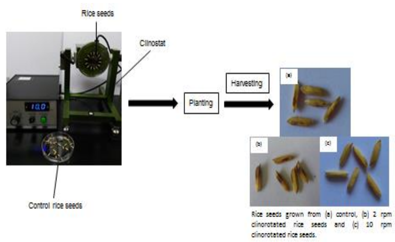
Graphical abstract



Abstract

Rice (*Oryza sativa* L.) is the staple food for many Asian countries and its demand is continuously increasing. However, the production of high quality rice seeds is still insufficient. Researches on plants growth in space related to the exposure of microgravity environment are rare, costly and limited by time. Similar experiments can be conducted on the ground to simulate microgravity condition using a 2-D clinostat which compensates unilateral influence of gravity. This study was conducted to establish a simple and cost effective technique to enhance the quality of the Malaysian rice seed variety MR 219 by using a 2-D clinostat and to determine the effects of simulated microgravity on the growth and yield of the rice seeds. The experiments were performed at different rotation speeds (2 rpm and 10 rpm) for 10 days at room temperature. The rice growth and yield parameters were measured every 2 weeks and at harvest time (day 110), respectively. The data were analysed using the MINITAB software. The mean values were compared using analysis of variance (ANOVA) and Tukey test were performed at 0.05 significance level. The significant differences in the number of tiller (44/plant), stem width (1.1 cm), chlorophyll content (42.7 SPAD value), weight of grains and panicles (65.8750 g) and total grain weight per plant (46.7500 g) were identified. The highest means were mostly found in 10 rpm clinorotated rice seeds. In general, plants grown from 10 rpm clinorotated seeds are also more resistance to the rice diseases (rice blast disease, rice tungro disease and hopper burn). These results suggest that simulated microgravity using a 2-D clinostat affected several rice (MR219) growth and yield parameters significantly.

Keywords: *Oryza sativa*, 2-D clinostat, simulated microgravity, growth and yield

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important cereal crop after wheat and has become the staple food nearly half of the world's population (Maclean *et al.*, 2002; Siwar *et al.*, 2013; Yogambigai *et al.*, 2015). Two species cultivated rice are *Oryza sativa* which is grown worldwide and *O. glaberrima* which is grown mostly in West Africa (Maclean *et al.*, 2002). Tiara *et al*. (2015) reported that rice is cultivated as a rainfed or irrigated lowland crop and mainly grown in Kedah, Perak and Kelantan in Peninsular Malaysia.

In Malaysia, Malaysian Agricultural Research and Development Institute (MARDI) as an important institution in breeding and producing rice seeds such as MR 84, MR 159, MR 167, MR 185, MR 211, MR 219, MR 220, MRQ 34, MRQ 50, MRQ 74, Pulau Siding and Pulut Hitam (Wan, 2006). Currently, MR 219 rice variety is currently the most commonly cultivated rice varieties in Malaysia due to its grain size and number of grains per panicle are greater than previous introduced local varieties (Yusuff *et al.*, 2014). Although the average yield of rice in Malaysia has been reported as 4.5 – 5 t/ha in 2015, but Malaysia still has to import the rice from the other countries (Tiara *et al.*, 2015).

Many investigations have been carried out involving clinorotation of various plants on the clinostat with the speed ranging from 0.25 to 3 rpm (Jagtap *et al.*, 2011a). For example, the study on rice seedlings variety of PRH-10 clinorotated continuously on a slow-rotating clinostat at the speed of 2 rpm for 3, 5 and 7 days which resulted in an increase in root length, shoot length, weights of seedlings and chlorophyll contents. Another study focuses on the rice yield attributes which demonstrated that the number of productive tillers, height of tiller from soil, number of tassels, length of panicles, number of grains per panicle, filled grains per panicle as well as number of filled grains per plant, total grain weight per plant and weight of 1000 grains increased significantly with clinorotation at 2 rpm for 12 days. Total chlorophyll content and protein content also increased in clinorotated samples, however decrease in carbohydrate and starch contents were also observed (Jagtap *et al.*, 2011b). Besides that, clinorotation of soybean seedlings at 1 rpm had influenced their growth, morphology and double ethylene production (Hilaire *et al.*, 1996). Clinorotation of rapeseed (*Brasicca napus*) on 2-D for 5 days using the speed of 1 rpm showed increase in fresh weight of root system, isocitrate lyase activity and ^{14}C-labeled sucrose in the cotyledons of clinorotated seedlings (Aarrouf *et al.*, 1999).

Therefore, the objectives of the study are to establish a simple and cost effective technique to enhance the quality of the rice seed MR 219 by using 2-D clinostat, and to determine the effects of simulated microgravity on the growth and yield of the rice.

EXPERIMENTAL

Rice seeds

One kilogram of the rice seeds of variety MR 219 were obtained from MARDI Seberang Perai, Pulau Pinang.

Experimental site and duration

This experiment was conducted in the glasshouse at the Engineering Research Center, MARDI Headquaters, Serdang from May 2016 to September 2016.

Simulated microgravity experiments

Before clinorotation, the rice seeds were screened in terms of size, uniformity and defects, and cleaned to remove dirt, husk, stones and other inert materials. Subsequently, 14 seeds were placed on a double-sided tape and mounted on a petri dish and placed at a distance of 1.5 cm from the center of the circle of a clinostat with one rotational axis (2-D clinostat) (United Nations Office for Outer Space Affairs, UNOOSA). The clinostat was rotated at 2 rpm for 10 days at room temperature with six replications. Similar procedure was applied to another set of seed using another 2-D clinostat at 10 rpm for the same period. The experimental set-up for the treatment is shown in Fig. 1.

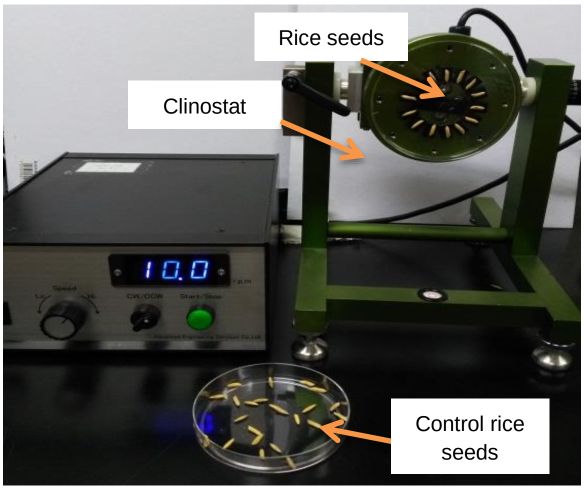


Fig. 1 Clinorotation of rice seeds (MR 219) on a 2-D clinostat and control.

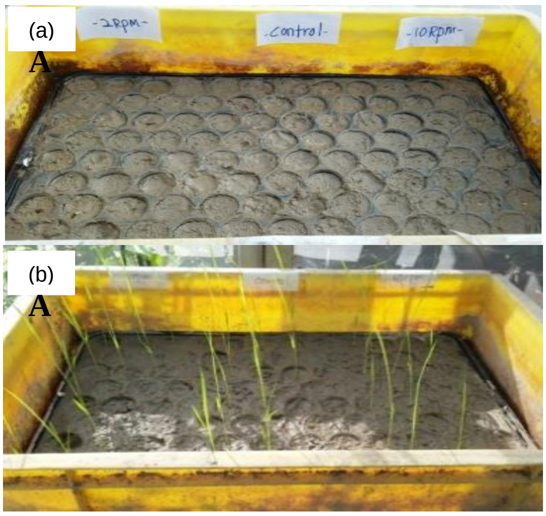


Fig. 2 Germinated seeds planted in trails (a) on the day of sowing (DAS) and (b) on 14th DAS.

Another 14 seeds were maintained at same environmental conditions but without clinorotation treatment and used as control. After clinorotation, the seeds were soaked for 1 day in a petri dish filled with distilled water for germination process. The germinated seeds were planted in a trail (64.5 cm × 41.3 cm × 14.5 cm) for two weeks (14 days) in an open area at the Engineering Research Center, MARDI. The trails were labeled as 2 rpm, 10 rpm and control (Fig. 2).

Each of the rice plants was transplanted into vases on 14th day after sowing (DAS). Number of tiller, height of tiller from soil, width of leaves, height of leaves and stem width were measured once in every two weeks using a ruler. Chlorophyll content of the leaves was measured as Soil-Plant Analyses Development (SPAD) value three times per week using a chlorophyll meter (SPAD-502, Minolta Corp.) during growth until 85th DAS (panicle initiation stage). The SPAD values were averaged from three SPAD readings taken from three different locations: top, middle and basal on the top of a leaf, based on modification of the method as described by Yuan *et al*. (2016). The numbers of panicle, length of panicle, size of seed, weight of grains and panicles, total grain weight per plant, filled grains per panicle, weight of 500 grains were measured on the day of harvest (110th day) after transplanting.

Fertilizers application

Fertilizers were applied at each vase using compound fertilizers including NPK blue and NPK green at a rate which was modified based on the fertilizers rate used by Elisa Azura *et al*. (2014). NPK green was top dressed at early stage (15th DAS) while NPK blue was applied during midtillering stage (35th DAS) and panicle initation stage (75th DAS).

Data collection

Data were collected on the number of tiller, height of tiller from soil, width of leaves, height of leaves and stem width once in every two weeks. Chlorophyll content was measured three times per week. The numbers of panicle, length of panicle, size of seed, weight of grains and panicles, total grain weight per plant, filled grains per panicle, weight of 500 grains were measured on the day of harvest (110th DAS).

Statistical analysis

Collected data were statistically analyzed using MINITAB software (version 17) to determine the significant difference among the treatments. The means difference between the treatments (2 rpm and 10 rpm) versus control and their 95% confidence interval were calculated accordingly. Analysis of variance (ANOVA) was used to compare the data of clinorotated seeds with those of the controls. Tukey test was used for pairwise comparisons between the mean values when the treatment effects were significant.

RESULTS AND DISCUSSION

Growth attributes of rice

(i) Number of tillers

Clinostat rotation at 10 rpm significantly affected the number of tillers positively versus control as indicated by *p*-value (≤ 0.05), where 10 rpm treated seeds had a significantly higher number of tillers (44/plant) as compared to that of control and 2 rpm (Table 1). In addition, the 95% confidence interval on the difference in the mean number of tillers of 10 rpm and control using Tukey's method are 1 and 10, respectively. The results agree fairly closely with the results obtained from ANOVA with *p*-value = 0.0226. This might due to higher ethylene levels in clinorotated seeds (10 rpm) as similar findings were reported by Carman *et al*. (2015). In addition, Harrison and Kaufman (1982) documented that ethylene play an important role in encouraging tiller swelling during the beginning of tiller emergence from apical dominance.

(ii) Height of tiller from soil

The height of tiller from soil was not varied significantly among treatments (2 rpm, 10 rpm and control) as indicated by *p*-value > 0.05. Since the *p*-value is large, there is no enough evidence to reject H_0: μ_1

= $\mu_2 = \mu_3$ and at least one of the statements $\mu_1 = \mu_2$, $\mu_1 = \mu_3$, $\mu_2 = \mu_3$ is true. However, 10 rpm had the highest height of tiller from soil (87.2 cm) (Table 1).

Table 1 Mean values of parameters of the rice seed variety MR 219 using rotation speed of 2 rpm and control (without clinorotation).

No.	Parameters	2 rpm	Control	10 rpm
1	Number of tiller per plant	43	39	44
2	Height of tiller from soil (cm)	86.1	85.9	87.2
3	Width of leaves (cm)	1.8	1.9	1.8
4	Height of leaves (cm)	39.8	42.2	42.5
5	Stem width (cm)	1.0	1.0	1.1
6	Chlorophyll content	35.9	33.1	42.7
7	Number of panicle	29	25	34
8	Length of panicle (cm)	25.9	26.2	25.0
9	Length of seed (cm)	0.9	0.9	0.9
10	Weight of grains and panicles (g)	51.750	44.125	65.875
11	Total grain weight per plant (g)	33.750	29.125	46.750
12	Filled grains per panicle	44	50	42
13	Weight of 500 grains (g)	13.2500	13.7500	13.2500

Bold = parameters that had significant difference between three means.

Table 2 F statistics values for test of significant differences.

No.	Parameters	F-value	*p*-value	Significant difference	Difference of level
1	Number of tiller per plant	4.00	0.0226	Yes	10 rpm and control
2	Height of tiller from soil (cm)	0.59	0.5561	No	-
3	Width of leaves (cm)	2.31	0.1064	No	-
4	Height of leaves (cm)	0.41	0.6638	No	-
5	Stem width (cm)	3.18	0.0473	Yes	10 rpm and control
6	Chlorophyll content	14.91	<0.0001	Yes	10 rpm and control; 10 rpm and 2 rpm
7	Number of panicle	2.40	0.1464	No	-
8	Length of panicle (cm)	0.69	0.5255	No	-
9	Length of seed (cm)	0.11	0.8960	No	-
10	Weight of grains and panicles (g)	4.55	0.0431	Yes	10 rpm and control
11	Total grain weight per plant (g)	14.00	0.0017	Yes	10 rpm and control; 10 rpm and 2 rpm
12	Filled grains per panicle	1.46	0.2821	No	-
13	Weight of 500 grains (g)	0.71	0.5191	No	-

Bold = parameters that had significant difference between three means.

(iii)Width of leaves

Based on the statistical analysis, there were no significant differences between the widths of leaves among the treatments (2 rpm, 10 rpm and control) since the *p*-value is larger than $\alpha = 0.05$. In this case, the null hypothesis is accepted at $\alpha = 0.05$. However, control plants had slightly higher widths of leaves (1.9 cm) compared to both treatments (Table 1).

(iv) Height of leaves

Similar trend was observed for height of leaves among the treatments (2 rpm, 10 rpm and control) as the *p*-value > 0.05 and it can be concluded that height of leaves was not affected by clinorotation. Nevertheless, 10 rpm showed the highest height of leaves (42.5 cm) (Table 1).

(v) Stem width

Stem widths was vary significantly (*p*-value $\leq$ 0.05) among the treatments (2 rpm, 10 rpm and control). The result was supported by the statistical analysis, where the confidence interval for the difference between the means of 10 rpm and control extends from 0.00225 and 0.10975 which showed that this range does not include zero, indicates that the difference is statistically significant. The results agree fairly with the results obtained from ANOVA (*p*-value = 0.0473) and Tukey test as shown in Table 2. Moreover, the stem width (1.1 cm) (Table 1) of 10 rpm treated sample was the highest compared to that of control and 2 rpm.

(i) Chlorophyll content

The highest chlorophyll content was showed by 10 rpm treatment (42.7) (as shown in Table 1). The differences are statistically significant since the *p*-value of the F test is so small (<0.0001) (Table 2), there was sufficient evidence to reject the null hypothesis. Moreover, Tukey test results indicated that confidence intervals of mean differences of 10 rpm *versus* control and 10 rpm *versus* 2 rpm did not contain zero, it can be concluded that at least one of the statements $\mu_1 = \mu_2$, $\mu_1 = \mu_3$ and $\mu_2 = \mu_3$ is false. Higher chlorophyll content in leaves may due to higher volume of stromal thylakoids per chloroplasts and increase in the number of chloroplast and mitochondria caused by modified chloroplast structures, significant bends and loose arrangement of thylakoids in plants rotated under slow clinorotation as reported by Jagtap *et al*. (2011a).

Rice yield parameters

(i) Number of panicle

From the result of one-way ANOVA (Table 2), it was found that *p*-value is higher than $\alpha = 0.05$ and there was an overwhelming evidence in accepting $H_0 = \mu_1 = \mu_2 = \mu_3$. Therefore there were no significant differences found between the numbers of panicle between the treatments (2 rpm, 10 rpm and control). However, 10 rpm showed the highest number of panicle (34) (Table 1).

(ii) Length of panicle

There were no significant differences (*p*-value > 0.05) found between the lengths of panicle between the treatments (2 rpm, 10 rpm and control). The fact is that the null hypothesis is true, so the decision was to accept the null hypothesis. Nevertheless, control showed the highest number of panicle (26.2 cm) (Table 1). Further test using Tukey's method also showed all pairs of means (2 rpm *versus* control; 10 rpm *versus* control; 10 rpm *versus* 2 rpm) are not significant.

(iii) Length of seed

The length of seed did not vary significantly (*p*-value > 0.05) among different treatments. In addition, all treated plants (2 rpm and 10 rpm) as well as control have similar lengths of seed (0.90 cm) as shown in Table 1. Therefore, there was not enough evidence to conclude that at least one mean is different from the others.

(iv) Weight of grains and panicles

There was statistical significance between the means of weight of grains and panicles. In addition, Tukey test results showed that the comparison between 10 rpm and control was significant at 0.05

significance level with non-overlapping confidence interval (1.314, 42.186). This result agrees with ANOVA statistical test (Table 2) with *p*-value = 0.0431. Based on these data, with an experiment-wise confidence of 95%, it can be concluded that there is insufficient evidence to accept the equality of the means for control, 10 rpm and 2 rpm. Table 1 shows that 10 rpm treated seeds had the highest mean of weight of grains and panicles (65.875 g) compared to control (44.125 g) and 2 rpm (51.750 g) treated seeds.

(v) Total grain weight per plant

Similarly, total grain weight per plant also did vary significantly at 0.05 significance level for at least one pair of mean. Further analysis using Tukey test showed the confidence interval for the difference between the means of pairs 10 rpm *versus* control (7.979, 27.271) and 10 rpm *vs* 2 rpm (3.354, 22.646) did not contain zero which indicated that the difference are significant at 95% confidence level. Higher total grain weight might be due to the increase in chlorophyll content of leaves as found in 10 rpm treated seeds, which led to higher photosynthetic rate, thus more photosynthates production (Chakrabortty *et al*., 2014).

(vi) Filled grains per panicle

Based on ANOVA results (Table 2), there were no significant differences (*p*-value > 0.05) found between the mean of filled grains per panicle among the treatments (2 rpm, 10 rpm and control). Therefore, the decision was to accept the null hypothesis as there were no differences among the pair means.

(vii) Weight of 500 grains

Treatments at 2 rpm and 10 rpm produced the plants that have similar weights of 500 grains (13.2500 g) which were lower than that of control (13.7500 g) (Table 1). Since the *p*-value > 0.05, thus the decision was to accept the null hypothesis. Furthermore, there was insufficient evidence to conclude that at least one mean was different from the others at 95% confident level.

Effects of simulated microgravity on disease resistance

Generally, plants grown from 10 rpm treated seeds were more resistant to rice diseases (rice blast disease, rice tungro disease and hopper burn) compared to the plants grown from 2 rpm treated seeds and control. The results can be seen in Table 2 since the rice growth and yield parameters including number of tiller per plant, stem width, chlorophyll content, weight of grains and panicles and total grain weight per plant were statistically significant in 10 rpm of clinorotation treatment. However, the rice diseases had attacked both plants grown from control and 2 rpm treated seeds. These diseases were the main problems found at the experimental site-which infected all rice plants in the vases of 2 rpm and control in different DAS.

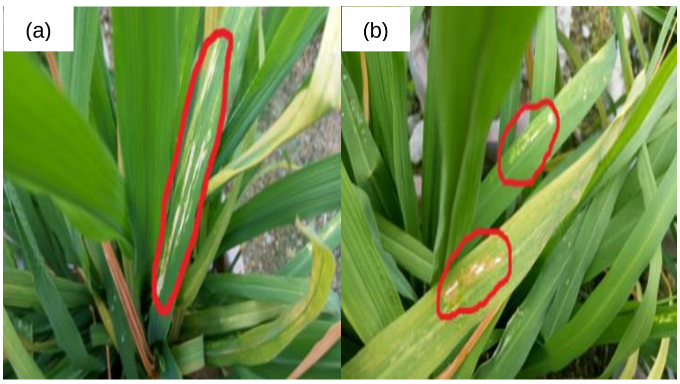


Fig. 3 Lesions on the rice leaves grown from (a) control and (b) 2 rpm clinorotated rice seeds on 83thDAS.

Rice blast disease, the most serious foliar fungal disease, caused by a seed-borne fungus *Mangnaporthe oryzae* (anamorph: *Pyricularia grisea*) which accounts for annual rice yield loss up to 30% (Joe *et al.*, 2015; Hubert *et al*., 2015; Marcel *et al.*, 2010). Based on the observations, the symptoms include lesions on leaves were observed on the rice leaves grown from control rice seeds and rice seeds clinorotated at 2 rpm and control on 83th DAS as shown in Fig. 3 (a) and (b), respectively. In addition, lesions were also found on rice seeds of the plants raised from control seeds and seeds exposed to slow clinorotation of 2 rpm, but not on 10 rpm on 110th DAS as shown in Fig. 4 (a), (b) and (c) respectively.

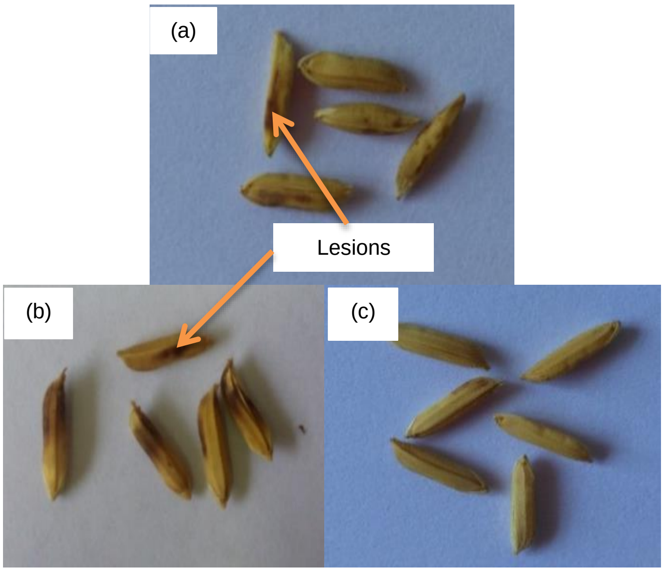


Fig. 4 Lesions on the rice seeds grown from (a) control and (b) 2 rpm clinorotated rice seeds but not on (c) 10 rpm clinorotated rice seeds on 110th DAS.

Invasion of brown planthopper (BPH) and green leaf hopper also affect rice growth and yield since these insects suck out the nutrients from rice plants which cause the plants had stunted growth during their development. Green leaf hopper (*N. vireseens*) transmitted two viruses, namely rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) (Azzam and Chancellor, 2002). Brown plant hopper (BPH), the most destructive pest of rice cause hopper burn which feeding near the base of tillers, usually occurs at tillering to panicle initiation stage. At early BPH infestation, the appearance of yellow patches and drying up of the plants known as "hopper burn" (Fig. 5) were observed on 36th DAS (tillering stage) which resulted in the stunted growth of rice plants grown from rice seeds clinorotated at 2 rpm. This infestation also affected rice plants grown from control rice seeds.

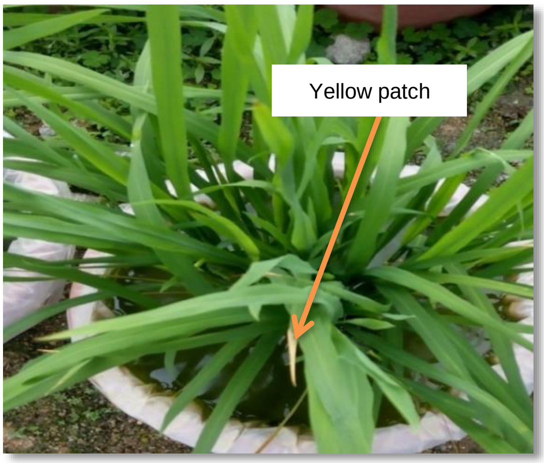


Fig. 5 Yellow patches formation at the end of the rice leaves of 2 rpm clinorotated rice seeds on 36th DAS.

Higher number of adult brown plant hoppers (BPH) were mostly found in rice plants grown from control seeds and 2 rpm treated seeds but few in rice plants grown from 10 rpm treated seeds (Fig. 6) on 82th DAS.

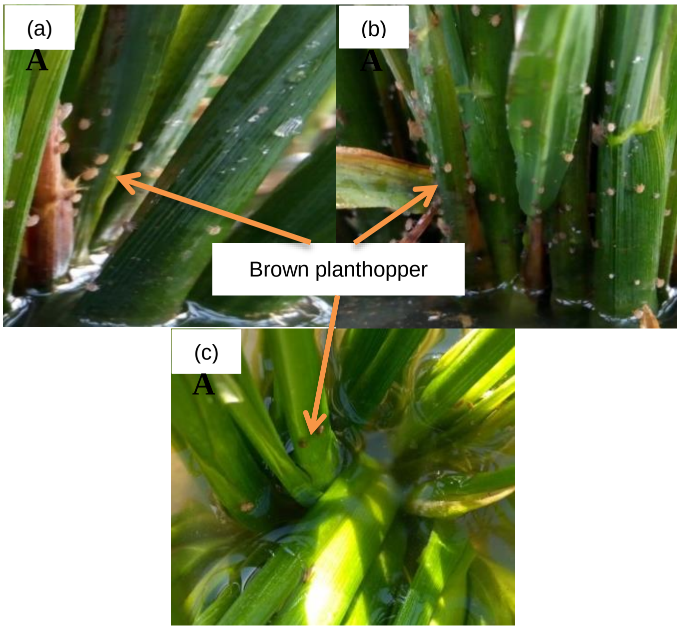


Fig. 6 Adult brown planthopper invasion at the tiller base (usually at leaf sheaths) of (a) control, (b) 2 rpm and (c) 10 rpm rice plants on 82nd DAS.

In addition, based on our observation, rice tungro disease started to invade the rice plants grown from control and 2 rpm treated seeds during the vegetative phase (at heading phase) on 75th DAS (Fig. 7). Consequently, yellow-orange discolouration of leaves was found on both plants (Fig. 8).

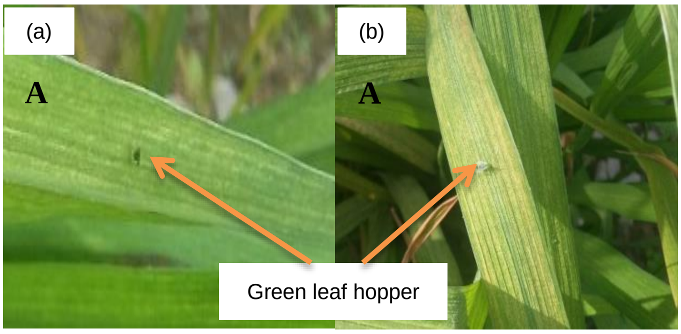


Fig. 7 Green leaf hoppers on leaves of plants grown from (a) control and (b) 2 rpm clinorotated rice seeds on 75th DAS.

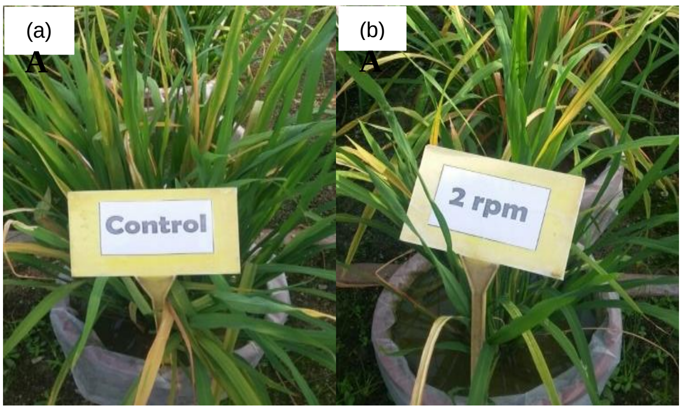


Fig. 8 Yellow-orange leaves discolouration on plants grown from (a) control and (b) 2 rpm treated rice seeds on 83rd DAS.

CONCLUSION

Rice seedlings were clinorotated and germinated after a few days of clinorotation and grown to observe their response towards simulated microgravity treatment in this study. The results of this study have demonstrated that short exposure to clinorotation did affect several rice growth and yield parameters significantly including the chlorophyll content and total grain weight per plant that were significantly affected by both treatments (2 rpm and 10 rpm), meanwhile the other parameters namely; number of tiller per plant, stem width and weight of grains and panicles were significantly affected only by 10 rpm. Contrarily, height of tiller from soil, width of leaves, height of leaves, number of panicle, length of panicle, length of seed, filled grains per panicle and weight of 500 grains did not affected by clinorotation treatments. These data suggest that the differences in rice growth and yield parameters between treated plants and control plants are caused by clinorotation of the rice seeds as all other aspects were controlled in each treatment. In general, plants grown from 10 rpm clinorotated seeds are more resistance to the rice diseases (rice blast disease, rice tungro disease and hopper burn). It can be concluded that simulated microgravity conditions provide a beneficial way in improving rice growth and yield.

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