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Effect of Simulated Microgravity on Growth and Yield Parameters of Rice Seeds MR 219

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Rice (*Oryza sativa* L.) is a staple food for majority of Asian people, therefore it is the most important crop in the region. However, the current rice production is unable to meet the continuously increasing demand due to increasing of the population. Rice seeds can be treated to improve the rice production, and one of the possible techniques is to treat the rice seeds under simulated microgravity conditions. In this study, a 2-D clinostat was used to treat Malaysian rice seed (MR 219) at 10 rpm for 10 days. The effects of simulated microgravity on growth and yield of this rice were evaluated. The following parameters were significantly affected by simulated microgravity: height of tiller from soil, leave length, leave width, stem width, chlorophyll content, number of panicles, filled grains per panicle, number of filled grains per plant and 1000-grain weight. Therefore, it can be said that better rice production could be achieved from the rice seeds treated under simulated microgravity conditions using a 2-D clinostat by a rotation of 10 rpm for 10 days.

Keywords: 2-D clinostat; simulated microgravity; MR 219; clinorotation; growth and yield

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for majority of Asian people. It is forecasted that the rice production has to be increased to at least 70% more due to the increase in human population by year 2025 (Kim and Krishnan, 2002). In addition, there was a decrease of 3% in rice production in 2016 as compared to 2015 (FAO, 2016). Factors such as unfavourable environmental conditions and limited material inputs, and inefficient cultural technologies and knowledge cause a huge gap between economically optimal and actual yields resulting in low yields (Faghani *et al.*, 2011; Vijayalaxmi *et al.*, 2016). Consequently, various approaches have been applied to improve the yield of a crop.

Research under altered gravity such as microgravity and hyper gravity has provided considerable findings that are related to the impact of gravity on the biological processes of plants, gravity-sensing mechanisms and on the organism's gravity-based orientation. The level of "microgravity" extends from 10^{-3} to 10^{-6} g, which dependent on the location in the spaceship and the vibration frequency (Tryggvason *et al.*, 2001; Penley *et al.*, 2002; Jules *et al.*, 2004).

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One of the common microgravity simulators that are used in gravitational biology is clinostat (Anken, 2013). Clinostat is a rotating device that rotates the samples perpendicularly to the gravitational field to avoid the biological system in recognizing the gravitational acceleration vector (Herranz *et al.*, 2013). Slow-rotating clinostat with a horizontal axis is the most extensively used as microgravity simulator that compensates the unilateral influence of gravity (Hoson, 2014; Kraft *et al.*, 2000).

The effect of gravity on plant growth and positive growth behaviours under the influence of clinostat have been emphasised by previous researchers (Mouhamad *et al.*, 2016). Many researchers have investigated its effect on the growth, development and metabolism of the plants including *Arabidopsis thaliana*, garden cress seedlings, rice, almond, apricot, apple, broad bean, white clover and many other plants (De Micco *et al.*, 2006; Jagtap *et al.*, 2011; Polinski *et al.*, 2017; Smith *et al.*, 1999; Yamada *et al.*, 1993). Nevertheless, to the best of our knowledge, this approach has not been investigated extensively in the open field of paddy in our country.

Hence, the main objective of this study is to determine the effects of simulated microgravity on growth and yield of Malaysian rice seed (variety of MR 219) using a 2-D clinostat.

II. MATERIALS AND METHODS

A. Rice Seeds

Ten kilograms of rice seeds (MR219) were collected from MARDI at Seberang Perai, Pulau Pinang, Malaysia.

B. Study Site and Duration

This study was conducted in open area at the Engineering Research Center, MARDI Headquarters, Serdang, Selangor, Malaysia from March to July 2016.

C. Treatments

The rice seeds (mean of length: 0.94 ± 0.067 cm) with minimum defect and uniform in size were used in this study. In order to expose the rice seeds with simulated microgravity condition, fourteen seeds were mounted on a petri dish which placed on a 2-D clinostat with a distance of 1.5 cm from the centre of clinostat. The rice seeds were rotated based on the optimum conditions (10 rpm for 10 days at 1.5 cm from the centre of clinostat) of 2^3 factorial design. Another 14 seeds without clinorotation were used as control. The experimental set-up of the clinorotation is presented in Figure 1.

Figure 1. The experimental set-up of the clinorotation of rice seeds MR 219

D. Germination and Plantings

The clinorotated and control seeds were soaked in 200 mL of distilled water in different plastic containers for 24 hours. Then, the seeds were placed on moist tissue paper for germination for a period of 24 hours. Germinated seeds were grown in a seedling tray with dimension of 64.5 cm × 41.3 cm × 14.5 cm for 20 days. The rice plants were transplanted into different pails on 20th day after sowing (DAS). The rates of NPK compound fertilizers and urea application were employed as suggested by Elisa *et al.* (2014) and Siavoshi *et al.* (2011), respectively.

The fertilizers were applied at week 5 (35 DAS), week 8 (55 DAS) and week 11 (75 DAS). The experiments were conducted in three replications. The image of the rice plants grown from control and treated seeds at 35 DAS is shown in Figure 2.

Figure 2. Rice plant samples inplastic pails (35 DAS)

E. Data Collection

Growth and yield parameters were measured in order to investigate effects of clinorotation on the growth and yield of the rice seed. For these purposes, all the tillers were counted and 30% of the tillers of rice plants were labelled and the height of the tiller from soil, leaves length, leaves width and stem width were measured in every two weeks. The chlorophyll content was recorded weekly using a Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502, Minolta Corp.).

On 130th DAS, the rice was harvested, and the following parameters were recorded: number of panicles, length of panicle, number of grains per panicle, filled grains per panicle, number of filled grains plant and 1000-grain weight.

F. Statistical Analysis

To evaluate the significant difference between control and treatments, the data of this study were subjected to statistical analysis including Student's t test at 0.05 significance level using Minitab software version 17.

III. RESULTS AND DISCUSSION

MR 219 rice seeds were developed by Malaysian Agricultural Research and Development Institute (MARDI) and chosen for this study due to its good yield and shape quality (Panjaitan *et al.*, 2009).

A. Growth Parameters

Various rotation speeds of clinostats (1-10 rpm) were applied to study gravitropism of plants (Anken, 2013; Herranz *et al.*, 2013). According to Hasenstein and van Loon (2015), parts of plants will respond to gravity and results in noticeable side effects such as spiral combination of movements caused by sedimentation and centrifugation when clinostat rotation is too slow. Thus, 2 and 10 rpm of rotation were applied in 2^3 factorial design in our study. However, the results reported in this study was the optimum results obtained from 2^3 factorial design, where 2 and 10 rpm were applied in the factorial design. The optimum rotation speed was found to be 10 rpm.

Table 1 shows that mean values of growth and yield parameters of clinorotated and control of MR 219 rice plants and their t-statistics values for test of significant differences after 90 DAS. The data shows that except for number of tillers per plant and number of grains per panicle, the other parameters for the clinorotated rice plants are greater than those of the control seeds. The *p*-value of less than 0.05 indicates that the height of the tiller improved significantly with the treatment.

The significant effect on leave length is probably due to elongation of microfilament in clinorotated rice cells as reported by Shevchenko *et al.* (2007).

Another important growth parameter namely chlorophyll content was also affected by clinorotation significantly. This observation is similar to the findings reported by Mouhamad *et al.* (2016). They found that amino acids which aid the increase of the chlorophyll content were present in two varieties of rice grown under clinorotation. The increase of the chlorophyll content in plants grown from clinorotated seeds might be also due to the increment in stromal thylakoids volume per chloroplast and modified chloroplast structures (Kordyum and Adamchuk, 1997) which cause higher number of chloroplast and mitochondria as well as bends and loose arrangement of thylakoids as reported by Popova (2006).

However, the number of tillers between rice plants grown from 10 rpm clinorotated treated and control seeds were not significant difference as indicated by the p-value = 0.824 which is greater than 0.05 (Table 1), where the treated seeds produced plants which have similar number of tillers as compared to that of the control.

B. *Yield Parameters*

Number of panicles per plant grown from treated seeds and control were 62 and 43, respectively, showing that the number of panicles was significantly affected by clinorotation at 5% significance level (Table 1) as the mean value of panicles increased significantly in plants grown from treated seeds.

However, clinorotation of rice seeds at 10 rpm did not affect the length of panicles significantly. The treated seeds produced rice plants which have slightly higher length of panicles (23.17 cm) compared to those from control (23.07 cm) as presented in Table 1.

Table 1. Mean values of growth and yield parameters of clinorotated and control of MR219 rice plants and their t-statistics values for test of significant differences after 90 days after sowing

No.	Parameters	Control	10 rpm	*p*-value	95% confidence interval
1	Number of tillers per plant	56	56	0.824	(-1.710, -2.141)
2	Height of tiller from soil (cm)	63.87	68.93	0.000	(-7.015, -3.054)
3	Leaves length (cm)	42.79	46.17	0.010	(-5.95, -0.81)
4	Leaves width (cm)	1.23	1.25	0.043	(-0.073, -0.001)
5	Stem width (cm)	1.02	1.15	0.000	(-0.234, -0.095)
6	Chlorophyll content	38.0	41.5	0.000	(-4.158, -2.826)
7	Number of panicles	43	62	0.026	(-34.30, -4.37)
8	Length of panicle (cm)	23.07	23.17	0.911	(-2.716, 2.516)
9	Length of seed (cm)	0.92	0.94	0.577	(-0.062, 0.035)
10	Number of grains per panicle	131	130	0.841	(-29.79, 33.12)
11	Filled grains per panicle	24	95	0.001	(-90.07, -53.26)
12	Number of filled grains per plant	957	5418	0.035	(-4994, -3926)
13	1000-grain weight (g)	23.0600	28.9601	0.009	(-10.36. -1.43)

Length of rice seeds did not significantly affect by the treatment as showed by *p*-value of 0.577 (Table 1). However, rice seeds grown from 10 rpm of clinorotation treatment were found to have less lesions as compared to that of control rice seeds (Figure 3). Lesions on rice seeds is a symptom of rice blast disease caused by a fungus, *Magnaporthe oryzae (*Faivre-Rampant *et al*., 2013).

The results from Table 1 revealed that treated seeds produced rice plants which have slightly lower number of grains per panicle (130), compared to the control (131), thus clinorotation of rice seeds at 10 rpm did not affect the number of grains per panicle.

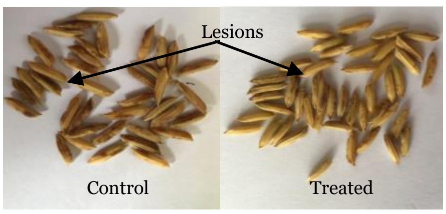

Figure 3. Lesions on treated and control rice seeds

This is supported by p-value of Student's t test which is larger than 0.05 and the value ($\mu1-\mu2$) = 0 was concluded at the confidence interval, where it was possible that these two means were equal.

However, significantly higher filled grains per panicle was recorded in treatment (95) compared to control (24) as indicated by *p*-value = 0.001 at 95% confidence interval. Consequently, significantly higher number of filled grains per plant (5418) from rice plants produced from treated seeds was also recorded as shown in Table 1 with non-overlapping confidence interval (-4994, -3926) at 0.05 significance level.

There was significant difference in 1000-grain weight between treatment and control at α = 0.05, since p-value (0.009) is less than 0.05. Table 1 shows significantly higher 1000-grain weight in treatment (28.9601 g) than control (23.0600 g).

Nevertheless, several growth and yield parameters (number of tillers per plant, length of panicle, length of seed and the number of grains per panicle) were not significantly affected by the clinorotation, might due to the fact that the slow-rotation by the clinostat can produce a stressful condition in the plant cell which cause statoliths continuously hit the cell wall, and resulted in unusual effects to the plant metabolisms at the ultrastructural and molecular levels due to the overloading of the gravitational stimulation (UNOOSA, 2013; Hader *et al.*, 2005).

IV. CONCLUSION

The study on the influence of simulated microgravity on the growth and yield of Malaysian rice variety MR 219 was conducted in an open field. The results revealed that height of tiller from soil, leaves length, leaves width, stem width, chlorophyll content, number of panicles, filled grains per panicle, number of filled grains per plant and 1000-grain weight were significantly affected by clinorotation at 10 rpm for 10 days using a 2-D clinostat. It can be said that slow clinorotation at 10 rpm for 10 days can improve several growth and yield parameters and potential to increase rice yield of MR219. The investigation on the relationship between the expression levels of rice yield-related genes and yield performances under altered gravity condition is recommended for future study.

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