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GREEN APPROACH OF RICE (MR 219) TREATMENT USING A 2-D CLINOSTAT: FACTORIAL DESIGN ANALYSIS

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Abstract: A sustainable and green approach was applied to treat rice (MR 219) in order to improve the rice yield (1000-grain weight) using a 2-D clinostat. A 2^3 full factorial experimental design technique was used to investigate the effects of distances of rice seeds from the center of clinostat (1.5 and 2.5 cm), rotation speeds (2 and 10 rpm) and rotation periods (5 and 10 days) on 1000-grain weight of rice (variety of MR 219) at two levels (low and high). The results were analyzed using Student's *t*-test, analysis of variance and *F*-test by Minitab software to determine the significant factors that affected the yield. Main effects and interaction effects of the factors were also analyzed statistically. The most significant variable was found to be the distance of rice seeds from the center of clinostat followed by two interaction effects of rotation period and distance of rice seeds from the center of clinostat. Higher rice yield (28.9601 g of 1000-grain weight) was achieved by the rice seeds treated with the clinorotation compared to that of control (23.0600 g).

Keywords: 1000-grain weight, 2-D clinostat, factorial design analysis, rice yield, sustainability.

Introduction

Rice is the staple food in Malaysia, thus sufficient rice supply is essential to ensure the food safety and sustainability. As a result, effective rice production is crucial and one of the alternatives to enhance rice production could be achieved by increasing the rice yield per unit area. Living organisms, including plants have evolved and adapted to Earth's gravity to grow and develop, however, external conditions, such as microgravity, bring a new environment for organisms to adapt and to live well (De Micco *et al.*, 2006). Research under altered gravity, such as microgravity and hypergravity, has provided many findings related to the impact of gravity on the biological processes of plants, gravity-sensing mechanisms and gravity-based orientation of the organism (Anken, 2013). Microgravity is low or small gravity, also known as weightlessness (Herranz *et al.*, 2013). Since real space microgravity experiments are expensive, scientists have developed mechanical devices that can simulate microgravity conditions on the ground. These microgravity simulators compensate real microgravity environment in space. There are a few common microgravity simulators, such as clinostats, random positioning machine and rotating-wall vessel (Anken, 2013). Clinostat is a rotating device that spins samples perpendicularly to the gravitational field to avoid the biological system recognizing the gravitational acceleration vector (Herranz *et al.*, 2013). The rotation speeds of 1-10 revolutions per minute (rpm) of clinostats have been used to study gravitropism of plants to hinder the gravity-triggered growth response (Anken, 2013; Herranz *et al.*, 2013).

Simulated microgravity techniques have been applied on several crops. Hilaire *et al*. (1996) reported that clinorotation of soybean seedlings at 1 rpm for 7 days influenced their growth, morphology and double ethylene production. The results showed significant increase in root length and fresh weight of clinorotated seedlings by 125% and 42%, respectively. From a study on the effect of clinorotation on rice seedlings variety of PRH-10 at the rotation speed of 2 rpm for 3, 5 and 7 days, increased root and shoot lengths and weights were observed (Jagtap *et al.*, 2011a). In another study, rice yield attributes, including 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 1,000 grains increased significantly with clinorotation of rice seeds at 2 rpm for 12 days. Total chlorophyll content and protein content also increased in the clinorotated samples (Jagtap *et al.*, 2011b). Moreover, treatment of tomato plantlets on a 3-D clinostat increases the activities of both peroxidase (PO) and superoxidase dismutase (SOD) (Chen *et al.*, 2015). A study by Akomolafe *et al.* (2016) showed that growth of wheat cultivars was enhanced under simulated microgravity condition using clinostat. Later, Yuan and Xu (2017) reported that there was a significant increase in starch content (approximately 2-fold up-regulation) in the early phase in a duckweed species (*Lemna aequinoctialis* and *Wolffia globosa)* under simulated microgravity conditions. Also, the relative growth rate of both duckweed species (*Lemna aequinoctialis* and *Wolffia globosa*) was enhanced under simulated microgravity condition. Interestingly, the results from the study by Oluwafemi and Olubiyi (2019) showed that the growth rate of roots of corn seeds was improved by 29.05% under simulated microgravity conditions. Recently, Nakajima *et al*. (2021) documented that fresh weight, water content and lengths in the clinostat seedlings of mung bean were enhanced. Additionally, aquaporin expression and the amylase gene were also upregulated as well as significant higher amylase activity under clinorotation.

Nowadays, development of sustainable and green technology has become the focus of numerous researchers. Therefore, using a simple, sustainable and green technology to increase rice yield is our main concern as it can reduce environmental pollution. However, appropriate rotation speed and rotation period as well as treating rice seeds at optimum distance of rice seeds from the center of rotation must be taken into considerations to treat the rice seeds under simulated microgravity condition for optimum local rice production. In a previous study, Jagtap *et al*. (2011a) explored the effect of rotation periods (3, 5 and 7 days) at constant rotation speed (2 rpm) and the distance of rice seeds from the axis in a circle (1.5 cm). Contrarily, Jagtap *et al*. (2011b) only clinorotated rice seeds at constant rotation speed (2 rpm), rotation period (12 days) and the distance of rice seeds from the axis in a circle (1.5 cm). In our study, the effects of rotation speeds (2 and 10 rpm), rotation periods (5 and 10 days) and the distance of rice seeds from the center of clinostat (1.5 and 2.5 cm) were investigated in simulated microgravity conditions. In this study, the distance of the rice seeds from the center of clinostat is one of the investigated factors because it is an important in generating microgravity as the magnitude of this gravity depends on the radius of the centrifuge and the rotation speed (van Loon, 2016). In addition, there is a lack of studies on the effect of the distance of rice seeds from the center of clinostat on rice yield.

Therefore, in this present study, we attempted to determine the significant factors (rotation speed, rotation period and the distance of rice seeds from the center of clinostat) to improve the rice (variety MR 219) yield in terms of 1000-grain weight.

Materials and Methods

Experimental Design

Factors of rotation speed (A), rotation period (B) and the distance of rice seeds from the center of clinostat (C) were constructed using factorial designs of 2^3 by Minitab 17 software in order to evaluate their effects on the responses of growth and yield parameters. The experimental design was performed using Minitab 17 software (Table 1).

Treatment of Rice Seeds

Clinorotation was performed to treat clean, uniform and minimum defect rice seeds MR 219 using a 2-D clinostat (United Nations Office for Outer Space Affairs, UNOOSA). The rice seeds were treated at different distances of rice seeds from the center of clinostat (1.5 and 2.5 cm), rotation speeds (2 and 10 rpm) and periods (5 and 10 days) as presented in Table 2. The petri dish which was mounted with the rice seeds were placed on the 2-D clinostat and rotated based on the conditions shown in Table 2. The rice seeds without clinorotation were used as a control in this study.

Germination and Cultivation of Rice

The control and treated seeds were placed in 200 mL of distilled water in different beakers. After 24 hours, the seeds were collected and placed on moist tissue paper for germination of 1 day (Figure 1).

Table 1: Design matrix of 2^3 full factorial design

StdOrder	RunOrder	CenterPt	Blocks	Rotation Speed (rpm)	Rotation Period (day)	Distance of Rice Seeds from the Center of Clinostat (cm)
6	1	1	1	10	5	2.5
1	2	1	1	2	5	1.5
8	3	1	1	10	10	2.5
5	4	1	1	2	5	2.5
4	5	1	1	10	10	1.5
2	6	1	1	10	5	1.5
7	7	1	1	2	10	2.5
3	8	1	1	2	10	1.5

Table 2: High and low level of factors

Factor	Symbol	Low (-1)	High (+1)
Rotation speed (rpm)	A	2	10
Rotation period (day)	B	5	10
Distance of rice seeds from the center of clinostat (cm)	C	1.5	2.5

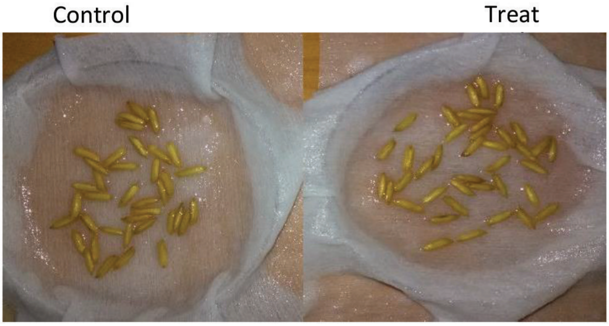

Figure 1: Control and treated rice seeds were placed on a moist tissue paper for germination

The germinated seeds were transferred and planted in a sowing tray (54 cm × 28 cm × 4.3 cm) placed in a plastic tray (64.5 cm × 41.3 cm × 14.5 cm) filled with 5 cm water for 20 days and then transplanted in different plastic pails (8 kg of loam clay/pail). After seedlings developed, the water level was maintained at 3 cm and 6 cm above the soil surface during early growth stage and at the later growth stage, respectively. Granular NPK compound fertilizers and urea were applied at 35, 55 and 75 days after sowing (DAS). A 2^3 full factorial experimental design was employed with a total number of 8 experimental runs (labeled as 1-8) to evaluate the effects of rotation speed, rotation period and the distance of rice seeds from the center of clinostat on 1000-grain weight of rice MR 219. The rates of NPK compound fertilizers and urea application were modified based on the rates reported by Elisa *et al*. (2014) and Siavoshi *et al*. (2011) respectively. The study was conducted in an open area at Engineering Research Centre, Malaysian Agricultural Research and Institution (MARDI), Selangor.

Data Collection and Analysis

On 130 DAS, the rice was harvested and 1000-grain weight were counted and the weight of the filled grains were measured using a balance. Statistical analyses of the experimental data were performed with Minitab software version 17 to compare the results between control and treatment at 0.05 probability level.

Results and Discussion

A 2^3 full factorial design was utilized to evaluate the significance and interactions of the factors including rotation speed (A), rotation period (B) and the distance of rice seeds from the center of clinostat (C) in this study. Low (denoted as -1) and high (denoted as +1) levels of each factor used in this study were based on the literatures (Aarrouf *et al.*, 1999; Jagtap *et al.*, 2011a; Jagtap *et al.*, 2011b; Yamada *et al.*, 1993). The experimental data was analyzed using statistical software Minitab version 17 including main effects and interaction effects. The mean values of the response (1000-grain weight) of the experiment were analyzed statistically and presented in Table 3. Higher rice yield (28.9601 g of 1000-grain weight) was achieved compared to that of control (23.0600 g) were documented in a previous report (Teoh *et al*., 2020).

Student's *t*-test and analysis of variance (ANOVA) was analyzed to determine the significant factors that affect the growth and yield of the rice parameters based on *p*-value with >95% confidence level. The small *p*-value (<0.05) indicates strong evidence in rejecting the null hypothesis which showed that there at least one mean is different from the others.

Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) was performed to determine the significant factors affecting the growth and yield of rice (MR 219) parameters. As shown in Table 4, F-test and *p*-values of this yield parameter showed no value (*) and since the Sum of Squares Errors is zero, indicating that the calculations of those terms were impossible to perform.

Therefore, the effects, regression coefficients, standard error, *t* and *p*-values were recalculated to refit the model after eliminating non-significant main effects (rotation period, A and rotation speed, B) and interaction effects (AB and AC). The results of ANOVA and statistical parameters for reduced model is presented in Tables 5 and 6, respectively. The effect with *p*-value less than 0.05 shows the significance of a factor on the response variable as the null hypothesis is rejected. Thus, distance

Table 3: Mean values of 1000-grain weight of rice grown from control and treated seeds

Parameter	Control	1	2	3	4	5	6	7	8
1000-grain weight (g)	23.0600	24.4030	25.3340	22.7005	24.9740	28.9601	27.0602	23.6283	28.2874

Table 4: Analysis of variance for 1000-grain weight – full model

Source	DF	Seq. SS	Adj. MS	F-test	*p*-value
Main effects	3	25.7826	8.5942	*	*
A	1	0.0329	0.0329	*	*
B	1	0.2679	0.2679	*	*
C	1	25.4755	25.4755	*	*
(2) way interactions	3	11.1192	3.7064	*	*
A*B	1	0.3828	0.3828	*	*
A*C	1	2.2451	2.2451	*	*
B*C	1	8.4913	8.4913	*	*
(3) way interactions	1	0.0158	0.0158	*	*
A*B*C	1	0.0158	0.0158	*	*
Error	0	*	*		
Total	7	36.9177			

*A: rotation speed; B: rotation period; C: distance of rice seeds from the center of clinostat

of rice seeds from the center of clinostat (C) and interaction between rotation period and the distance of rice seeds from the center of clinostat (BC) significantly affected 1000-grain weight as their *p*-values were 0.001 and 0.013, respectively. According to Chen *et al.* (2019) sugar translocation rate and carbohydrate transport are important during early grain filling. Moreover, growth hormones (Zhang *et al.*, 2016; Cui *et al.*, 2020) and genes (Kim *et al.*, 2017) are also responsible for grain filling. The distance of rice seeds from the center of clinostat (main effect C) and the interaction effect of the rotation period and the distance of rice seeds from the center of clinostat (BC) might affect the sugar translocation rate, carbohydrate transport, growth hormones and the related genes for grain filling, which influence the 1000-grain weight, as the 1000-grain weight is determined during grain filling (Baillot *et al.*, 2018).

Table 5: Analysis of variance for number of 1000-grain weight – reduced model

Source	DF	Seq. SS	Adj. MS	F-value	*p*-value
Main Effects	1	25.476	25.4755	43.17	0.001
C	1	25.476	25.4755	43.17	0.001
BC	1	8.491	8.4913	14.39	0.013
Error	5	8.491	8.4913	14.39	0.013
Total	7	36.918	0.5902		

Table 6: Statistical parameters for 2^3 full factorial design for 1000-grain weight – reduced model

Terms	Effects	Coefficient	SE Coefficient	*t*-value	*p*-value
Constant		25.626	0.272	94.35	0.000
C	-3.570	-1.785	0.272	-6.57	0.001
BC	-2.060	-1.030	0.272	-3.79	0.013

The following codified model employed for 2^3 factorial design is expressed as Equation 1:

$$Y = X_0 + X_1A + X_2B + X_3C + X_4AB + X_5AC + X_6BC + X_7ABC \quad (1)$$

where Y is the predicted response, X_0 represents the global mean and X_i represents the regression coefficient corresponding to the main and interaction effects. Since A, B, AB, AC and ABC were non-significant effects and removed, thus the reduced model equation of 1000-grain weight was obtained by substituting the constant and regression coefficients of the significant effects (C and BC) into the Equation 1. Therefore, the reduced model equation of 1000-grain weight can be expressed as:

$$\text{1000-grain weight} = 25.626 - 1.785C - 1.030BC \quad (2)$$

Pareto Chart

Pareto chart draws a reference line on the chart at *t*-value limit by which the effect of each variable is converted to *t*-statistic by dividing the variable value by its standard error (Timma *et al.*, 2014). The values that exceed the reference line showed the minimum statistically significant effect magnitude at 0.05 confidence level (Ponnusami *et al.*, 2007). From the Pareto chart, 1000-grain weight was significantly affected by the distance of rice seeds from the center of clinostat (C) and the interaction effects of rotation period and the distance of rice seeds from the center of clinostat (BC) as indicated by *t*-value = 2.571 at 0.05 confidence level, as illustrated in Figure 2. This might be due to several biological factors affected by the distance of rice seeds from the center of clinostat (main effect C) and the interaction effect of the rotation period and the distance of rice seeds from the center of clinostat (BC), including sugar translocation rate and carbohydrate transport (Chen *et al.*, 2019), growth hormones (Zhang *et al.*, 2016; Cui *et al.*, 2020) and genes (Kim *et al.*, 2017).

Main Effects Plot

Mean of 1000-grain weight was similar at both levels of rotation speed (A), but slightly higher at high level of rotation period (B) (25.8088 g), as depicted in Figure 3. The results revealed that high level of rotation period is preferable, whereas low or high level of rotation speed can be applied. Nonetheless, increase the distance of rice seeds from the center of clinostat (C) cause a reduction in the mean of 1000-grain weight from 23.8412 to 27.4102 g. The results also demonstrated that the distance of rice seeds from the center of clinostat was the most significant factor, which indicated by the highest gradient among the plots.

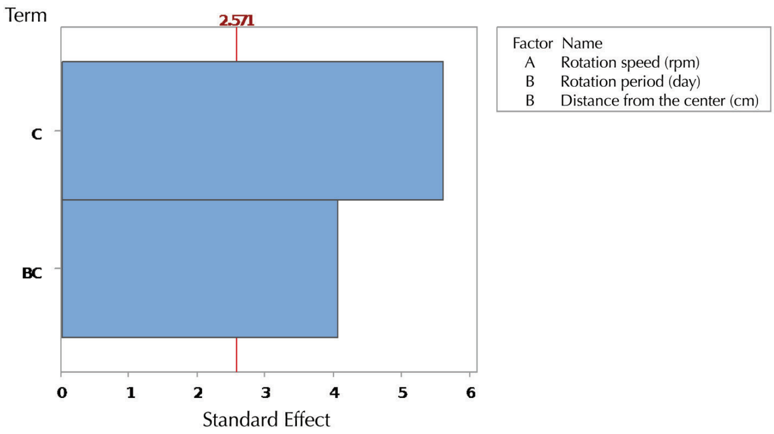

Figure 2: Pareto chart of standardized effects on 1000-grain weight—reduced model

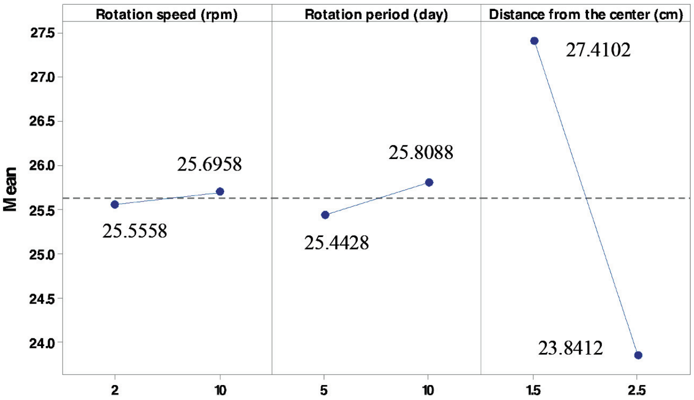

Figure 3: Main effects plot of 1000-grain weight

Interaction Effects Plot

An effective interaction plot, indicated by non-parallel lines of interaction effects, occurs when a change in response from a low to a high level of one factor is dependent on different levels of another factor (Mathialagan & Viraraghavan, 2005). Figure 4 shows that there are non-parallel lines forming between all studied factors; therefore, there are interaction effects between rotation speed and rotation period (A*B), rotation speed and the distance of rice seeds from the center of clinostat (A*C) and rotation period and the distance of rice seeds from the center of clinostat (B*C). Slightly higher 1000-grain weight (25.9575 g) was obtained at low level (2 rpm) of rotation speed (A) while similar 1000-grain weight was observed at high level (10 rpm) of rotation speed (A) when the rotation period (B) changed from low to high level. Increasing the distance of rice seeds from the center of clinostat (C) caused reductions in 1000-grain weight by 2.5094 g at 2 rpm and 4.6285 g at 10 rpm. The interaction effects between the rotation period and distance of rice seeds from the center of clinostat (B*C) indicate that 1000-grain weight decreased by 1.5085 g at low level of rotation period (B) when the distance of rice seeds from the center of clinostat (C) was changed from -1 to +1. Similarly, 1000-grain weight again decreased by 5.6291 g at high level of rotation period (B) when the distance of rice seeds from the center of clinostat (C) was changed from -1 to +1.

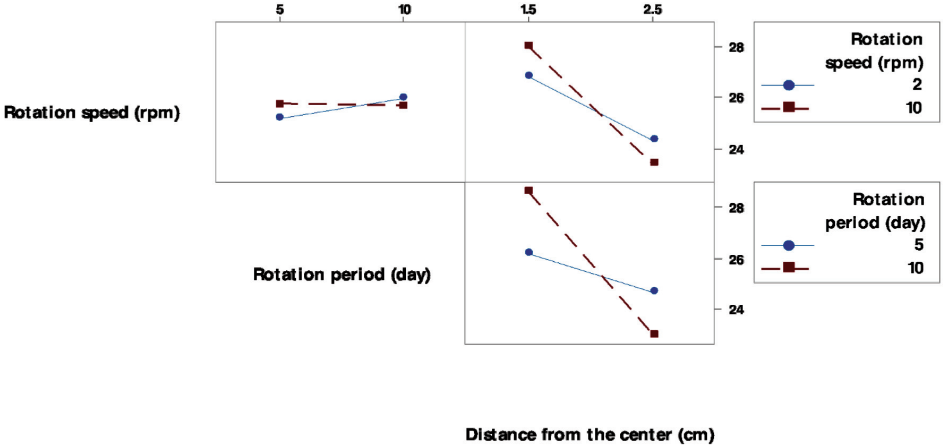

Figure 4: Interaction effects plot of 1000-grain weight

Conclusion

The results of present study demonstrated that rice (MR 219) treated with a 2-D clinostat is effective in enhancing the rice yield in terms of 1000-grain weight. Higher rice yield (28.9601 g of 1000-grain weight) was achieved compared to that of control (23.0600 g). Among the factors investigated, distance of rice seeds from the center of clinostat played the most significant role in enhancing rice yield, where low level of distance of rice seeds favors the yield. The interaction effect between rotation period and distance of rice seeds from the center of clinostat also significantly affected the rice yield. This technique can provide a sustainable, economical and green approach to improve rice yield.

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