AIMS Agriculture and Food, 5(4): 649–665.
DOI: 10.3934/agrfood.2020.4.649
Received date: 13 July 2020
Accepted date: 07 September 2020
Published date: 30 September 2020

http://www.aimspress.com/journal/agriculture

Research article

Enhancing germination and early seedling growth of Malaysian *indica* rice (*Oryza sativa* L.) using hormonal priming with gibberellic acid (GA_3)

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Abstract: A common practice to enhance seed quality for direct seeding of rice is priming under unfavorable designated conditions. This study aims to increase germination performance and early seedling growth of the MR219 rice by determining optimum priming times and ideal concentrations of gibberellic acid (GA_3). Seeds were primed with GA_3 in concentrations ranging from 20, 40, 60, 80 and 100 mg/L for 12 and 18 hours separately, and unprimed seeds served as control. The germination test was measured using a completely randomized design (CRD); meanwhile, pot cultivation was carried out using a randomized complete block design (RCBD) for four weeks. In germination studies, it was found that seeds primed with GA_3 for 12 hours at 60 mg/L significantly enhanced the germination performance, and early seedling growth of MR219 compared to unprimed seeds. The primed seeds had a germination percentage between 90-100%, increased seed vigor and germination indexes 3-fold and 2-fold respectively, and reduced the mean germination time by 24 hours. Correspondingly, in pot cultivation, the establishment and early seedling growth of primed MR219 was significantly increased as the seedling height, and total fresh weight was two times higher than unprimed seedlings. In addition, the biochemical attributes of GA_3 primed seedlings, including total soluble sugar, carbohydrate, and total soluble protein, were significantly increased by 2-, 1.6- and 4-folds, respectively, compared to unprimed seedlings. Priming MR219 seeds with GA_3 at 60 mg/L for 12 hours was found to significantly enhance the germination performance and early seedling growth in pot cultivation of direct-seeded rice as it promoted vigorous seedling growth of the MR219 rice cultivar.

Keywords: early seedling growth; germination; hormonal priming; gibberellic acid; *Oryza sativa*

1. Introduction

Rice (*Oryza sativa* L.) is a staple food in many Asian countries with a regional consumption of more than 80% of global rice production. The world's five largest rice producers and consumers are China, India, Indonesia, Bangladesh, and Vietnam. Therefore, rice demand is increasing as consumption increase with growing population [1]. In 2017, the world's rice production was 756.7 million tons, and rice production increased by 1.4%, which is equivalent to 769.9 million tons in 2018 [2]. The cultivation practice of rice usually occurs through direct seeding or seedling transplanting as germination is a critical phase in the plant's life cycle and is affected by an array of environmental factors and the viability of seed structure in determining the potential growth of the embryo [3].

Rice cultivation by transplanting is more effective in improving yields as only invigorated seeds are transplanted into the field. Seedlings need to be transplanted between 30 to 35 days; otherwise, missing the optimum transplanting time will cause weak tillering, which contributes to a decline in production [4]. Furthermore, the transplanting of rice seedlings manually into the paddy field is not only tedious for farmers but also labor-intensive as it involves working and moving in a stooped posture [5]. The method of direct seeding is the oldest rice-growing practice, which requires less time and labor, yet the success depends mainly on several factors, including a proper stand establishment of the crop [6].

Several invigoration techniques of seeds are well-known for reducing emergence time, synchronizing germination, and results in a better seedling establishment [7]. Improving seed germination and seedling establishment are both critical in the direct seeding method for maintaining rice production sustainability [8]. Seed priming is a technique found to be effective in improving the germination performance of direct-seeded rice under controlled conditions [9]. Priming is a low-risk technique [10] and a low-cost solution for poor germination performance and seedling establishment [11]. Seed priming is a technique recognized to be practical and easy to improve speed and uniformity of seed emergence, elevate seedling vigor, and yield in many field crops under adverse environmental conditions [12].

Seed priming triggers pre-germinative metabolism and activates the physiological processes during the early stage of seed imbibition, including the activation of DNA repair pathways and antioxidant mechanism to conserve genome integrity, assuring better germination and seedling establishment [12]. Various techniques of seed priming have been developed, including hydropriming, halopriming, osmopriming, and hormonal priming. Through hormonal priming, plant growth regulators including gibberellins (GA), abscisic acid (ABA) and salicylic acid (SA) have been regularly used to increase the uniformity of seed germination, seedling establishment and yield of various field crops such as rice, corn, safflower, wheat, beet and sunflower [13]. Exogenous application of plant hormones specifically GA_3 counteract ABA effects and promote dormancy release leading to seed germination. Moreover, GA_3 can also stimulate the expression of hydrolytic enzymes involved in the conversion of starch to sugar. By controlling starch accumulation and use, GA can affect overall plant growth [14].

Different strategies of seed priming could be used to improve germination and stand

establishment as it is likely to be species- and dose-dependent [15]. In recent years, studies have been focusing on the use of hormonal priming, particularly GA_3 in rice germination under unfavorable conditions [16]. However, the benefits of GA_3 priming in enhancing germination under normal growth conditions are scarce, especially with Malaysia's favorite *indica* rice, the MR219 cultivar. The MR219 rice was selected in this study as it is popular for its high potential yields and has lasted for 20 years and still receives demands from farmers. Unfortunately, there are reports about the existence of a germination problem for the MR219 rice variety [17]. Optimizing the priming method using GA_3 of MR219 variety is essential to maximize germination potential and stand establishments in direct-seeded MR219 rice. Thus, the present study aims to investigate the effect of different concentrations of GA_3 and priming time on germination performance and early seedling growth of the MR219 rice variety under direct seeding.

2. Materials and methods

The study is divided into two parts; (1) the first part is the germination test to measure the performance of seeds subjected to priming, the ideal concentration and priming time is determined, (2) the second part of the study focuses on the early seedling growth and biochemical response of pot-cultivated seedlings. Figure 1 shows an overview of the methodology.

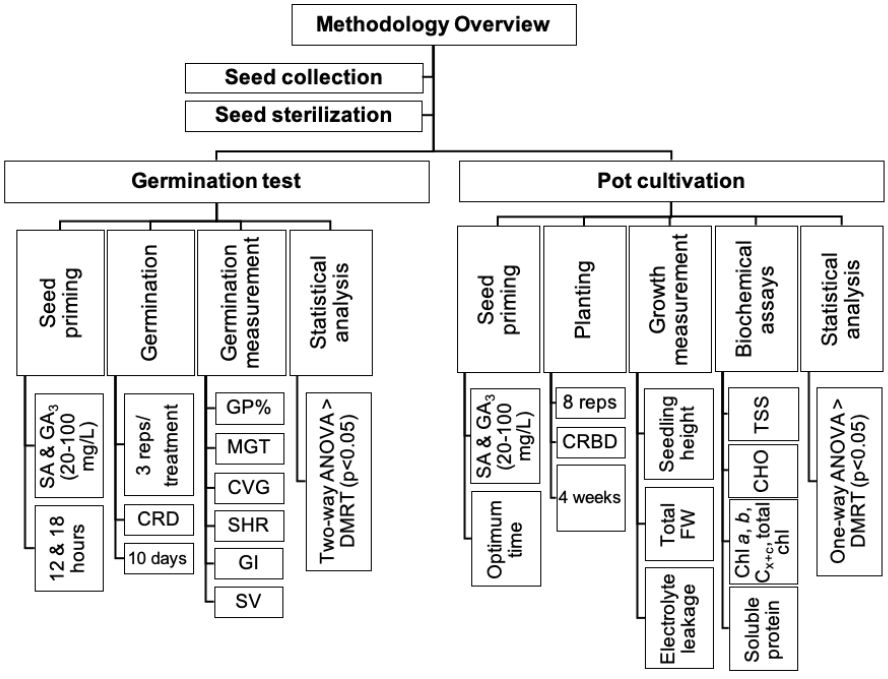

Figure 1. Overview of the methodology.

2.1. Plant material and seed surface sterilization

Malaysian *indica* rice cv. MR219 (accession no.: MRGB11633) seed was obtained from the Malaysian Agricultural Research and Development Institute (MARDI) Serdang, Selangor, Malaysia. Viable seeds were chosen randomly. In order to minimize contamination during priming, these seeds

were surface sterilized using 70% (v/v) ethanol for a minute, then swirled continuously in 20% (v/v) NaOCl for 30 minutes, and rinsed three times with sterilized distilled water and air-dried following the methods of [18], with a slight modification.

2.2. Effect of GA_3 hormonal priming on germination performance of MR219 seed

Sterilized seeds were primed in a range of GA_3 concentrations (20, 40, 60, 80, 100 mg/L) for 12 and 18 hours separately, with the ratio of seed weight to solution volume of 1: 5 [19]. After 12 or 18 hours of priming, the seeds were blotted dry using a clean paper towel. Five seeds were sown in a petri dish (90 mm × 15 mm) lined with two sheets of filter paper (Whatman No. 1) containing 5 mL of sterilized deionized water. Four replicates of each treatment were arranged in a completely randomized design (CRD), with unprimed seeds used as control. The germination was performed in a controlled growth room with a temperature of 24 ± 1 ℃ and 12 hours of photoperiod. The germination was monitored daily for 10 days. The germination performance was evaluated.

Seed germination was marked by the emergence of a 2 mm radicle length [20]. At ten days after sowing (DAS), the number of germinated seed was recorded, and the germination percentage (GP) was calculated using the following formula [21]:

$$\text{GP\%} = \frac{\text{no. of germinated seeds}}{\text{no. of seed with viability}} \times 100\% \tag{1}$$

Seed vigor index (SVI) was determined using the following formula [22]:

$$\text{SVI} = \frac{\text{hypocotyl length + radicle length}}{100} \times \text{GP\%} \tag{2}$$

Mean germination time (MGT) was calculated using the formula by [23]:

$$\text{MGT} = \sum \frac{\text{dn}}{\text{n}} \tag{3}$$

where n is the number of seeds newly germinated at time d; d is days from the beginning of the germination test.

Germination index (GI) was determined by using the following formula [24]:

$$\text{GI} = \sum \frac{\text{no. of germinated seeds}}{\text{days of final/last count}} \tag{4}$$

Coefficient of the velocity of germination (CVG) was calculated described by [25] using the formula:

$$\text{CVG} = \left[\frac{(n_1 + n_1 + \ldots + n_x)}{(n_1t_1 + n_2t_2 + \ldots + n_xt_x)}\right] \times 100\% \tag{5}$$

where n is number of seeds germinated on each consecutive day; t is number of days from seeding corresponding to n.

The following formula was used to calculate seedling height reduction (SHR) [26]:

$$SHR = \frac{\text{height of control seedling} - \text{height of primed seedling}}{\text{height of control seedling}} \times 100\% \quad (6)$$

All the data were analyzed with IBM SPSS Statistics Version 23 (Mac OS X) using Two-way ANOVA at $p < 0.05$ to test the significant difference among treatments followed by a post-hoc test of Duncan's Multiple Range Test (DMRT) at $p < 0.05$ for mean comparison.

An ideal concentration of GA_3 and optimum priming time were determined based on the statistically evaluated germination performance. Following this, pot cultivation was carried out using the same range of GA_3 concentration (20, 40, 60, 80, 100 mg/L) with the selected optimum priming time.

2.3. Effect of GA_3 hormonal priming on early seedling growth and biochemical responses in pot cultivation

After germination studies, MR219 seeds were primed for 12 hours, which was determined to be the optimum priming time. Therefore, the seeds were primed for 12 hours with GA_3 (20, 40, 60, 80, 100 mg/L) and sown in pots (8 cm × 8 cm) containing soil, then arranged in a randomized complete block design (RCBD). Direct-seeded seeds were used as control (unprimed). The soil medium used was compost soil, Impra Bio Soil—a well-balanced mix of sand, coconut husk, rice husk, river sand, and humus. The pots were sub-flooded with 3 cm of water and irrigated daily with 50 mL of tap water [27]. The early seedling growth of the primed MR219 was measured, and the biochemical assays were performed.

2.4. Early seedling growth measurement

Seedling height and total biomass of primed MR219 seedlings were measured at week four. For seedling height, primary tillers were selected at random in each pot, and measurements were taken from base to the spike tip using a ruler. For its total fresh weight, the MR219 seedlings were washed under running tap water to remove soil and debris from the roots. The seedlings were patted dried using clean paper towels to remove excess water, and the seedlings were weighed using an analytical balance [28].

2.5. Biochemical measurement

Chlorophyll content: chlorophyll content was measured, following the methods of [29]. A four-week-old leaf sample (1.0 g) was extracted in 1 mL of 80% ethanol for 20 minutes at 70 °C in a dry bath incubator. The extract was collected, and the leaves samples were extracted again until the leaves turned whitish and were then measured using a spectrophotometer at 665, 649, and 470 nm. 80% of ethanol was used as a blank solution. The chlorophyll content was calculated using formulas as follow:

$$\text{Chlorophyll } a\ (C_a) = 13.95\ (A_{665}) - 6.88\ (A_{649}) \quad (7)$$

$$\text{Chlorophyll } b\ (C_b) = 24.96\ (A_{649}) - 7.32\ (A_{665}) \quad (8)$$

$$\text{Total chlorophyll} = 20.2A_{649} + 8.02A_{665} \quad (9)$$

Total soluble protein: the total soluble protein was determined by using a method by [30] with modifications. Four-week-old MR219 seedlings were weighed (0.1 g) and cut into small pieces. The leaves were ground using pestle and mortar in liquid N_2, and 2 mL of 62.5 mM Tris-HCl at pH 6.7 was added. The homogenate was then centrifuged at 20,000 g for 30 minutes at 4 ℃. The supernatant (20 μL) was mixed with 830 μL distilled water and 150 μL Bradford reagent to a final volume of 1000 μL and incubated at room temperature (24 ℃) for 30 minutes. After incubation, the mixtures were quantified using a spectrophotometer at 595 nm. The total soluble protein content was calculated using a linear equation ($y = 0.412x + 0.0481$; $R^2 = 0.999$) generated from the standard graph plotted using a series of known concentrations of Bovine Serum Albumin (BSA).

Total soluble sugar: total soluble sugar was measured following the methods of [31] with slight modifications. Four-week-old MR219 seedlings (0.2 g) were weighed and homogenized with chilled 80% ethanol. The homogenates were then centrifuged at 5,000 g for 10 minutes at 4 ℃. One-tenth mL of the supernatant was added into 3 mL of freshly prepared anthrone reagent (150 mg anthrone; 100 mL 72% w/v sulphuric acid). The reaction mixture was placed in 100 ℃ water bath for 10 minutes and then taken out immediately to stop the reaction in an ice bath. The mixture was then measured using a spectrophotometer at 620 nm and calculated using a linear equation based on the standard curve of glucose ($y = 0.9843x + 0.0397$; $R^2 = 0.998$).

Total carbohydrate: total carbohydrate content was measured following the methods of [32] method with slight modification. Leaf sample (0.2 g) was extracted using 1 mL of 80% ethanol at 70 ℃ for 20 minutes; the extraction was repeated 3 times. 3 mL of the alcoholic extract was mixed with 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4. The final volume of the mixture was adjusted to 10 mL with distilled water. Then, the mixture was vortexed and left to cool at room temperature. The absorbance of the solution was measured using a spectrophotometer at 485 nm. The estimation of the total carbohydrate content was calculated using a linear equation based on the standard curve of glucose ($y = 0.9843x + 0.0397$; $R^2 = 0.998$).

Electrolyte leakage: electrolyte leakage (EL) was determined using young leaves and root tissues following the method [33]. Four-week-old samples were weighed (0.1 g) and cut into 0.5 cm length, then placed in test tubes containing 10 mL of deionized water and incubated at 24 ℃ for 12 hours. Afterward, the initial electrical conductivity (EC_1) was measured using an electrical conductivity meter (Model: Thermo Scientific; EUTECH CON 450 Conductivity Kit). The samples were autoclaved at 120 ℃ for 20 minutes to destroy the tissues and release all electrolytes. The samples were then cooled to room temperature (24 ℃), and the final electrical conductivity (EC_2) was measured. The relative EL was calculated using the following formula: $EL = EC_1/EC_2$; where EC refers to a mean of electrical conductivities, and subscripts 1 and 2 refer to initial and final conductivities, respectively.

All data were analyzed with IBM SPSS Statistics Version 23 (Mac OS X) using One-way ANOVA at $p < 0.05$ to test the significant difference among the treatments followed by the post-hoc test of Duncan's Multiple Range Test (DMRT) at $p < 0.05$ for mean comparison.

3. Results and discussion

3.1. ANOVA of GA_3 priming effects on germination

The ANOVA shows that the response of the MR219 variety to GA_3 priming treatment was significant ($p < 0.05$) in terms of GP, GI, MGT, CVG, and SVI (Table 1). The ANOVA also shows a significant difference among the GA_3 treatment in terms of SHR at a 5% probability level. Accordingly, the interaction between GA_3 concentration and priming time for all traits were found to be significant at 5% level (Table 1).

Table 1. ANOVA of GA_3 priming effect on germination performance of MR219 variety.

Source of variation	df	GP	GI	MGT	CVG	SVI	SHR
GA_3 concentration (GC)	5	718.525*	9.637*	0.427*	2.379*	112.091*	2839.698*
Priming time (PT)	2	5557.449*	66.127*	1.072*	6.228*	744.201*	38020.939*
GC × PT	10	659.631*	6.139*	0.181*	1.018*	91.171*	3626.702*
Error	36	124.972	0.226	0.038	0.218	0.662	53.725
Total df	54	-	-	-	-	-	-

*Significant at the 0.05 probability level. GP: germination percentage, GI: germination index, MGT: mean germination time, CVG: coefficient of the velocity of germination, SVI: seed vigor index, SHR: seedling height reduction.

3.2. Germination performance of MR219 in different concentrations of GA_3 and priming times

Complete germination was obtained after priming for 12 h with 100% GP except in 80 mg/L (86.7%). On the other hand, seeds primed for 18 h were observed to have significantly reduce the GP as concentrations of GA_3 increased (Figure 2(A)). Besides, it was found that seeds primed for 18 h reduced GP by 50% when primed in 60 mg/L (Figure 2(A)) and more. The lowest GP of MR219 recorded was the seed primed for 18 h, in 80 mg/L, with a reduction of 61% compared to control.

SVI showed improvements in 12 h; by increasing the GA_3 concentration, the trend was observed to rise before the decline in higher concentration (Figure 2(B)). The highest SVI was found to be the seed primed in 60 mg/L, for 12 h (31.4), registering a nearly 4-fold improvement to the control (8.3). Contrarily, the seeds primed for 18 h diminished the SVI with the lowest value belonging to the seeds primed in 100 mg/L (1.5), which decreased by 5.5 times compared to control (Figure 2(B)).

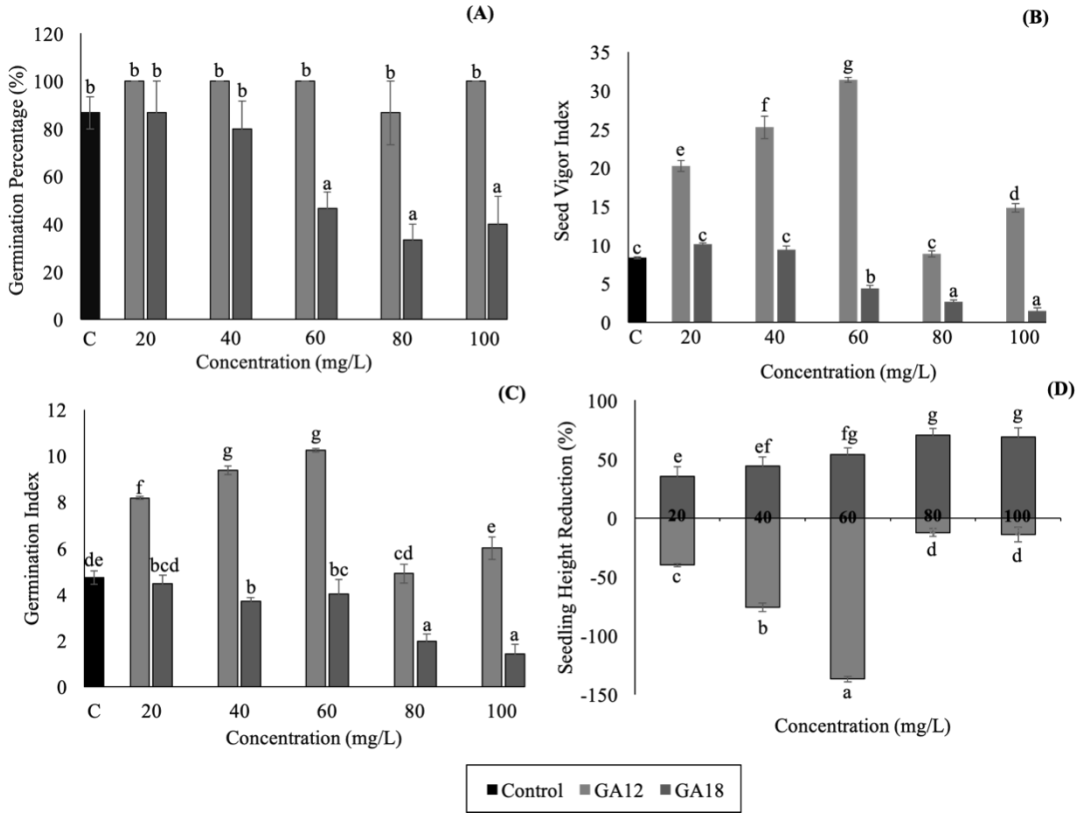

Figure 2. Germination percentage (A), seed vigor index (B), germination index (C), and seedling height reduction (D) of MR219 seed primed in different concentrations of GA_3 and priming time. Values are mean ± SE. Bars labeled with the different alphabet(s) are significantly different (Duncan's Multiple Range Test, $p < 0.05$).

The MR219 also showed improvements in GI primed for 12 h; the highest mean recorded was for seeds primed in 60 mg/L (10.2) and this increased by 2-fold when compared to the control (4.7). Seeds in a higher concentration of GA_3 exceeding 60 mg/L, primed for 12 h, found that GI was not significant to control (Figure 2(C)). On the other hand, the seeds primed for 18 h decreased the GI continuously as the concentration increased. The lowest GI belonged to seeds primed in 100 mg/L, for 18 h (1.4) with a reduction of more than 3-fold compared to the control (4.7), Figure 2(C).

The germination time and velocity of MR219 significantly reduced in 12 h. The shortest MGT was recorded in 60 mg/L, 12 h (6.0 days), which took a day less than the control to complete the germination (Table 2). For the 18 h, MGT also showed a reduction in a lower concentration of GA_3; however, germination was delayed in 100 mg/L by 0.1 days (2.4 h) compared to the control (Table 2).

Correspondingly, the CVG resulted in an acceleration in 12 h in all concentrations with the fastest belonging to 60 mg/L (16.6%) with an increase of 2.3% compared to control (Table 2). Similarly, 18 h showed that the CVG increased in low concentrations and decelerated at 0.3% in 100 mg/L (Table 2).

At ten DAS, SHR of MR219 in 12 h reduced as concentrations increased and reached the lowest in 60 mg/L (-136.8%), Figure 2(D). In addition, a continuous increase of GA_3 above 60 mg/L further increased the SHR (Figure 3(A)). Contrarily, SHR of MR219 in 18 h progressively increased as concentrations increased and reached a peak at 100 mg/L (Figure 2(D)) with a 68.8% reduction compared to the control (Figure 3(B)).

Table 2. Mean comparison of MGT and CVG of MR219 rice variety in different concentrations of GA_3 and priming time.

Concentration	MGT (Day)		CVG (%)	
(mg/L)	12 h	18 h	12 h	18 h
Control	7.0 ± 0.0^{d}		14.3 ± 0.1^{a}	
20	6.4 ± 0.0^{ab}	6.4 ± 0.2^{ab}	15.7 ± 0.0^{cd}	15.7 ± 0.5^{cd}
40	6.1 ± 0.0^{a}	6.3 ± 0.2^{a}	16.3 ± 0.0^{d}	15.9 ± 0.5^{d}
60	6.0 ± 0.0^{a}	6.8 ± 0.1^{bcd}	16.6 ± 0.0^{d}	14.7 ± 0.2^{abc}
80	6.5 ± 0.2^{abc}	6.4 ± 0.2^{ab}	15.4 ± 0.5^{bcd}	15.7 ± 0.5^{cd}
100	6.9 ± 0.1^{cd}	7.1 ± 0.2^{d}	14.5 ± 0.2^{ab}	14.0 ± 0.3^{a}

Values are means and standard errors of measurement made on three replicates (n = 3). Means within the same column/row in one parameter with the different alphabet(s) are significantly different (Duncan's Multiple Range Test, $p < 0.05$).

Generally, under normal and stress conditions such as salinity, matric water stress, and low temperature, the vigor of primed seeds increases. This study confirms that different concentrations of GA_3 behave differently from all the investigated traits depending on the priming duration. The duration of treatment obligatorily cannot be longer than the "safe limit", a maximum time of priming, without the risk of seed or seedling damage caused by premature germination. Prolonging the duration of priming may cause loss of seed tolerance to desiccation and reduce seed's viability [34].

The relationship between priming duration and concentration of priming solution was observed to be proportional as an increase in both factors causes severe inhibition on seed germination and plant growth. Therefore, precise regulation of time and water during the hydration process is critical to ensure an adequate and uniform level of seed moisture. MR219 seeds primed with high concentration of GA_3 for 18 h exhibited advent inhibition on germination. Following the results of other studies proves that different factors, such as the chemical composition of seed, seed cotyledon, and endosperm, can affect the seed permeability [35].

The results of some studies comply with the positive effect of priming on the acceleration of germination and improving the germination-related traits, which leads to increased plant growth. The better ability of primed seeds to complete the final germination process in a short time might be attributed to a readily available substance for germinating seedlings [36]. Furthermore, the primed seeds registered the lowest time needed to germinate as a result of acceleration in the germination process due to early initiation of metabolic activities, rapid breakdown, and mobilization of endosperm reserves [37].

GA_3 plays a vital role in the endosperm cap weakening. A high level of gibberellin is needed for the counteraction of ABA activity in seeds to promote dormancy release and radical protrusion during seed germination [14]. The embryo produces bioactive which are then transported to an aleurone layer, triggering the expression of α-amylase [38]. During seed germination, the aleurone layer is unable to synthesize GA_3 but perceives the GA_3 signals. Through the exogenous application of GA_3, the expression of α-amylase gene is upregulated, increasing seed vigor, thus leading to seed germination [39].

3.3. *Selection of ideal concentration and priming time based on the germination performance of MR219*

ANOVA and Duncan's Multiple Range Test of the GA_3 priming confirmed that the effects of time, different concentrations, and the interactions between them are significant on the germination performance of MR219. The best GA_3 treatment for the MR219 variety was determined to be 60 mg/L in 12 h (Table 3) by using a scoring index of germination performance. Consequently, the 12 h treatment was selected as the optimum priming time for the MR219 variety and used in pot cultivation.

Table 3. Scoring index of germination performance of MR219 in different concentration of GA_3 and priming time at ten days.

Treatment	Conc. (mg/L)	Time (h)	Germination Parameters						Total Score (*x*)	Germination Performance Index (x/6)
			GP (%)	MGT (Day)	CVG (%)	SVI	GI	SHR (%)		
Control	-	-	0	0	0	0	0	0	0	0
GA_3	20	12	1	0	0	0	0	0	1	0.17
		18	0	0	0	0	0	0	0	0
	40	12	1	0	0	0	0	0	1	0.17
		18	0	0	0	0	0	0	0	0
	60	12	1	1	1	1	1	1	6	1.0
		18	0	0	0	0	0	0	0	0
	80	12	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0	0
	100	12	1	0	0	0	0	0	1	0.17
		18	0	0	0	0	0	0	0	0

A score of "1" was given to the highest mean value observed in each accession, and "0" was given to the contender.

3.4. ANOVA of GA_3 priming effects on early seedling growth and biochemical attributes in pot cultivation

ANOVA showed that the effects of different concentrations of GA_3 were significant ($p < 0.05$) in 12 h on MR219 early seedling growth in terms of SH and TFW (Table 4). ANOVA also showed a significant difference among the studied biochemical attributes, including chl b, total chl, TSS, TC, TP, and LEL at a 5% probability level (Table 5). Nevertheless, the result showed that there were no significant effects of GA_3 concentration on chl a and REL at a 5% probability level (Table 5).

Table 4. ANOVA of GA_3 priming effect on early seedling growth of MR219 variety.

Source of variation	Df	SH	TFW
Between groups	5	99.799*	0.011*
Within groups	30	1.103	0.000
Total df	35	-	-

* indicate significant at the 0.05 probability level. SH: seedling height, TFW: total fresh weight.

Table 5. ANOVA of GA_3 priming effect on biochemical attributes of MR219 variety.

Source of variation	df	Chl a	Chl b	Total chl	TSS	TC	TP	LEL	REL
Between groups	5	11.058**	10.803*	56.596*	2.566*	0.670*	43.616*	0.070*	0.013**
Within groups	12	5.773	1.633	18.018	0.148	0.009	7.628	0.007	0.028
Total df	17	-	-	-	-	-	-	-	-

* and ** indicate significant and nonsignificant at the 0.05 probability level, respectively. Chl a: chlorophyll a, Chl b: chlorophyll b, Total chl: total chlorophyll, TSS: total soluble sugar, TC: total carbohydrate, TP: total protein, LEL: leaf electrolyte leakage, REL: root electrolyte leakage.

3.5. Early seedling growth and biochemical attributes of MR219 in pot cultivation

The establishment and early seedling growth of MR219 increased significantly with GA_3 priming in 12 h. At four weeks, 60 mg/L recorded the highest average of SH by 1.7-fold compared to unprimed seedlings (Table 6, Figure 3(C)). Similarly, TFW significantly increased in 60 mg/L by 2-fold compared to unprimed seedlings (Table 6).

Table 6. Mean comparison of early seedling growth and biochemical attributes of MR219 rice variety in different concentration of GA_3, 12 h**.**

Concentration (mg/L)	Control	20	40	60	80	100
SH (cm)	14.65 ± 0.20^{a}	14.42 ± 0.54^{a}	17.42 ± 0.30^{b}	24.25 ± 0.38^{e}	22.55 ± 0.65^{d}	19.83 ± 0.33^{c}
TFW (g)	0.11 ± 0.005^{a}	0.13 ± 0.002^{b}	0.15 ± 0.005^{c}	0.22 ± 0.008^{e}	0.20 ± 0.002^{d}	0.18 ± 0.003^{d}
Chl *a* (mg.g FW^{-1})	5.36 ± 0.20^{a}	8.94 ± 1.95^{ab}	8.75 ± 0.65^{ab}	11.39 ± 2.26^{b}	8.51 ± 0.52^{ab}	8.64 ± 1.37^{ab}
Chl *b* (mg.g FW^{-1})	2.96 ± 1.33^{a}	7.33 ± 0.96^{b}	6.97 ± 0.51^{b}	8.51 ± 1.17^{b}	7.05 ± 0.20^{b}	7.10 ± 0.82^{b}
Total chl (mg.g FW^{-1})	9.61 ± 0.35^{a}	18.74 ± 3.36^{b}	18.10 ± 1.33^{b}	22.94 ± 3.97^{b}	17.92 ± 0.84^{b}	18.12 ± 2.53^{b}
TSS (mg.g^{-1})	2.17 ± 0.11^{a}	2.79 ± 0.29^{ab}	3.14 ± 0.16^{b}	4.94 ± 0.12^{c}	3.45 ± 0.34^{b}	3.39 ± 0.21^{b}
TC (mg.g^{-1})	1.66 ± 0.05^{a}	1.75 ± 0.05^{a}	2.15 ± 0.05^{b}	2.95 ± 0.06^{d}	2.46 ± 0.07^{c}	2.22 ± 0.05^{b}
TP (mg.g^{-1})	3.86 ± 1.77^{a}	6.32 ± 2.23^{ab}	6.70 ± 0.32^{ab}	14.43 ± 0.26^{c}	10.61 ± 1.77^{bc}	10.47 ± 1.96^{bc}
LEL (μS)	0.56 ± 0.06^{a}	0.73 ± 0.01^{b}	0.88 ± 0.05^{c}	0.73 ± 0.07^{b}	0.91 ± 0.04^{c}	0.97 ± 0.01^{c}
REL (μS)	0.57 ± 0.18^{a}	0.51 ± 0.03^{a}	0.47 ± 0.03^{a}	0.49 ± 0.02^{a}	0.59 ± 0.09^{a}	0.64 ± 0.11^{a}

Values are means and standard errors of measurement made on at least three replicates ($n \geq 3$). Means with the different alphabet(s) indicate significant differences among accessions, following Duncan's Multiple Range Test ($p < 0.05$).

The current study shows that the GA_3 priming increased chlorophyll a, b and total chlorophyll by 2-, 2.8-, 2.4-fold, respectively, compared to the control (Table 6). Studies also showed that the amount of TSS in 60 mg/L (4.94 mg/g) was twice higher when compared to unprimed seedlings (2.17 mg/g). Furthermore, TC recorded the highest mean value in 60 mg/L (2.95 mg/g) by 1.3–fold compared to unprimed seedlings (1.66 mg/g), Table 6.

Correspondingly, at four weeks of cultivation, the TP showed significant improvement by GA_3 priming with the highest TP belonging to 60 mg/L (14.43 mg/g) with a 3-fold increase compared to unprimed seedling (3.86 mg/g), Table 6.

At four weeks, studies on the LEL found that priming for 12 h in GA_3 increased the amount of electrolyte leaked in all concentrations of the solution compared to unprimed seedlings with the highest LEL belonged to 100 mg/L (Table 6). Contrarily, the current finding showed there was no significant difference between the seeds primed in GA_3 for 12 h on the REL (Table 6).

Plant height is an essential trait of rice that directly relates to biomass production and its potential yield. The current study showed that seedlings grown in pots retained a height difference at 4 weeks after sowing. A similar study on the different varieties of rice, including MR219 by [40], reported that GA_3 priming lost its effect 2–3 weeks after sowing as the seedling height was not significant. In contrast, [41] reported that the priming effect is perceived up to 3–5 weeks after sowing in maize. This shows that various priming treatments depend on several factors, such as sowing time, the interaction between plant growth regulators, and priming stages.

The current finding confirmed that GA_3 priming increased the tiller height through its role in the regulation of shoot expansion. GA_3 plays an essential role in stem/internode elongation by stimulating cell division and expansion [39]. The elongation of cells requires recognition of GA_3 by a receptor molecule, the interaction of the activated receptor with the cell produces a wall-loosening factor or inhibition of a wall-stiffening factor and causes the cell to expand [42]. Higher tillering in primed rice seedlings increased the total biomass as a result of faster emergence, which allowed the seedlings to develop longer.

Application of GA_3 facilitated the growth rate of indoor/greenhouse cultivated plants by increasing the synthesis of photosynthetic pigments [43]. Current study showed chlorophyll content increased in GA_3-primed seedlings possibly donated by its role in fixation of CO_2. GA_3 treated plants show enhanced carbonic anhydrase activity, which play a major role in photosynthetic CO_2 fixation as it takes part in the hydration of CO_2 and is strictly associated with chloroplast [44]. This ensured adequate CO_2 supply, thus increase the net photosynthetic rate and consequently, accumulation of biomass [45] as indicated in the present findings. Nonetheless, the response of chlorophyll content to GA_3 application is still disputable and may depend on GA_3 dose and timing of application [44].

Higher soluble sugar content observed in GA_3-primed seedling is likely to be associated with the improvement of starch metabolism and respiration rate in both rice seed and its seedling. Wang et al. [36] assessed starch metabolism in primed rice seedlings in terms of α-amylase activity. Accordingly, activity of α-amylase was enhanced and causes the activation of starch degradation and rapid mobilization of food reserves. The plant's ability to degrade starch into soluble sugars plays a fundamental role in the plant growth and survival in a range of environments and provides the substrates required to generate energy to grow and its maintenance processes [46]. This is in agreement with current finding, where GA_3-primed seedlings recorded an increase in soluble sugar content, showing better seedling establishment and growth compared to unprimed seedlings (Figure 3(C)).

As priming elevates starch metabolism in primed rice seedling, an increase in the respiration rate creates more ATP for the growth of radicle and coleoptile [36]. Gibberellin increased the accumulation of soluble sugar and carbohydrate mainly through changes in the activities of carbohydrate metabolism enzymes [47]. GA_3 induces α-amylase activities in early post-germination growth of rice, which is consistent with the increasing growth of coleoptile, mesocotyl, and first leaf [48]. The application of GA_3 is believed to facilitate a simultaneous production and consumption, where starch degradation in the seed and utilization of soluble sugars for seedling growth occur concurrently [49].

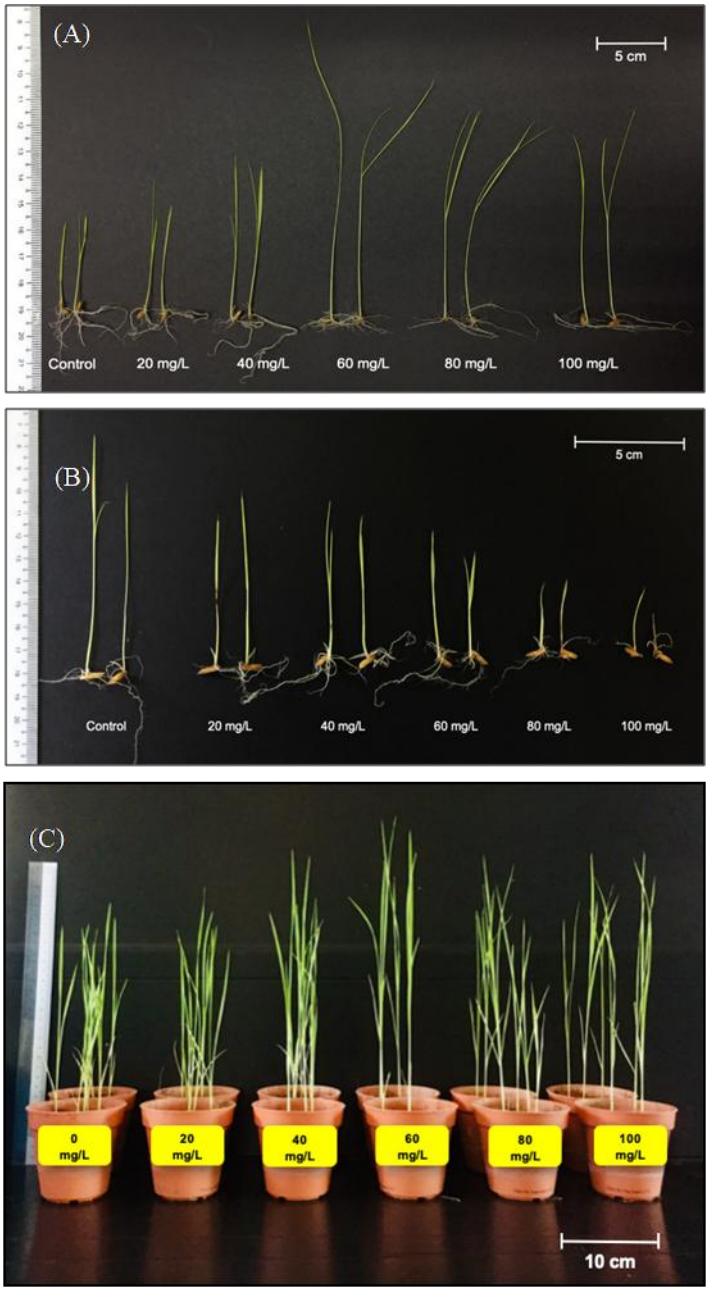

Figure 3. MR219 seedlings primed with different concentrations of GA_3 for 12 h (A), 18 h (B) at ten days, and four weeks for 12 h (C).

Besides when mitochondria are restored, the synthesis of protein starts at the beginning of the germination phase using existing mRNA and DNA. Priming the rice seeds helps to activate the enzymes responsible for germination and initiates the physiological activity related to germination along with the synthesis of new proteins by translation of new mRNAs. The activation or synthesis of enzymes (proteins) catalyzing the storage reserves breakdown and mobilization in post-germination leads to a better emergence and establishment [50].

Priming-induced protein synthesis is through upregulation of genes encoded for a protein involved in energy production through the mitochondrial electron transport chain and chemical defense mechanism and reinforces the protein-synthesizing machinery [51]. An increase in the

integrity of the ribosome was observed through improved in rRNA synthesis. It is also found priming increases the RNA levels of genes encoding the components of translation apparatus such as ribosomal subunits and translation initiation and elongation factors, hence promoting protein synthesis by improving the protein synthesis machinery function [52].

Priming is suggested to alleviate membrane damage induced by stress [53]. Current finding shows that electrolyte leaked in GA_3 primed seedlings under normal condition was not prominent as plant cultivated under designated stress conditions. Increased in electrolyte leakage following the application of GA_3 is likely due to the rise in membrane permeability [44]. GA_3 increase permeability of membrane to facilitate the absorption and utilization of nutrients along with the transportation of photoassimilates [54].

4. Conclusion

The current study suggests that hormonal priming using GA_3 for 12 h can be successfully applied to achieve higher seed germination performance and seedling vigor in MR219 variety. Primed seed profoundly exhibited higher vigor in both germination tests, and pot cultivation indicated that the physiological activities of primed seed start ahead of the final germination. Accordingly, hormonal priming using GA_3 enhanced the biochemical properties of direct-seeded MR219 variety. As the success of priming is species-dependent, more precise invigoration techniques can be employed to enhanced rice performance in direct-seeded cultures of a great number of different rice varieties. Moreover, mechanisms of rice seed priming, particularly related to enzymatic activities such as α–amylase should be study and revealed.

Acknowledgement

Acknowledgement are made to Universiti Putra Malaysia for the research grant [Geran Putra (GP-IPS/9556400)] and the Malaysian Agricultural Research and Development Institute (MARDI) through the Genebank and Seed Centre (GB) for MR219 seed supply (accession no. MRGB 11633).

Conflict of interest

The authors declare no conflict of interest.

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