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Growth and Yield of Rice Under Different Priming Media

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Abstract

Improvement in the yield of rice is a pre-requisite to having self-sufficiency in rice production which has not yet been achieved with pre-germination technology. This study was, therefore, conducted to determine the efficacy of seed priming over pre-germination treatment in improving rice yield. Seed priming with 100mM calcium chloride, 40% (w/v) polyethyl glycol 6000 (PEG_{6000}) and 100ppm kinetin were tested against pre-germination. The experimental design used was randomized complete block design (RCBD) with three replications. The plants were assessed using leaf area index (LAI), absolute growth rate (AGR), relative growth rate (RGR), crop growth rate (CGR), net assimilation rate (NAR), leaf area duration (LAD), plant height, number of tillers, productive tillers, rate of photosynthesis, stomatal conductance, intercellular carbon dioxide, transpiration rate, spikelets per panicle, number of filled spikelets, 100-grain weight, grain yield, harvest index and days to heading. It was found that kinetin priming was 18.24%, 10.32% and 28.31% better in number of spikelets per panicle, grain yield and harvest index respectively than pre-germination that was used as the check. This finding implies that 100ppm kinetin priming could be effectively used for better yield improvement of MR219 rice under optimum conditions and can successfully replace pre-germination with better yield.

1. Introduction

Rice (*Oryza sativa* L.) which comes mainly from irrigated lowland constitutes the major staple food for about two-thirds of the people of the world [1]. This rice is lowland rice and is produced at subsistence levels. Despite the scale of production, high yield is highly needed and should be sustained. This could be achieved through pre-planting seed treatment called seed priming.

There has been wide use of seed priming to achieve higher germination of seeds and better uniformity of seedling establishment [2]. Furthermore, seed invigouration (seed priming) is also used for reduction of emergence time during germination with uniformity of emergence as well as improvement of crop stands [3]. In the same vein, mean germination time could be reduced with

osmo-priming using sodium chloride and polyethyl glycol 8000 [4]. Similarly, priming with growth regulators and organic chemicals could guarantee higher uniformity in germination, better stand establishment, growth enhancement and higher crop yield [2]. Seed priming like hydro-priming can increase yield and yield components of crops like pinto bean (*Phaseolus vulgaris* L.) as a result of improvement of seedling vigour [5]. Similarly, osmo-priming using zinc sulphate could improve crop yield as found in maize [6]. Furthermore, osmo-priming (calcium chloride priming) led to improvement of yield and yield attributes of rice [7]. Also, priming of wheat seeds for 12 hours increased yield and harvest index of the crop [8]. In the same vein, seed priming improves quality of seeds produced. For instance, osmo-priming resulted in increase in calcium content of rice grain [7] as a result of earliness in development of roots which in turn led to better and effective absorption of nutrients needed for grain filling and development [9].It should, however, be noted that optimum concentration of priming solutions must be considered to forestall retardation of germination or killing of treated seeds as a result of toxicity of the priming solution [10]. Similarly, priming duration should be considered to avoid exceeding the safe limit for expected result to be at optimum.

Farmers have been using pre-germination as a suitable pre-planting seed treatment in lowland rice production because it results in 100% germination because in only germinating seeds will be selected and planted. However, the problem of keeping pre-germinated seeds for future use remains unsolved. In addition to that, pre-germination does not guarantee high yield of rice and other crops despite it caters for uniformity of seedlings. Therefore, there is dire need of having a seed treatment that will guarantee higher germination, higher yield and quality along with the ability of the treated seeds to be kept for future use. Therefore, this experiment was conducted to determine the best priming treatment for replacement in production of MR219 rice under normal condition.

2. Methodology

2.1. Experimental Site

This study was conducted in Field 15 glass house of Universiti Putra Malaysia (UPM), Serdang, Selangor on Latitude 3^{o} 02' N and longitude 101^{o} 42' E. The average monthly maximum and minimum temperatures are 33.5^{o}C and 21.5^{o}C respectively while the relative humidity is 92.5%. The sunshine hour is 6.6 h /day while the average rainfall and evaporation are 9.8 mm/day and 4.6 mm/day respectively.

2.2. Plant Materials and Experimental Design

The seeds used in this experiment were collected from Department of Crop Science, Faculty of Agriculture, UPM. The rice variety used was MR219 because it is the widely grown variety in the rice producing areas of Malaysia. The four experimental treatments were 48-hour priming with 100 mM calcium chloride dihydrate, 48-hour priming with 40% (w/v) polyethyl glycol 6000, 24-hour priming with 100 ppm kinetin and pre-germination as the control. The experiment was laid out in randomized complete block design (RCBD).

2.2. Seed Treatment

Kinetin priming was achieved by soaking dry rice seeds in 100 ppm kinetin solution for 24 hours. For calcium chloride and polyethyl glycol priming, dry seeds were soaked in solutions of 100 mM calcium chloride dihydrate and 40% (w/v) polyethyl glycol 6000 respectively for 48 hours. After that, the seeds were drained of the priming agents, washed three times with water to remove all the

traces of the priming chemicals from the seeds. The seeds were then air-dried on filter paper for three days to have 11% moisture content and then kept between 4 and 8^{o}C in the refrigerator until sowing. Pre-germination was achieved by putting dry seeds on well soaked tissue paper in a covered Petri-dish until radicle protrusion (germination). Ten pre-germinated seeds (seedlings) were planted immediately after their removal from the petri-dish where they were pre-germinated.

2.3. Planting and Cultural Practices

An equal mass (23 kg) of clay-loamy soil was used to fill each of the experimental pots with an area of 780 cm^2. The soil was puddled to create an impervious layer which prevents uncontrolled percolation of water and leaching of nutrients. The soil was kept saturated to pave way for seed-soil-water contact. Ten primed or pre-germinated seeds were planted per pot. The seedlings were regularly irrigated and finally reduced to four plants per pot. The plants were kept weed–free using hand pulling till the end of the experiment. The plants were flooded till the end of the experiment by maintaining at least 1cm layer of water above the soil surface.

2.4 Data Collection and Analysis

2.4.1 Tiller Characteristics

At grain filling stage, number of tillers and productive tillers were counted per pot for each treatment. Plant height was measured from the ground level of the plants to the tip of the longest leaf using a measuring tape. Subsequently, tiller efficiency was calculated using Equation 1.

$$\text{Tiller Efficiency (\%)} = \frac{Number\ of\ \ panicle\ bearing\ tillers\ per\ pot}{Total\ number\ of\ tillers\ per\ pot} x100 \quad (1)$$

2.4.2 Growth Analysis

Two destructive samplings at an interval of seventeen days were done at vegetative stage. The leaf areas of the sampled plants were measured using leaf area meter Licor, inc. Lincoln (Nebraska, USA). The sampled plats were oven-dried at 65^{o}C until constant mass was achieved. The dry masses of the sampled plants (whole) were measured using analytical weighing balance. The area occupied by each plant was determined by calculating the area of the pot and dividing it by the number of plants occupying it using equation 2.

$$\text{Area occupied by each plant} = \frac{Area\ of\ the\ pot\ (\pi r^2)}{Number\ of\ plants} \quad (2)$$

Where r is the radius of the plastic pot used and π is a constant which is equal to 3.142

Leaf area index (LAI), absolute growth rate (AGR), crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR) and leaf area duration (LAD) were calculated using equations 3 to 8.

$$\text{Leaf area index (LAI)} = \frac{Leaf\ Area}{Ground\ Area} \quad (3)$$

$$\text{Absolute growth rate (AGR)} = \frac{W2-W1}{t2-t1} \quad (4)$$

W_1 and W_2 are plant dry masses at times t_1 and t_2 respectively.

Crop growth rate (CGR) = $\frac{1}{P} \times \frac{W2-W1}{t2-t1}$ (5)

W_1 and W_2 are plant dry masses at times t_1 and t_2 respectively and P is the area of land covered by the plant

Relative growth rate (RGR) = $\frac{ln\, W2-ln\, W1}{t2-t1}$ (6)

W_1 and W_2 are plant dry masses at times t_1 and t_2 respectively.

Net assimilation rate (NAR) = $\frac{(W2-W1)(lnL2-lnL1)}{(t2-t1)(L2-L1)}$ (7)

Where L_1 and W_1 are leaf area and dry masses of plants at time t_1 and L_2 and W_2 are leaf area and dry masses at time t_2.

Leaf area duration (LAD) = $\frac{L1+L2}{2} \times (t_2+t_1) + \ldots + = \frac{L1+L2}{2} \times (t_n + t_{n-1})$ (8)

Where L_1 is the leaf area at time t_1, L_2 is the leaf area at time t_2, L_n is the leaf area at time t_n and L_{n-1} is the leaf area at time t_{n-1}

2.4.3. Days to Heading and Yield Data

Days to heading was counted from the day of germination to the day of panicle appearance. After harvesting, number of spikelets per panicle and filled grains were counted. Mass of 100 grains was determined by counting 100 grains and weighing them on a balance. Final yield per treatment was determined by weighing threshed grains per pot on a balance. The harvest index was calculated using equation 9.

Harvest Index = $\frac{Weight\ of\ Economic\ yield}{Weight\ of\ Biological\ yield} \times 100$ (9)

2.4.4. Chlorophyll Determination

The leaf chlorophyll content was determined using the procedure of Coombs et al.[11]. Fresh leaves were collected from each replication from each treatment. A leaf disc (1 cm²) was cut from the leaf sample of each replicate using a leaf puncher and was put in a scintillation vial containing 20 ml of 80% acetone. The vial was capped and covered with aluminium foil and then kept in the dark for 7 days. Absorbance at 647 and 664 nm wavelengths was measured with UV spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll were calculated using equations 10 to 12.

Chlorophyll a = (13.19 x A_{664})-(2.57 x A_{647}) (10)

Chlorophyll b = (22.1 x A_{647})-(5.26 x A_{664}) (11)

Total chlorophyll = Chlorophyll a + chlorophyll b (12)

A_{664} and A_{647} are absorbance at wavelengths 647 and 664nm respectively.

2.5. Leaf Gas Exchange Characteristics and Proline Determination

2.5.1. Leaf Gas Exchange

Data on net photosynthetic rate (μmol m^{-2} s^{-1}), stomatal conductance (μmol H_2O m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}) and intercellular carbon dioxide (μmolCO_2m^{-1}) were taken with a closed infra-red gas analyser LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA) following the method of Ibrahim and Jaafar [12]. Leaf surfaces were cleaned and dried using tissue paper before being enclosed in the leaf cuvette. Optimal conditions were set at 400 μmol mol^{-1} CO_2, 30°C cuvette temperature, 60% relative humidity with air flow rate set at 500 cm^3 min^{-1}, and modified cuvette conditions of 225, 500, 625 and 900 μmolm^{-2} photosynthetic photon flux densities (PPFD) respectively were used for the measurements. Gas exchange measurements were carried out when the sun was fully bright using fully expanded young leaves.

2.5.2. Proline Determination

Fresh leaf samples (0.5 g) were collected from each experimental pot and were homogenized in 3% (w/v) sulfosalicylic acid. The mixture was then filtered using whattman filter paper. The filtrate was kept while the residue was discarded. The proline content was estimated colorimetrically using the acid ninhydrin method of Bates et al. [13]. The reacting mixture contained 2ml glacial acetic acid, 2 ml ninhydrin (2.5% w/v ninhydrin in 60% v/v 6 M phosphoric acid) and 2 ml filtrate. The reacting mixture was incubated in a water bath at 95^oC for 1 hour. The reaction was then stopped by incubating the reaction tube in an ice bath. After bringing the temperature of the tube to ambient the temperature, 4 ml of toluene was added to reaction mixture and mixed thoroughly by vortexing. The upper phase was carefully pipetted into a glass cuvette and absorbance was measured at 520 nm using spectrophotometer.

2.6. Data Analysis

All the data collected were subjected to analysis of variance following RCBD procedure using SAS 9.2 package. Significant means were separated using least significant difference (LSD) at 5% probability level.

3. Results and Discussion

3.1 Effects of Priming Agents on Classical Growth Analysis

3.1.1 Effects of Priming Agents on Leaf Area Index

Polyethyl glycol (PEG) priming produced plants with the highest leaf area index (5.03) followed by kinetin (3.57) while the least was from calcium chloride priming (2.61) (Table 1). Leaf area index (LAI) describes the size of assimilatory apparatus of plants. It also serves as the primary factor for determination of the rate of dry matter production in plant system. Moreover, Addo-Quaye et al.[13] quoting Kvet et al. (1971) established that this trait explains disparities in production efficiency among varieties of crops. The result here might have stemmed from the fact that PEG priming enhanced better vegetative life which led to increase in leaf area despite the small area occupied by the plant in the pots. It should be recalled that when measuring leaf area, mutual shading is not considered and, therefore, higher LAI may not predict the yield because not all the leaves could trap enough solar radiation for photo-assimilate production. The implication is that as LAI increases, mutual shading of the leaves increases and consequently net assimilation rate is reduced. This finally reduces the final grain yield (Table 6).

Table 1: Effects of priming agents on leaf area index and absolute growth rate

Treatments	Leaf Area Index	Absolute Growth Rate(g day^{-1})
Calcium chloride	2.61d	0.72a
Polyethyl glycol	5.03a	0.46b
Kinetin	3.57b	0.36c
Pre-germination	3.29c	0.31c

Means with the same letter are not significantly different at 5% level.

3.1.2. Effects of Priming Agents on Absolute Growth Rate

The highest value of absolute growth rate (AGR) was from calcium chloride priming followed by PEG while the lowest rate was recorded from the control (Table 1). Absolute growth rate (AGR) is the rate of change in size of plant per unit time. It can be easily used to compare growth rate of two plants at a time without giving consideration to their initial weight. In this work, the result of AGR followed the pattern of relative growth rate (RGR) as explained below. This suggests that the two characters could be interchangeably used to quickly assess the growth rate of a particular plant species or crop. It should be noted that RGR is more specific than AGR because it considers unit weight of the initial plant part and measures the increment above it unlike AGR which considers the whole plant at once. There is strong relationship between these two traits (Table 6). Furthermore, AGR can be used to predict the trend of net assimilation rate (NAR) and net photosynthesis. So, if AGR is high, RGR, NAR and net photosynthesis will be high too. There is strong relationship among all these traits. The betterment of calcium chloride priming could be the result of enhancement of better root growth for better absorption of useful materials for luxuriant growth per unit time which was revealed by its high AGR. Contrary to the expectation in plants from pre-germination treatment (the control) which started life before the plants from the rest treatments, plants' growth rate was not better than the priming treatments except in leaf area duration where pre-germination was better than all the priming treatments.

3.1.3. Effects of Priming Agents on Crop Growth Rate

The fastest rate of dry matter accumulation was mostly favoured by calcium chloride priming because it had the highest value of crop growth rate (CGR) while the slowest rate was from kinetin priming (Table 2). Crop growth rate (CGR) measures dry matter accumulation per unit area and is considered a reasonable approximation for rate of canopy photosynthesis per unit ground area [15]. Its values vary according to the growth stage at which the data is taken [14]. The result observed here could, in part, be linked to photosynthetic rate because it has been established that dry matter accumulation has direct relationship with photosynthetic rate despite the fact that grain production is not associated with it (photosynthetic rate) [16]. However, when there is judicious assimilate partitioning and mobilization to the economic part of the plant, photosynthesis could be attributed to grain yield.

Table 2: Effects of priming agents on crop growth rate and relative growth rate

Treatments	Crop Growth Rate (g (crop)$m^{-2}day^{-1}$)	Relative Growth Rate (mg $g^{-1}day^{-1}$)
Calcium chloride	27.66a	0.15a
Polyethyl glycol	17.58c	0.09b
Kinetin	13.82d	0.09b
Pre-germination	19.93b	0.03c

Means with the same letter are not significantly different at 5% level.

3.1.4 Effects of Priming Agents on Relative Growth Rate

The results here showed that calcium chloride priming had the highest relative growth rate (RGR) while the control had the lowest rate (Table 2). Relative growth rate (RGR) is used to determine the rate of increase in plant mass per unit of plant weight already present. Having higher RGR in this work means higher net assimilation rate (NAR) which in turn depicts high photosynthesis as could be found in this work (Table 8). This could be attributed to the fact that calcium chloride priming enhanced absorption of the needed nutrients from the soil which enhanced higher relative weight gain in plants compared to other priming agents and the control. Furthermore, the machineries of mass gain which are net assimilation rate and net photosynthesis could be the ones responsible for the highest rate recorded from calcium chloride priming in this work. Finally this trait can be used to predict crop growth rate in case it is not measured because there direct relationship between them (Table 6)

3.1.5. Effects of Priming Agents on Net Assimilation Rate

Calcium chloride priming had the highest net assimilation rate (NAR) value (22.62) followed by PEG priming (19.94) while the least was from the control (9.75) (Table 5.3). Net assimilation rate is a useful trait for measuring photosynthetic rate. It also measures the rate of increase in plant mass per unit leaf area. The trend of NAR was perfectly followed by photosynthetic rate as found in this work (Table 8). When LAI increases, it is expected that leaf mutual shading would result. This will consequently lead to decline in net photosynthesis and subsequently NAR. This occurs because greater part of the leaves will receive light intensity below the required threshold for photosynthetic activities to take place. There is no difference whether the sunshine is bright or dull. The betterment found in calcium chloride priming could be attributed to better leaf architecture which allowed for greater part of the leaves to be available for trapping solar energy as revealed by the value of NAR and corroborated by the result on net photosynthesis (Table 8). As earlier explained, increase in LAI will result in mutual shading and consequent reduction in NAR. This is confirmed by the result of this work in which calcium chloride priming had the lowest LAI with highest values of NAR and net photosynthesis.

Table 3: Effects of priming agents on net assimilation rate and leaf area duration

Treatments	Net Assimilation Rate(g (crop)$m^{-2}day^{-1}$)	Leaf Area Duration ($m^2day\ m^{-2}$)
Calcium chloride	22.62a	0.38c
Polyethyl glycol	19.94b	0.40b

Kinetin	12.25c	0.48b
Pre-germination	9.75d	0.57a

Means with the same letter are not significantly different at 5% level

3.1.6. Effects of Priming Agents on Leaf Area Duration

The highest leaf area duration (LAD) (0.57) was from pre-germination (control) followed by kinetin priming (0.48) while calcium chloride priming had the least (0.38) (Table 3). Leaf area duration is measure of retention of photosynthetically active leaf over a period of time. It was derived from integration of leaf area index with time. It takes care of both duration and the extent of activeness of photosynthetic tissue of the canopy. The result of this work has shown that LAD and net photosynthesis have strong indirect relationship (Table 6). This implies that when there is high photosynthetic rate, LAD will be low and vice-versa. The result of pre-germination (control) could be attributed to leafiness of the plants which permitted retention of much more green leaves over time by the plants. It has been quoted by Abayomi et al.[17] that leaf area and leaf area duration are the main causes of yield differences not photosynthesis or net assimilation rate. However, the result of this study showed that kinetin priming which had lower LAD value than pre-germination had the highest grain yield. This could have resulted from better remobilization and partitioning of photo-assimilates to the filling grains after anthesis.

3.2 Effects of Priming Agents on Tiller Characteristics

3.2.1. Effects of Priming Agents on Number of Tillers

Production of highest number of tillers was favoured by calcium chloride and PEG priming. The lowest number of tillers came from both kinetin and the control (pre-germination) (Table 4). Tillering is an equivalent of branching in non-grass species. It shows success of vegetative growth of grass families like rice and can predict the yield of the plants to an appreciable extent because it relates with the number of productive tiller that the plants will eventually produce. From the results of this work, it is clear that all the priming treatments with the exception of kinetin were better than pre-germination in tiller production. Better performance of the priming treatments could be the result of biochemical and physiological repairs that occurred during the priming operation. The maintenance of this development could lead to better production of effective tillers (panicle bearing tillers). The completion of metabolic activities during priming period [18] gives the resulting plants a head start for harnessing available growth resources to produce higher number of tillers as well as panicle-producing tillers [19]. Furthermore, rapidity of germination gained from priming as well as growth uniformity of seedlings could well explain the increase in number of tillers gained by plants from seed priming.

Table 4: Effects of priming agents on number of tillers and productive tillers of rice

Treatments	Number of Tillers (no/pot)	Productive Tillers (no/pot)
Calcium chloride	43.00a	41.00a
Polyethyl glycol	43.00a	27.00b
Kinetin	41.00b	40.00a
Pre-germination	41.00b	40.00a

Means with the same letter are not significantly different at 5% level.

3.2.2. Effects of Priming Agents on Number of Productive Tillers

The highest number of effective tillers per pot was from plants primed with calcium chloride while plants from PEG priming had the lowest number. Both kinetin priming and pre-germination had the same number of panicle bearing tillers (Table 4). Panicle bearing tillers are the important tillers because of their link with the final yield. If panicle productivity is high, there is high probability of getting high yield at the end of a production cycle. Better performance of calcium chloride priming here could be attributed to the repair done to the embryo at the priming stage which then translated to better seedlings after germination and produced higher number of productive tillers. Moreover, the performance of calcium chloride priming might have stemmed from the fact that it aided earlier and uniform emergence of the seedlings [20]. Despite the fact that kinetin priming produced the same number of productive tillers with the control (pre-germination), it is evident that its performance was still better because pre-germinated seeds started growth and development before kinetin primed seeds that were dry seeded. The performance of kinetin priming might be because it regulates the physiology of plant and enhances its growth and development. This performance is not for kinetin priming alone because the use of salicylic acid (a plant growth regulator like kinetin) has also been found to result in betterment of rice growth and development when applied exogenously through priming process [21]. The keen competitive ability of the priming agents with pre-germination might be attributed to increase in number of actively reproducing cells in inflorescence meristem which resulted in increase in number of effective tillers and reduction in the level of tiller sterility [22]. Furthermore, mobilization of nutrients by the plants from seed priming [23] could have highly contributed to the betterment in the performance of the plants from priming process. Finally, biochemical repairs at the priming stage during imbibition [24] might have well enhanced the performance of all the priming treatments.

3.2.3. Effects of Priming Agents on Plant Height

The tallest plants were from calcium chloride priming followed by kinetin priming while the shortest plants were from the control (Table 5). The desirability of having tall plants is hinged on avoidance of intra- or inter-species shading which might make a plant prone to etiolation and reduction in photosynthetic efficiency when light harvesting apparatus receives solar energy below the threshold for efficient production of photo-assimilates. Furthermore, plant height and the angle of inclination of the leaves are major factors affecting light interception by plants. It should be noted that the three uppermost leaves which are the sink sources for filling grains after fertilization require better orientation through appreciable height to achieve the purpose for which they are meant for (efficient interception of light for better photo-assimilate production). Nevertheless, excessive height could make a plant prone to lodging and reduction in number of tillers which is believed is believed to have been compensated for by height gain. The consequences of excessive height constitute limitations to plant productivity because higher number of tillers is a pre-requisite for having higher number of productive tillers. This in turn is a very important yield determinant and, therefore, it becomes a target trait for all agronomic, physiological and genetic manipulations. In this work, all the priming agents produced plants that were taller than the ones from the control despite the fact that the control plants started growth and development before the plants from seed priming. The advantage that calcium chloride and kinetin priming had over the control might be the result of enhancement of meristematic activities [25] by the concerned priming treatments because meristematic cells at the apices are responsible for increase in plant height. Furthermore, it could be attributed to betterment of physiological activities of the plants during morphogenesis which is believed to have been induced by priming treatments [26]. In the same vein, height gain could be

linked to better absorption of water and nutrients enhanced by development of efficient roots as a result of priming treatments. The absorption of the required growth materials then led to luxuriant growth of the shoot and consequent increase in height.

Table 5: Effects of priming agents on plant height and days to heading

Treatments	Plant Height (cm)	Days to heading (days)
Calcium chloride	98.25a	76.00a
Polyethyl glycol	88.75b	77.00a
Kinetin	95.50a	76.00a
Pre-germination	86.75b	72.00b

Means with the same letter are not significantly different at 5% level.

3.2.4. Effects of Priming Agents on Days to Heading

The longest duration to heading was taken by plants from PEG priming followed by both calcium chloride and kinetin priming while the shortest duration was taken by the control treatment (Pre-germination) (Table 5). Earliness in beginning of plant' life tells a lot about the duration for completion of its life cycle. Stress can affect plant's life and lead the plant to completing its life cycles late. However, earliness or lateness in completion of plant's life cycle has little or no relationship with the final yield. Earliness in heading of plants from pre-germination could be attributed to the fact that the plants started their lives earlier than the plants from priming treatments. Since other factors of growth were not limiting, the reproductive lives of the plants started undisturbed. This further elucidates the fact that that keen competition at the vegetative stage has little or no effect on the interval between germination and heading. However, reaching heading stage earlier by pre-germination treatment did not result in higher yield as found in this work (Table 11). Number of days to heading has inverse relationship with the final grain yield (Table 6). For instance, pre-germination that took 72 days to reach heading stage had 78.35 g of grain yield while kinetin priming that took 76 days to reach heading stage had 87.37 g of grain yield. Therefore, it could be inferred that earliness to heading or completion of life cycle might not be advantageous to higher yield which is the target of the farmers. However, this could be useful in production of the same crop many times a year.

Table 6: Correlation co-efficients of some pairs of rice traits

Correlated Trait	Correlation Co-efficient
Leaf area index vs. grain yield	-0.515
Absolute growth rate vs. relative growth rate	0.506
Crop growth rate vs. relative growth rate	0.498
Net photosynthesis vs. net assimilation rate	0.871
Leaf area duration vs. net photosynthesis	-0.871
Days to heading vs. grain yield	-0.342

Stomatal conductance vs. net photosynthesis	0.756
Net photosynthesis vs. grain yield	-0.478
Intercellular carbon dioxide vs. net photosynthesis	-0.887
Stomatal conductance vs. transpiration rate	0.863
Biological yield vs. grain yield	-0.377
100-grain weight vs. grain yield	-0.162
Grain yield vs. harvest index	0.691

3.3. Effects of Priming Agents on Leaf Chlorophyll Content

3.3.1. Effects of Priming Agents on Leaf Chlorophyll a, b and Total Chlorophyll Content

This experiment revealed that priming agents enhanced production of higher concentration of chlorophyll-a than the control with the exception of calcium chloride priming. As for chlorophyll-b, none of the priming agents was better than the control. Finally, total leaf chlorophyll followed the trend of chlorophyll-a content (Table 7). This result in part indicates that the total chlorophyll content in the leaf is majorly determined by the level of chlorophyll-a. The chlorophyll level also determines the photosynthetic rate of the plants. This is inferred from this work because PEG priming that had the highest level of chlorophyll produced leaves with the highest photosynthesis rate (Table 8).

Leaf chlorophyll level indicates the level of nitrogen in plant [27] because nitrogen is an integral part of chlorophyll structure. This is why people quickly detect nitrogen deficiency in the soil through leaf chlorosis. It also determines the source strength of the plant especially at the grain filling stage [28]. So, high chlorophyll content results in high assimilate production and high harvest index [29] provided photo-assimilate produced is judiciously partitioned into the grains. Furthermore, leaf chlorophyll content has been established to be positively and significantly correlated with both yield and harvest index [30]. Having very high chlorophyll content to guarantee high yield [30] was not the case with the performance of kinetin priming in this experiment. Although the chlorophyll content was not low, it was below what PEG priming produced (Table 7). The observation of some researchers that higher chlorophyll content is a prelude to production of high rice yield [31] could be upheld partly because of the variation in the production environment as well as the type of inputs used. Moreover, photosynthesis is directly linked to dry matter yield not the grain yield except if the assimilate partitioning to the grains is effective. Also, there will be increase in the harvest index of the crop when there is better remobilization of assimilate from the shoot to the developing grains. Finally, there has been inconsistence in the establishment of the fact that that higher chlorophyll content is the hallmark of high grain yield in rice. This might be attributed to the existence of other yield contributing factors which vary from one production area to the other.

Table 7: Effects of priming agents on chlorophyll-a, chlorophyll-b and total chlorophyll

Treatments	Chlorophyll-a ($\mu mol\ cm^{-2}$)	Chlorophyll-b ($\mu mol\ cm^{-2}$)	Total chlorophyll ($\mu mol\ cm^{-2}$)
Calcium chloride	1.39d	2.09c	3.48c
Polyethyl glycol	2.35a	3.04b	5.39a
Kinetin	2.11b	3.09b	5.20a
Pre-germination	1.89c	4.76a	4.76b

Means with the same letter are not significantly different at 5% level.

3.4. Effects of Priming Agents on Leaf Gas Exchange Characteristics

3.4.1. Effects of Priming Agents on Stomatal Conductance

Calcium chloride priming was the best in enhancing stomatal conductance followed by both kinetin priming and pre-germination. The treatments were significantly different from one another at p=0.05 (Table 8). Stomatal conductance regulation is very important in plants because it plays a vital role in CO_2 assimilation on which photosynthesis depends and controls evapotranspiration on which cooling as well as desiccation of plants depends [32]. Stomata remain open during the day except for CAM plants which have them open only at night to adapt plants to drought-stricken environment. Similarly, stomata could become closed if there are moisture stress, decrease in atmospheric vapour pressure, decrease in leaf turgor and low root-generated chemicals [33]. To have photosynthetic rate reduction, there should be stomatal closure and suppression of mesophyll conductance [34]. Therefore, photosynthetic rate enhancement could come through enhancement of mesophyll conductance and stomatal openings because they have strong relationship (Table 6). The result of this work shows the potential of seed priming in keeping plant stomata wide open for better gas exchange to occur though this might be detrimental to the plant sustenance if the plants are in a moisture-stressed environment. This is because water will be lost into the atmosphere through transpiration as a result of wide-open stomata.

Table 8: Effects of priming agents on net photosynthesis and stomata conductance

Treatments	Net Photosynthesis ($\mu molCO_2 m^{-2}s^{-1}$)	Stomatal Conductance ($molH_2O\ m^{-2}s^{-1}$)
Calcium chloride	13.17a	0.14a
Polyethyl glycol	10.39b	0.11c
Kinetin	9.75c	0.12b
Pre-germination	7.35d	0.12b

Means with the same letter are not significantly different at 5% level.

3.4.2. Effects of Priming Agents on Net Photosynthesis

The highest photosynthesis rate was recorded from PEG priming followed by calcium chloride while the lowest was from the control (pre-germination) (Table 8). It could be inferred that PEG priming did enhance photosynthetic rate. There is strong relationship between photosynthetic rate and stomatal conductance (Table 6). Despite this, there are other important determining factors that finally dictate how fast the rate of photosynthesis will be. It has been made known that photosynthetic rate does not determine the final grain yield as found in this work. So, there is very weak relationship between the two traits (Table 6). So enhancement of photosynthesis alone cannot guarantee better yield in rice production [16]. The contribution of photosynthesis is directly on the dry matter production which can be at the detriment of the filling grains except if there are better assimilate partitioning and remobilization of photo-assimilate from the vegetative parts to the filling grains. This could be clearly shown by harvest index which reveals the proportion of the economic yield to the whole biological yield.

3.4.3. Effects of Priming Agents on Intercellular Carbon Dioxide

The highest volume of intercellular CO_2 in this experiment was found in plants from pre-germination followed by kinetin priming while the least volume was from calcium chloride priming (Table 9). Despite the fact that calcium chloride priming enhanced higher stomatal conductance, plants from it had the lowest intercellular CO_2 volume. With the highest volume of intercellular CO_2 in the control, the photosynthetic rate was so low and incommensurate with the amount of the useful gas found in the inter-cellular space. This might be because the two of them have indirect relationship (Table 6). So, if the volume of intercellular CO_2 is increased, there will be low photosynthetic rate which is our utmost aim. This is simply because when carbon dioxide is higher in the intercellular space, further assimilation of such gas is hampered because of saturation. This is linked to photosynthesis which is directly responsible for the utilization of the gas for manufacturing of photo-assimilates. So, when less volume of the gas is available in the intercellular space, assimilation by the process of photosynthesis occurs and better conductance results. In the same vein, having higher photosynthetic rate does not lead to higher grain yield except if better partitioning of assimilate is found [16]. Therefore, the reliance on photosynthetic parameters should be directed towards interpretation of dry matter production and less on other physiological aspects. Nevertheless, it should be realized that dark respiration and photorespiration determine the net dry matter production which is the leftover after removal of the consumption of both dark respiration and photorespiration from the total photo-assimilate produced.

Table 9: Effects of priming agents on intercellular carbon dioxide and transpiration rate

Treatments	Intercellular Carbon Dioxide ($\mu molCO_2 m^{-1}$)	Transpiration Rate ($mmolH_2O\ m^{-2}s^{-1}$)
Calcium chloride	215.30d	3.72a
Polyethyl glycol	217.16c	2.98d
Kinetin	233.94b	3.46b
Pre-germination	270.31a	3.37c

Means with the same letter are not significantly different at 5% level.

3.4.4. Effects of Priming Agents on Transpiration Rate

PEG priming had the highest transpiration rate followed by calcium chloride priming while the lowest rate was from the control (Table 9). Transpiration determines how cool the plants are. This is based on the basic Physics principle that evaporation causes cooling. This depends on the stomatal opening, the level of moisture in the rhizosphere, ambient temperature and wind speed. In this experiment, water availability was normal for rice production. We majorly depended on the contribution of stomatal opening because wind speed and ambient were not part of the assessed traits. We expected calcium priming to have the highest transpiration rate because it had the highest level of stomatal conductance. However, it was the PEG which was the best for photosynthetic rate that had the highest transpiration rate. Nevertheless, calcium chloride followed PEG in rate ranking. There is strong relationship between stomatal conductance and transpiration rate (Table 6). Despite the advantage of cooling the plants through evapotranspiration, it predisposes plants to wilt when the rate of evapotranspiration exceeds absorption as a result water deficit in the rhizosphere.

3.5. Effects of Priming Agents on Rice Yield and Its Attributes

3.5.1. Effects of Priming Agents on Total Spikelets and Filled Grains

Kinetin priming enhanced production of more spikelets than other priming treatments. For this experiment, kinetin priming had 137 spikelets per panicle as against PEG priming which had 79 (Table 10). The number of spikelets produced by a panicle determines the maximum number of grains that could be produced per panicle. However, panicle architecture determines grain mass and quality because the superior spikelets get filled first while the inferior ones are either poorly filled or remains blank (empty). It should be understood that increasing panicle size or height to increase the number of spikelets and consequently the number of grains could be detrimental to light interception and photosynthesis rate of the source leaves that are positioned beneath the panicle for the supply of assimilates to the grains during grain filling [35]. It has been established that the number of grains per panicle in rice is determined by panicle length and the filled grains per panicle length [36]. Therefore, final grain yield per unit area is dependent on the population of spikelets produced by panicles per unit area. The result here reveals the inherent potential of seed priming especially with the use of growth regulators in increasing the number of spikelets per panicle. It further confirms the role of growth regulators on growth and development of plant reproductive phase. The number of filled grains per panicle did not follow the same pattern as that of spikelet production. Instead, calcium chloride priming produced the highest number of filled grains per panicle (94) while kinetin was next to it with 88 filled grains. The treatment with the least number of filled grains was still PEG priming like the case of spikelet production. The highest percentage of filled grain was from calcium chloride priming while the lowest percentage was recorded from PEG priming (Table 4).

Although kinetin priming had the highest number of spikelets, it had lower number of filled grains than calcium chloride priming (Table 10). This is because having large panicle size with higher number of spikelets will significantly increase the number of poorly filled grains while most of the grains in the inferior spikelets will become source-limited [37]. This might be because poor partitioning and translocation of assimilates from the source leaves and stems during grain filling could not sustain the development and filling of a large number of spikelets [38]. Moreover, starch synthesis in the endosperm cells of inferior spikelets is poor [39] and assimilates partitioned to them (inferior spikelets) remain unused. In addition to that, superior spikelets on the upper part of the panicle flower early, exert dominance, accumulate higher level of starch, and produce better quality grains than inferior spikelets that flower late [40].

Table 10: Effects of priming agents on total spikelets and filled grains

Treatments	Total Spikelets (no/panicle)	Filled Grains (no/panicle)	Per cent Filled Grain (%)
Calcium chloride	104.00c	94.00a	90.38a
Polyethyl glycol	79.00d	37.00d	46.84d
Kinetin	137.00a	88.00b	64.23c
Pre-germination	112.00b	77.00c	68.75b

Means with the same letter are not significantly different at 5% level.

3.5.2. Effects of Priming Agents on One Hundred Grain Mass, Grain Yield and Harvest Index

Pre-germination (the control) had the highest mass (4.10g) for 100 grains followed by kinetin priming (4.09g) while the lowest mass (3.91g) was recorded from PEG priming. For the final grain yield, the highest (87.37g) was realised from kinetin priming while the lowest (25.44g) was from PEG priming. Coincidentally, the highest harvest (HI) (47.12) was still from kinetin priming while the lowest (23) was from PEG priming in accordance with the yield production (Table 11).

Table 11: Effects of priming agents on 100-grain weight, yield and harvest index

Treatments	100- Grain Weight (g)	Yield (g/pot)	Harvest Index (%)
Calcium chloride	3.94b	64.82c	38.98b
Polyethyl glycol	3.91b	25.44d	23.00d
Kinetin	4.09a	87.37a	47.12a
Pre-germination	4.10a	78.35b	33.78c

Means with the same letter are not significantly different at 5% level

The mass of individual grain is the major contributor to the final yield. The size and mass of the grain depends on the spikelet position on the panicle. If the spikelet is a superior one, the grain size will be better filled. Otherwise, the grain is either poorly filled or the spikelet remains blank. This aspect is not directly affected by photosynthesis because its contribution is not yet really understood [41]. Photosynthesis has been confirmed to contribute immensely to biomass production. It has been made evident that many spikelets still remain poorly filled or blank even when we have very heavy panicles [42]. Higher grain mass observed in this work with kinetin priming could be attributed to better assimilate partitioning as depicted by higher HI (Table 11). The final yield could predict the HI because there is strong direct relationship between the two traits (Table 6). This could have been the result of better assimilate partitioning with very little or no relationship at all with light saturated-photosynthesis [16]. This is because photosynthesis is not affected by antisense which suppresses sucrose transporter gene that impairs rice grain filling [43].

4. Conclusion

From this work, it was found that 24-hour priming with 100 ppm kinetin was 12% better than pre-germination in grain yield. Therefore, 24-hour priming with 100 ppm kinetin could be used for better grain yield of MR219 rice produced under normal condition.

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