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Optimization of priming duration for rice production under drought stress

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Abstract

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The type of priming chemical and the duration of priming are important factors determining the performance of plants resulting from priming treatment. Therefore, this research was conducted to determine the effects of different priming agents and soaking durations on growth and yield of drought-stressed rice.

To achieve this objective, a pot experiment was conducted where three priming agents (100 mM calcium chloride dihidrate ($CaCl_2$), 40% (w/v) polyethylene glycol (PEG) 6000 and 100 ppm kinetin) combined with two soaking (priming) durations were tested on drought stressed rice plants in a 3x2 factorial experiment. The experiment was laid out in a completely randomized design (CRD) with three replications.

Parameters on germination percentage, number of tillers, number of productive tillers, tiller efficiecy, shoot fresh and dry weight, yield, seed length to seed width ratio (seed size), 100-seed weight and harvest index were taken. Also, data on photosynthetic rate, stomatal conductance, intercellular carbon-dioxide and transpiration rate and leaf water were taken. It was found that the highest yield was got when PEG was used for priming for 48 hours. It was found that number of tillers, shoot dry mass, 100-seed mass, rate of photosynthesis, stomatal conductance, transpiration and leaf area water were increased when seeds were primed for 48 hours with kinetin solution. However, tiller efficiency, harvest index and seed size were favoured by 24-hour priming with PEG while number of productive tillers, shoot fresh mass and grain yield were favoured by 48 hour priming with PEG. It was concluded that 48 hour priming with PEG was effective alleviate moisture stress in MR219 rice. This implies that to avoid wastage of priming chemicals and circumvent undue prolongation of priming period or duration which will result in harming the seeds (toxicity) and poor performance of the resulting plants, 40% (w/v) PEG 6000 should be used for 48 hours for priming MR219.

Key words: moisture stress, flooding, photosynthetic activities, yield, yield attributes.

Introduction

Moisture availability is an important environmental factor which determines the habitat of different plants and animals as well as their survival. When this factor becomes limiting, ways out will be the next line of action to have success in the production of any crop of interest. This problem is perennial in some places while in others it is ephemeral. Throughout the period of growth and development of plants, they experience different environmental stresses as a result of their exposure (Zhao et al., 2007) of which extreme temperature; drought and salinity are the major ones. The stresses play significant roles in causing unpredictable and substantial yield loss in agricultural productions (Jakab et al., 2005). Not only that, these stresses also cause varieties of biochemical, physiological and metabolic changes (Xiong & Zhu, 2002) that cause oxidative stress which disturbs plant performance and metabolism as well as the yield (Shafi et al., 2009).

Among the physiological activities of plants affected by drought or moisture stress photosynthetic rate, stomatal conductance, intercellular carbon-dioxide, leaf carbon dioxide, transpiration rate and leaf temperature, leaf water and relative humidity. The photosynthetic rate decreases when plants are moisture-stressed. This comes partly because the transpiration rate should be lowered through stomatal closure. This, therefore, results in having low stomatal conductance values. So, if the stomatal conductance is high, there will be higher photosynthetic rate (Carmo-Silva et al., 2008). The chain effect is felt in the intercellular carbon dioxide which is dependent on the stomatal opening or closure. The leaf temperature determines the relative humidity of this aerial part of the plant. They are indirectly related. These two have summative effects on the rate of transpiration too. At higher temperature and low relative humidity, the transpiration rate is high and vice versa.

Finally, the photo-assimilate production decreases as photosynthesis equally decreases during stress. Therefore, there is decrease in yield of the plant under consideration. This could be because the produced assimilates were not enough to have the largest percentage being directed towards the filling grains or the little produced was directed toward the vegetative parts for their survival at the expense of yield production.

Based on these problems, all modern agricultural strategies now aim at increasing yield per unit land area and reducing production losses both before and after harvesting caused by environmental stresses (Gust et al., 2010). With the problems of drought stress on ground, simple way-out that is cost effective and easily adoptable will be acceptable by all farmers whether literate or less-educated groups.

From physiological perspective, the way-out that is line with the aforementioned qualities is seed priming. As defined by Beckere and Conrath (2007), primed state is the physiological condition at which plants can better activate their defence response. Seed priming induces a particular physiological state in plants which is acquired through pre-sowing treatment with natural or synthetic compounds in solution. Because priming does not affect plant fitness with its ability to protect them against diseases and abiotic stress, it now emerges as a promising strategy for alleviating biotic and abiotic stresses in the modern stress management. Despite the advantages of this technology, only chemicals that can reduce the adverse effects of different environmental stresses should be given prime consideration (Uchida et al. 2002). For instance, calcium chloride is an example of priming chemicals that can alleviate drought stress. This has been used in alleviating drought stress in barley (Kaczmarek et al., 2017). Polyethylene glycol (PEG) 6000 has equally been used for stimulating germination and early seedling growth in Chinese cabbage (Yan, 2015). Finally, kinetin priming has been successfully used to alleviate drought stress in *Silybum marianum* L. (Zavariyan, 2015)

The loss caused by drought in rice production is so significant that it has led to cancellation of a whole season and loss of millions of Malaysian Ringgit in 2012 which was mainly due to cessation of rain in the main granary areas of Malaysia. So, better preparation against future occurrence is inevitable since the predictability of another one lies only in the hand of the creator.

The right combination of priming agent and appropriate soaking duration play a very important role in priming efficiency and its ability to improve yield and alleviate moisture stress. Therefore, this experiment was conducted to determine the best combination of priming agents and duration on performance of rice under drought stress.

Materials and Methods

Experimental Site

These experiments were conducted in the glass house of the Rice Research Centre of the Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (30 02’ N, 1010 42’ E; elevation 31 m). 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.

Experimental Treatments, Priming Procedure and Experimental Design

The seeds used in this experiment were collected from the gene bank of the Faculty of Agriculture, UPM. Rice variety researched on was MR219. The experiment was a factorial experiment comprising three priming chemicals (100 mM calcium chloride di-hydrate, 40% w/v polyethylene glycol and 100 ppm kinetin) combined with two priming durations (24 and 48 hours). The priming chemicals and their concentration were from our previous study (Kareem et al., 2019). Priming was carried out by soaking rice seeds in 100 mM calcium chloride dehydrate, 40% (w/v) polyethylene glycol 6000 and 100 ppm kinetin for 24 and 48 hours. After the designated priming durations, the soaked seeds were drained of the priming chemicals and washed three times with water to free the seeds of the traces of the priming chemicals. The seeds were then air-dried on filter paper for three days to have final moisture content of 11% and then kept between 4 and 8° C in the refrigerator until sowing. So, there were six treatment combinations as shown in Table 1. The experiment was laid out in randomized complete block design (RCBD) with three replications.

Crop Husbandry and Stress Treatment

The primed seeds were directly sown in pots of area of 780 cm^2 filled with 18 kg of clay loamy soil. After seedling establishment, the seedlings were thinned to two per pot. To impose drought stress, irrigation was withdrawn for 15 days at tillering (vegetative) stage and the final soil moisture content was 8% for the stress condition. Hand weeding was used to free the crop of weeds throughout the period of the study.

Data Collection

Leaf Gas Exchange Characteristics

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 (μmol CO_2m^{-1}) were taken with a closed infra-red gas analyser LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA) following Ibrahim and Jaafar (2014). 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 $\mu molm^{-2}$ 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 to record net photosynthesis rate (A).

Tiller Characteristics, Yield, Shoot Weight, Harvest Index and Seed Dimension

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 as follows:

Tiller Efficiency (%) = (Number of panicle bearing tillers per pot)/(Total number of tillers per pot) x100

At harvest, weight of threshed grains per pot was measured using weighing balance to determine yield per pot. The whole shoot was then cut from the ground level and weighed fresh per pot and recorded. The shoot was then dried in the oven at 65o C until a constant mass was recorded. Thereafter, harvest index was calculated using the following formula:

Harvest Index = (Economic yield)/(Total biological yield) x100

After threshing, seed length and width were measured using Nikon E600 compound microscope and the images were captured with a Nikon DXM1200 digital imaging system equipped with Nikon ACT-1 software. Then, seed dimension was calculated using the following formula:

Seed Size (Seed length to width ratio) = (Seed Length)/(Seed width)

Data Analysis

All the data collected were analysed using analysis of variance (ANOVA) with the aid of SAS 9.2 package while the treatment means were separated using Least Significant Difference (LSD).

Results

Number of Tillers

The highest number of tillers was from kinetin priming for all priming durations tested. On average, kinetin priming produced the highest number of tillers. The lowest number of tillers was produced by calcium chloride in all the priming durations tested except for 48-hour duration. On average basis, calcium chloride still produced the lowest number of tillers (Table 2).

Priming for 48 hours resulted in higher number of tillers in all priming agents used except polyethylene glycol. On average, higher number of tillers was produced when seed priming was for 48 hours. Lower number of tillers was from 24-hour priming in all the priming agents used except polyethylene glycol. On average, lower number of tillers was from 24-hour priming (Table 2).

Productive Tillers and Tiller Efficiency

The highest number of productive tillers was from polyethylene glycol priming for all priming durations tested. On average, polyethylene glycol priming produced the highest number of tillers. The lowest number of tillers was produced calcium chloride in all the priming durations tested except for 48-hour duration. On average basis, calcium chloride still produced the lowest number of productive tillers (Table 3).

Priming for 48 hours resulted in higher number of productive tillers in all priming agents used except kinetin priming. Lower number of productive tillers was produced when 24-hour priming was used in all the priming agents used except kinetin priming. On average, both priming durations tested had the same number of productive tillers (Table 3).

Tiller Efficiency (%)

Ppolyethylene glycol priming produced plants with highest tiller efficiency in all the priming durations tested. On average, the highest tiller efficiency was from plants resulting from polyethylene glycol priming while the lowest was from plants resulting from kinetin priming. The use of 24-hour priming resulted in plants with higher tiller efficiency in all the priming agents tested except polyethylene glycol. On average, 24-hour priming resulted in plants with higher tiller efficiency while 48-hour priming resulted in lower tiller efficiency (Table 4).

Shoot Fresh Mass (g/hill)

The heaviest fresh shoot was from polyethylene glycol priming on average basis. While the lightest fresh shoot was from calcium chloride priming. Heavier fresh shoot was produced when priming duration was 48 hours while lighter fresh shoot was produced when the priming was done for a period of 24 hours. A 48-hour priming produced the heaviest fresh shoots in all the priming agents tested with the exception of calcium chloride priming (Table 5).

Shoot Dry Mass (g/hill)

The heaviest dry shoot was produced by kinetin priming on average basis while the lowest was from poly ethyl glycol. Kinetin priming also produced the heaviest dry shoot in all the priming duration tested. The used of 48 hours for priming resulted in production of heavier dry shoot in all the priming agents tested while lighter dry shoot was from 24-hour priming. On average, 48-hour priming had heavier dry shoot while 24-hour priming produced lighter dry shoot (Table 6).

Yield (g/hill)

The highest yield was produced by polyethylene glycol priming on average basis while the lowest was from kinetin priming. polyethylene glycol also produced the highest yield in all the priming durations tested. Priming for 48 hours resulted in higher yield in all the priming agents tested with the exception of kinetin priming. On average, 48-hour priming produced higher yield while lower yield was from 24-hour priming (Table 7).

Harvest Index (%)

Plants with highest harvest index were from polyethylene glycol on average while the lowest was from kinetin priming. Polyethylene glycol produced plants with higher harvest index in all the priming durations tested. On average, 24-hour priming favoured higher harvest index while lower harvest index was favoured by 48-hour priming (Table 8).

Length-width ratio (seed size)

On average, the biggest seed size was from polyethylene glycol priming while the smallest seeds were from plants resulting from calcium chloride priming. The use of 24-hour priming resulted in bigger seeds in all the priming agents tested except polyethylene glycol. On average, 24-hour priming also resulted in bigger seeds while smaller seeds were from plants resulting from 48-hour priming (Table 9).

100-Seed mass (g)

Mass of 100seeds was, on average, highest in kinetin priming while the lowest was from calcium chloride priming. The use of 48-hour priming resulted in higher mass of 100seeds in all the priming agents used with the exception of polyethylene glycol. On average, higher mass of 100 seeds was from 48-hour priming while lower mass was from 24-hour priming (Table 10).

Rate of Photosynthesis ($\mu mol\ CO_2 m^{-2}s^{-1}$)

The rate of photosynthesis was highest in plants resulting from kinetin priming in all the priming durations tested. On average basis, kinetin priming still had the highest photosynthetic rate. The lowest rate on average basis as well as individual priming duration was from calcium chloride priming. The highest photosynthetic rate for all the priming agents tested was from 48-hour priming while the opposite was true for 24-hour priming. On average basis, 48-hour priming resulted in plants with higher photosynthetic rate while 24-hour priming resulted in plants with lower photosynthetic rate (Table 11).

Stomatal conductance ($mol\ H_2O\ m^{-2}s^{-1}$)

The highest stomatal conductance was found in plants resulting from kinetin priming in all the priming durations tested. On average, the highest stomatal conductance was also found in plants resulting from kinetin priming. The lowest stomatal conductance was from plants resulting from calcium chloride priming. Higher stomatal conductance was found in plants resulting from 48-hour priming in all the priming agents tested. On average basis, 48-hour priming still produced plants with higher stomatal conductance. The lowest stomatal conductance was from plants resulting from 24-hour priming (Table 12).

Transpiration rate ($mmol\ H_2O\ m^{-2}s^{-1}$)

The highest transpiration rate was from plants resulting from kinetin priming in all the priming durations tested. On average, the highest transpiration rate was from kinetin priming. However, the lowest transpiration rate was from calcium chloride priming in all the priming durations tested and on average basis. Higher transpiration rate was from plants resulting from 48-hour priming in all the priming agents tested. On average basis also, 48-hour priming resulted in higher transpiration rate. Lower transpiration rate was from plants resulting from 24-hour priming for all the priming agents tested and on average basis (Table 13).

Intercellular CO_2 ($\mu mol\ CO_2 m^{-1}$)

On average, the highest intercellular CO_2 was from plants resulting from calcium chloride priming. However, the lowest intercellular CO_2 was from plants resulting from kinetin priming. On average, plants resulting from 24-hour priming had higher intercellular CO_2 while plants from 48-hour priming had lower intercellular CO_2. Priming for 24 hours produced plants with higher intercellular CO_2 in all the priming agents tested while the opposite is true for 48-hour priming (Table 14).

Leaf water ($mmol\ H_2O\ m^{-1}$)

The highest leaf water was found in plants resulting from kinetin priming in all the priming durations tested while the lowest was from plants resulting from calcium chloride priming. On average, the highest leaf water was from kinetin priming while the lowest was from calcium chloride priming. A 48-hour priming resulted in plants with higher leaf water in all the priming agents tested while lower leaf water was found in plants resulting from 24-hour priming. On average, 48-hour priming resulted in plants with higher leaf water while 24-hour priming resulted in plants with lower leaf water (Table 15).

Discussion

The edge of kinetin priming in producing the highest number of tillers in a stressful condition could be linked to enhancement of meristematic activity by the growth regulator (kinetin) (Werner et al., 2001) as well as nutrient mobilization improvement (Sakakibara, 2005). The result could further be attributed to ability of plants in early usage of available resources for better growth and development which resulted higher number of tillers produced by the plants (Harris et al., 2002). In the same vein, growth regulator (kinetin) priming leads to growth repair which might have resulted in production of higher number of tillers than the rest priming agents.

Panicle bearing was least affected by drought in plants resulting from PEG 40% (w/v) priming. This could be attributed to the adaptation of the plants to moisture stress from the seed stage. In this case the seed has the memory of change in physiology which has conferred on it the ability to withstand future stress by reducing the effect of to bare minimum that cannot be so detrimental to its overall performance (Chen & Arora, 2013). This also led to best tiller efficiency. So, the issue of panicle blanking has been appreciably arrested with this osmotic treatment. These enhanced parameters finally affected the yield because the parameters are parts of yield determinants in rice.

The performance of poly ethyl glycol(PEG) priming in producing plants with highest number of tillers might be attributed to increase in number of actively reproducing plant cells in inflorescence meristem which led to increase in number of effective tillers (Leibfried et al., 2005). Consequently, there was higher number of productive tillers despite being subjected to moisture stress. Similarly, mobilization of nutrient by PEG (Sakakibara, 2005) could have contributed immensely to having the present result from PEG priming. Finally, achievement of biochemical repairs during imbibition (Shakirova et al., 2003) might have well enhanced the performance of PEG priming. It should be noted that higher number of tillers does not count in rice production if the number of panicle bearing ones among them is low. In that case, tiller efficiency will be low and it will not pay a crop producer. So, higher tiller efficiency from plants resulting from PEG priming shows that under drought stress, higher number of productive tillers could be produced with employment of PEG priming.

The result of fruit fresh mass might be attributed to better conservation of water by plants resulting from PEG priming. However, the rate of transpiration was moderate not that it was low as it would have been expected that because of very low transpiration rate, there was high water conservation with consequent higher fresh mass. The gain in succulence resulted in lower dry matter accumulation as found in this work. This implies that moisture content in crops is inversely related to dry matter accumulation. This implies that the more the moisture content, the less the dry matter accumulation.

Dry matter production was best with kinetin priming. It is known that higher photo-assimilate production is the basis of dry matter accumulation. So, it might be that kinetin priming has enhanced crop growth rate, net assimilation rate and leaf area index to have increased the final dry shoot weight (Farooq et al., 2006). It should be noted that higher biological yield at the expense of economic yield is detrimental to the target of the farmers whose target is the grain yield. This is the case of yield in this experiment. Nevertheless, higher biological yield could be advantageous if the objective of production is fodder production because biological yield will be the economic yield in that case. With less water in plants from kinetin priming, more dry matter was accumulated.

Priming with 40% (w/v) PEG still produced plants that could put up some tolerance to moisture stress they were subjected to and produced the highest grain yield among all the treatments. This could be the result of stand establishment improvement which confers better drought tolerance, pest damage reduction and crop yield increase (Harris et al, 1999). In the same vein, Thakur et al., (2005) established that increase in physiological activities and yield components that accompany seed hydro-priming and pre-germination are the essentials of better survival of crops in facing terminal moisture stresses. This could be due to effective partitioning of assimilate to the developing grain resulting from more production of spikelets. This in turn then created a big assimilate sink.

Moisture stress reduces grain size and the reduction is cultivar dependent (Mostajeran & Rahimi-Eichi, 2009). It has been found and reported that moisture deficit contributes to photosynthetic reduction as well as decrease in assimilate translocation to the grains which finally resulted in lower grain mass (Liu et al., 2008). Furthermore, water stress might lead to considerable increase in secondary rachis branch abortion which leads to reduced number of spikelets per panicle in addition, moisture stress could lower kernel sink potential by decreasing the number of endospermic cells and the amyloplast formed. Therefore, the level of grain mass correlates with the capacity of starch accumulation in the endosperm (Yang & Zhang, 2006). Increased number of filled grain could be as a result of increase in photosynthetic rates for assimilate production which is effectively translocated to the developing grains and thus increased in the final yield (Xu & Zhou, 2007). Recent proposal was that there is a link between grain yield and the senescence process of the whole plant (Mi et al., 2002). Slow and poor filling of the inferior spikelets which may even result in sterile spikelets or non-consumable grains are major contributors to low yield in rice which can be the result of moisture deficit (Ishimaru et al.,2005). This poor filling has been attributed to carbohydrate supply limitation (Yang & Zhang, 2006).

The harvest index is a measure of assimilate partitioning which may be judicious or not. PEG priming which favoured production of higher yield could be attributed to the fact that PEG priming aided better partitioning of assimilates to developing grains. That reason was revealed by having higher harvest index than other priming treatments used. Despite the fact that kinetin favoured production of higher dry matter (shoot dry mass) (Table 6), partitioning of assimilates to the developing grains was not favoured. Therefore, it had the lowest harvest index which implies kinetin priming was least effective in that aspect.

The grain size is determined by cell enlargement and elongation. It could be said that PEG priming aided production of more seed cells through mitotic division and followed it through better cell elongation and enlargement which manifested in grain size. So, the biggest grains were produced by plants resulting from PEG priming. However, the grains produced through PEG priming were not as heavy as those from kinetin priming despite their big sizes (Table 10) because grain mass depends on the amount of assimilates partitioned into the grains. This situation resembles the case of a tall and thin man with low body mass and a short and sturdy man with higher body mass. Higher yield from plants resulting from PEG priming could not be attributed to higher grain size as found in this work (Table 10). However, higher yield through PEG priming could be attributed to higher number of grains produced by the plants resulting from PEG priming as revealed by number of productive tillers (Table 3) and tiller efficiency (Table 4).

Mass of individual seeds is depicted by 100- or 1000-grain mass. Individual grain masses should be part of yield determinants. It should have been expected that heaviest grains should come from PEG priming. Contrary to the expectation, plants resulting from kinetin priming produced the heaviest seeds. This might be attributed to production of more photosynthesis as a result of higher photosynthetic rates in plants resulting from kinetin priming (Table 11). Despite the fact that grain size is a component of yield determinants in rice, there was more yield from plants resulting from PEG priming because the grains produced were more than those from kinetin priming (Tables 3 and 4). So, what was lost in mass was gained in number.

The highest photosynthetic rate recorded from kinetin priming could be linked to the level of stomatal conductance found in plants from kinetin priming (Table 12). This implies that stomatal conductance is directly related to photosynthetic rate because it is responsible for exchange of gases. This further establishes the relationship between photosynthesis and stomatal conductance (Flexas et al., 2002). Photosynthesis is the primary process that can lead to significant yield reduction when it is affected by drought stress (Chaves et al., 2009). Photosynthetic rate is lowered by only stomatal limitation in mild stress while both stomatal and non-stomatal (metabolic) limitations reduce its net value when plants are subjected to severe moisture stress (Sengupta et al., 2011).

Higher net photosynthesis through seed priming under moisture stress is an indication of less limitation from both stomatal and non-stomatal (metabolic) factors. The lower values recorded for calcium chloride priming indicates that there was non-stomatal (metabolic) limitation for decrease in photosynthetic activities. This is because the intercellular CO_2 was less affected. Decrease in net photosynthesis found in kinetin priming might result in production of higher level of reactive oxygen which may consequently cause oxidative damage to lipid, proteins, DNA and photosynthetic apparatus (Xiong et al., 2002). These reactive oxygen species cause lipid peroxidation which is measured by malondialdehyde (MDA) content in the tissues (Wang et al., 2014). Similarly, reduction in net photosynthesis under moisture stress is the result of adverse effect of stress on electron transport among photosystems I and II, enzymes of carbon metabolism like ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) as well as those enzymes related to its synthesis (Medrano et al., 2002). However, net photosynthesis has not been associated with grain filling (Murchie et al., 2002) because many poorly filled or blank spikelets still exist with existence of high value of photosynthesis. To explain this, it has been suggested that antisense which suppresses gene for sucrose transportation which is responsible for better grain filling does not affect photosynthesis (Scoefield et al., 2002).

Stomatal conductance regulation is very important in plants because of its role in CO_2 assimilation and prevention of desiccation (Medici et al., 2007). During moisture stress, decrease in atmospheric vapour pressure, reduction in leaf turgor and root-generated chemical signals lead to stomatal closure (Chaves et al., 2009). Therefore, both stomatal closure and suppression of mesophyll conductance result in photosynthesis reduction whether the stress is mild or severe (Flexas et al., 2004). In this work the maintenance of stomatal opening which kept the conductance at higher rate in kinetin priming might have been because priming treatments did not allow the plants to produce enough abscicic aid (ABA) that is responsible for stomatal closure. This is because the mechanism of stomatal closure during moisture stress is closely linked with ABA and because it is amply produced by plants under stress, it is called stress hormone (Melcher et al., 2009). The stomatal closure is achieved by increasing cystosolic Ca^{2+} production and activating plasma membrane–localized channels (Kohler & Blatt, 2002). Consequently, there will be efflux of potassium, depolarization of guard cells, loss of turgor and guard cell volume, high production of hydrogen peroxide with eventual closure of the stomata (Wang et al., 2012).

Transpiration rate under moisture stress was better regulated by kinetin priming in this work. Kinetin priming was the best in regulating the rate of water loss through transpiration. The role of kinetin priming is distinct and evident because it led to having lower transpiration rate. The rate of transpiration determines the speed at which plants desiccate. The more the water loss from the leaves, the quicker it is for plants to wilt. In the same vein, the impact of water loss through transpiration is directly felt in net photosynthesis (Peri et al., 2009). To forestall desiccation problem, stomatal closure comes into operation and serves as a signal of moisture stress (Harb et al., 2010). If moisture stress is not checked, the period of stomatal closure becomes lengthened and such reduces the rate of carbon assimilation in addition to regulation of water loss (Pan et al., 2011). So, plant maintenance hinges on regulation of water loss. Transpiration is taken as a necessary evil because accent of water up the plant will not be possible without it. Furthermore, the process leads to cooling of the plant system. This is based on the fundamental principle of physics that evaporation causes cooling.

Moisture stress affects gas exchange (intercellular CO_2) activities of plants and consequently their photosynthetic ability. Reduction in carbon assimilation rate relates directly to decrease in leaf water potential and relative water content (Lawlor & Cornic, 2002). Moreover, CO_2 assimilation is controlled by stomatal activities. This is because when drought persists, stomatal closure is experienced for longer hours of the day and consequently there will be reduction in carbon assimilation rate and loss of water through transpiration so as to maintain carbon assimilation in the presence of low water (Pan et al., 2011).

In this work, leaves of plants from calcium chloride priming had the highest volume of intercellular CO_2 despite the fact that they had the lowest stomatal conductance and net photosynthesis. This is a departure from the earlier established fact that strong correlation exists between stomatal conductance and inter-cellular CO_2 along with net photosynthesis (Flexas et al., 2002). This might be because the absorbed CO_2 was judiciously used and that consequently left a significant amount in the intercellular space. This is because stomatal conductance that should have led to higher accumulation of inter-cellular CO_2 was low and the photosynthesis that should consume the gas was lower compared to other treatments. As for low stomatal conductance, the coping strategy under water stress is to have stomatal closure to cut down the rate of water loss through transpiration so as to maintain plant metabolic activities which can only occur in the presence of transporting fluid. The outcome of this work suggests that the amount of intercellular CO_2 does not determine the value of net photosynthesis, stomatal conductance and transpiration contrary to the earlier established discovery that reduction in photosynthetic rate is more influenced by intercellular CO_2 than leaf water potential and leaf water content (Galmés et al., 2011).

Succulence of leaf (leaf water) influences stomatal opening and stomatal opening in turn determines the rates of photosynthesis and transpiration as found in this research. The fact that kinetin priming resulted in plants with higher leaf water explains why there were higher rates of photosynthesis and transpiration. So, kinetin priming would have led to production of higher yield than PEG priming but assimilate partitioning was judiciously done as depicted by the value of harvest index (Table 8).

As for priming duration, optimum concentration of any priming media used and priming duration are very important factors that determine germination success and seedling establishment. This is because water imbibition of the seeds during priming process is directly related to priming duration. It is an established fact that effectiveness of seed priming on seed invigoration and final yield are dependent on the treatment duration (Ghassemi−Golezani et al., 2008). The period of priming may be beneficial or detrimental to the seeds or resulting plants from the treatment. For instance, increased germination, better seedling establishment and higher yield were realized through 7 to 14 hour priming of pinto seeds while longer duration was detrimental to pre- and post-germination lives of the plant (Ghassemi−Golezani et al., 2010). Furthermore, priming for shorter duration has been found to result in low leachate leakage and electrical conductivity as a result of seed protection and minimization of cell wall damage. It has been explained that primed seeds imbibe water until a plateau is reached and then there would be a little change to circumvent the protrusion of radicle (Farahani & Maroufi, 2011). This has been confirmed by Ghasesemi-Golezani et al., (2010) and they added that the process will lead to positive response of the primed seeds while priming beyond the described level would be disastrous to the seeds and the resulting plants. The longevity of priming duration favoured production of higher number of tillers. This could be attributed to the fact that effectiveness of seed priming on seed invigouration and final yield are dependent on the treatment duration (Ghassemi−Golezani et al., 2008). The period of priming may be beneficial or detrimental to the seeds or resulting plants from the treatment. In this work, it was found that the highest yield was got when PEG was used for priming for 48 hours. Also, number of tillers, shoot dry mass, 100-seed mass, rate of photosynthesis, stomatal conductance, transpiration and leaf area water were increased when seeds were primed for 48hours with kinetin solution. However, tiller efficiency, harvest index and seed size were favoured by 24-hour priming with PEG while number of productive tillers, shoot fresh mass and grain yield.

Nevertheless, variations do occur on the basis of seed types and the presence of growth inhibitors. This is because thickness of the seed coat varies from one plant to another. The problem of seed coat thickness is mainly evident in tree crops where the seeds have to be treated first for removal of dormancy. In priming codia seeds (Cordia millenni) which is a species of timber tree, a three or four day osmo-priming was found most effective in breaking the seed coat to give the highest emergence percentage while hydro-priming for two days gave better results than osmo-priming for the same duration (Adebisi et al., 2011). In the same vein, when seeds of bread wheat were primed with PEG for a period of 12 hours, emergence percentage as well as economic and biological yield parameters were enhanced (Yari et al., 2011). It has also been found that priming rice seeds with 100 mM calcium chloride or 40% PEG 6000 should not exceed 48 hours while priming with 100 ppm kinetin should not exceed 24 hours to achieve effectiveness in the priming treatment under normal and drought stress conditions and avoid wastage of resources (Kareem et al., 2020b).

Similarly, in wheat cultivars (Cross-Alborz and Sardari), Ghobadi et al., (2012) discovered that a 12 hour priming with PEG6000 (-0.3MPa) resulted in higher germination percentage, appreciable root and shoot lengths as well as higher weight of roots and shoots. The result of higher germination was attributed to production of super oxide dismutase (SOD) and peroxidase (POD) (Jie et al.,2002).

Table 1. Treatment Combinations

	Priming Agent		
Priming Duration	Calcium Chloride Dihydrate (P1)	Polyethylene Glycol 6000 (P2)	Kinetin(P3)
24 Hours (T1)	TIP1	T1P2	T1P3
48 Hours(T2)	T2P1	T2P2	T2P3

Table 2. Effect of priming agents and soaking durations on number of tillers of rice under drought stress

Number of Tillers(Tillers/Hill)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	58.00	62.00	62.00	60.67
48 Hours	62.00	60.00	63.00	61.67
Average	60.00	61.00	62.50	

Table 3. Productive Tillers (Tillers/Hill)

Productive Tillers(Tillers/Hill)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	46.00	50.00	50.00	48.67
48 Hours	48.00	51.00	47.00	48.67
Average	47.00	50.50	48.50	

Table 4. Tiller Efficiency (%)

Tiller Efficiency(%)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	79.44	80.14	80.14	79.91
48 Hours	78.24	84.75	75.41	79.47
Average	78.84	82.45	77.77	

Table 5. Shoot Fresh Mass (g/hill)

Shoot Fresh Mass(g/hill)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	329.67	306.33	306.33	314.11
48 Hours	313.67	345.00	340.00	332.89
Average	321.67	325.67	323.17	

Table 6. Shoot Dry Mass (g/hill)

Shoot Dry Mass(g/hill)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	96.21	96.31	96.31	96.28
48 Hours	106.92	103.38	109.07	106.46
Average	101.57	99.85	102.69	

Table 7. Yield (g/hill)

Yield(g/hill)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	73.11	74.24	74.24	73.86
48 Hours	76.78	78.88	70.63	75.43
Average	74.95	76.56	72.44	

Table 8. Harvest Index (%)

Harvest Index (%)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	43.33	43.00	43.00	43.11
48 Hours	42.00	43.33	39.00	41.44
Average	42.67	43.17	41.00	

Table 9. Length-width ratio

Length-width ratio(seed size)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	4.22	4.19	4.40	4.27
48 Hours	4.17	4.34	4.04	4.18
Average	4.20	4.27	4.22	

Table 10. 100-Seed mass (g)

100-Seed mass (g)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	2.39	2.43	2.41	2.41
48 Hours	2.41	2.38	2.48	2.42
Average	2.40	2.41	2.45	

Table 11. Rate of Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)

Rate of Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)				
		Priming Agents		
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	2.90	27.77	45.59	25.42
48 Hours	58.13	54.06	71.20	61.13
Average	30.52	40.92	58.40	

Table 12. Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)

Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	0.24	2.15	3.03	1.81
48 Hours	2.44	2.64	3.77	2.95
Average	1.34	2.40	3.40	

Table 13. Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)

Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	6.15	29.84	35.61	23.87
48 Hours	32.40	32.95	39.08	34.81
Average	19.28	31.40	37.35	

Table 14. Intercellular CO_2 (µmol CO_2 m^{-1})

Intercellular CO_2 (µmol CO_2 m^{-1})				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	362.79	351.74	342.04	352.19
48 Hours	321.69	329.37	324.60	325.22
Average	342.24	340.56	333.32	

Table 15. Leaf water (mmol H_2O m^{-1})

Leaf water (mmol H_2O m^{-1})				
	Priming Agents			
Priming Duration	Calcium Chloride	Polyethylene Glycol 6000	Kinetin	Average
24 Hours	30.28	34.83	35.98	33.70
48 Hours	35.35	35.46	36.66	35.82
Average	32.82	35.15	36.32	

Conclusion

It was found that number of tillers, shoot dry mass, 100-seed mass, rate of photosynthesis, stomatal conductance, transpiration and leaf area water were increased when seeds were primed for 48 hours with kinetin solution. However, tiller efficiency, harvest index and seed size were favoured by 24 hour priming with polyethylene glycol (PEG) while number of productive tillers, shoot fresh mass and grain yield were favoured by 48 hour priming with PEG. It was concluded that 48 hour priming with PEG was effective for alleviating moisture stress in MR219 rice. This implies that to avoid wastage of priming chemicals and circumvent undue prolongation of priming period or duration which will result in harming the seeds (toxicity) and poor performance of the resulting plants, 40% (w/v) PEG 6000 should be used for 48 hours for priming MR219.

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