



IMPROVEMENT OF RICE SEED GERMINATION AND SEEDLING GROWTH USING SEED PRIMING

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INTRODUCTION

There is need for rapid germination of the seeds with uniform emergence and luxuriant growth are prerequisite to improved yield and better quality of annual crops like rice (Parera and Cantliffe, 1994). As a result of this need, researchers have tried technology of seed priming and found that it resulted rapid germination with better growth synchronization, high seedling vigour, high germination percentage and

ABSTRACT

Background: Improvement of rice seed germination as well as growth of seedlings which will eventually result in higher yield is highly needed.

Objectives: In a study was conducted to determine the influence of seed priming on germination and seedling growth of rice.

Methods: The osmotic priming media used were 100mM CaCl₂, 40% (w/v) polyethylene glycol (PEG) 6000 and 100 mg/kg kinetin solutions. Rice seeds (50g) of variety MR 219 were soaked in each of the priming media for the duration of 24 and 48hours respectively to have a total of seven treatments with inclusion of the control. The experiment was laid out in completely randomized design (CRD) with three replications. Data collection was on root fresh and dry masses, shoot fresh and dry masses, root length and shoot length, final germination percentage, root volume, electrical conductivity, days to 50% germination, germination index, seedling vigour index, succulence index and root to shoot ratio.

Results: The results showed that priming treatments improved root dry mass, shoot fresh and dry masses, shoot length, final germination percentage, root volume, electrical conductivity, days to 50% germination, germination index and seedling vigour index. From the priming media used, priming with 40 % (w/v) PEG₆₀₀₀ for duration of 24hours improved root and shoot dry masses, shoot length and root volume.

Conclusions: It could, therefore, be concluded that priming with 40 % (w/v) PEG₆₀₀₀ for duration of 24hours be used for improving seedling growth of MR219 rice variety.

Keywords: Rice, germination, seedling growth, osmotic priming and hormonal priming

better yield especially in vegetable (Bruggink et al., 1999) and other field crops (Farooq et al., 2006). This technology involves soaking seeds of interest in solutions of low water potential to allow controlled hydration of the seeds with eventual prevention of radicle protrusion (Giri and Schilinger, 2003). The mechanism of its operation is that it gives room for some necessary

germination metabolic processes to occur up to the second stage of germination. The use of high osmotic potential solutions obstructs the soaked seeds from imbibing enough water to allow radicle protrusion and, therefore, the seeds are left in the lag phase of germination called germination *sensu stricto* (Taylor et al., 1998).

Success in priming is influenced by some factors like species, type of priming media, temperature, concentration, priming duration, seed viability, oxygen and the storage condition of the seeds (Mubshar et al., 2006). So, priming results differ for different chemicals used and the consistence of such results is not guaranteed in other crops. Priming could be done through the use of osmotic salts, growth regulators, vitamins and so on. Based on the chemical used, the priming process is named. For instance, priming with osmotic salt is called osmotic priming or osmo-priming. Similarly, the use of hormone gives hormonal priming and so on. The use of growth regulators in priming different crops has equally led to improved germination and better performance of the resulting seedlings (Miyoshi and Sato 1997). For instance, GA₃ activates α -amylase to break down stored starch in the seeds which will be consumed by the growing embryo during germination. Furthermore, GA₃ and ethylene are involved in the stimulation and elongation of coleoptile, internodes and mesocotyl of rice seedlings after germination. In the same vein, abscisic acid promotes elongation of the mesocotyl of rice seedlings (Lee et al., 1999). Application of kinetin and gibberellin by Miyoshi and Sato (1997) on dehusked seeds of Indica and Japonica rice revealed the stimulatory effects of gibberellin under aerobic and anaerobic rice cultivation and its better influence on the treated seeds.

The duration of soaking seeds in priming media is very important and should not exceed the safe limit. Otherwise, there will be seed or seedling damage as a result of premature germination (Harris et al., 2000). It should be noted that primed seeds do not germinate until they get adequate moisture from their environments. Based on this fact, they can be stored like normal seeds till the time they will be needed. But when priming exceeds the safe limit, the treated seeds proceed to the third stage of germination for protrusion of radicle after the cessation of the priming process. This can still occur when there is moisture

inadequacy in the environment. So, they become pre-germinated seeds not primed seeds. Pre-germinated seeds are only useful for immediate planting without any delay.

Researches have been conducted on the effect of seed priming on seedling production in other rice varieties and not MR219. In addition to that, most of the researches did not consider variation in the priming duration. Therefore, the present experiment was conducted to determine the effect of seed priming and varying duration on seed germination and seedling growth of MR219 rice variety.

MATERIALS AND METHODS

Experimental site

This experiment was conducted in the glass house of the Rice Research Centre of Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (3^o02' N, 101^o42' E; elevation 31 m). During the experiment, monthly average maximum and minimum temperature and were 33.5^oC, 21.5^oC respectively while relative humidity was 92.5 % while rainfall, evaporation and sunshine hours were 9.8 mm/day, 4.6 mm/day and 6.6 hrs/day respectively.

Experimental treatments and design

Seven priming treatments were used in this experiment (Table 1). Osmo-priming was achieved using calcium chloride at 100mM and PEG at 40% (w/v) while hormonal priming was through 100ppm of kinetin. Unprimed seeds were used as the control. The primed seeds were then drained of the priming chemicals and washed three times to free the seeds of the priming chemicals. Then, the seeds were air dried on filter paper to restore them to their initial moisture levels. The experiment was laid out in Completely Randomized Design (CRD).

Crop husbandry

The prepared seeds were directly sown in pots of diameter 31.5cm and area 779.625cm²

Table 1: Description of Treatments

Treatment	Priming Chemical	Concentration	Priming Duration (Hours)
CaCl ₂ 1	Calcium chloride dihydrate	100 mM	24
CaCl ₂ 2	Calcium chloride dihydrate	100mM	48
PEG 1	Polyethylene glycol 6000	40 % (w/v)	24
PEG 2	Polyethylene glycol 6000	40 % (w/v)	48
Kinetin1	Kinetin	100 ppm	24
Kinetin2	Kinetin	100ppm	48
Control	-	-	-

and kept in anaerobic condition throughout the experimental period. Regular hand weeding was used to free the seedlings weed-free and prevent inter-specific competition between them and the seedlings.

masses, shoot fresh and dry masses, root length and shoot length, root to shoot ratio, seedling succulence index, root volume and electrical conductivity of the leachates from the primed seeds.

Data collection

Data collection was on germination percentage, germination index, root fresh and dry

Final germination percentages (GP) were calculated according to AOSA (1983) using the following formula:

$$GP = \frac{\text{Number of germinated seeds at final count}}{\text{Total number of planted seeds}} \times 100 \dots\dots\dots 1$$

Germination index (GI) was also calculated according to AOSA (1983) using the following formula:

$$GI = \frac{\text{Number of germinated seeds}}{\text{Day of the first count}} + \dots + \frac{\text{Number of germinated seeds}}{\text{Day of the final count}} \dots\dots\dots 2$$

Day to 50% germination (T₅₀) was calculated according to the modified formula by Farooq *et al.* (2005) as follows:

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i} \dots\dots\dots 3$$

Where N is the final number of germinated seeds and n_i and n_j are cumulative numbers of seed germinated by adjacent counts at time t_i and t_j (days) respectively. N_i<N/2<n_j. This is expressed in days.

Ten days after planting (DAP), three seedlings were uprooted from each pot. From the seedlings, root and shoot lengths were measured using a ruler. The root volume was measured with a root scanner. The partitioned seedlings were then dried in a forced-air oven at 70⁰C until constant mass and the root and shoot dry masses were measured using top pan balance. Seedling vigour index (SVI) was calculated according to AOSA (1983) using this formula:

$$SVI = \frac{\text{seedling length (cm)} \times \text{Germination percentage}}{100} \dots\dots\dots 4$$

Finally, seedling succulence index (SSI) was calculated as follows:

$$\text{Seedling Succulence Index (SSI)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight}} \dots\dots\dots 5$$

Statistical analysis

All the data collected were analysed using Analysis of Variance (ANOVA) and significant treatment means were identified with Duncan Multiple Range Test (DMRT) at 5% probability level using the SAS statistical software package version 9.2

RESULTS

Table 2: Effect of seed priming on germination percentage and days to 50 % germination of rice seeds

Treatment	Germination percentage (%)	Days to 50 % germination (Day)
CaCl ₂ 1	70.00b	1.75 b
CaCl ₂ 2	73.33a	1.85b
PEG 1	56.67d	1.53c
PEG 2	70.00b	1.77b
Kinetin1	63.33c	1.84b
Kinetin2	36.67e	1.50c
Control	73.33a	3.92a

Means with the same letter(s) in the same column are not significantly different from one another.

Table 3: Effect of seed priming on mean germination time, germination index and seedling vigour index of rice

Treatment	Germination index	Seedling vigour index
CaCl ₂ 1	10.98a	32.53b
CaCl ₂ 2	9.40 b	34.57a
PEG 1	10.77a	27.26d
PEG 2	10.27a	32.40b
Kinetin1	9.20b	29.61c
Kinetin2	8.36c	17.42c
Control	7.84d	35.99a

Means with the same letter(s) in the same column are not significantly different from one another.

Table 4: Effect of seed priming on root and shoot fresh and dry masses of rice seedlings

Treatment	Root fresh mass (g)	Root dry mass (g)	Shoot fresh mass (g)	Shoot dry mass (g)
CaCl ₂ 1	1.09b	0.16a	1.05a	0.15b
CaCl ₂ 2	0.89c	0.12b	0.87c	0.11d
PEG 1	1.01b	0.17a	0.92b	0.13c
PEG 2	0.80c	0.10b	1.04a	0.17a
Kinetin1	0.67d	0.08d	0.89c	0.13c
Kinetin2	0.73c	0.10c	0.60d	0.09e
Control	1.14a	0.12b	0.97b	0.14c

Means with the same letter(s) in the same column are not significantly different from one another.

Table 5: Effect of seed priming on root length, shoot length, root-shoot ratio and succulence index of rice seedlings

Treatment	Root Length(cm)	Shoot Length(cm)	Root-Shoot ratio	Succulence index
CaCl ₂ 1	11.00a	34.78c	0.32a	5.90c
CaCl ₂ 2	10.50a	36.00b	0.29b	6.60b
PEG 1	9.95b	39.61a	0.25c	5.43c
PEG 2	9.89b	35.00c	0.28b	5.81c
Kinetin1	10.28a	36.18a	0.28b	5.81c
Kinetin2	10.17a	35.61c	0.29b	6.00b
Control	12.00a	37.11b	0.32a	7.12a

Means with the same letter(s) in the same column are not significantly different from one another

Effect of seed priming on Germination percentage, days to 50% germination, germination index of rice seeds and vigour index of rice seedlings

All the priming treatments resulted in lower germination percentage than the un-primed control with the exception of CaCl₂ 2 which was the same with the control (Table 2). The lowest germination percentage was recorded from kinetin2. CaCl₂ 2 was 49.99% better than kinetin 2 which had the least germination percentage. The extension of germination percentage is seedling vigour index (SVI) which measures uniformity of seedling growth. In this research, all the priming treatments exhibited lower values compared with the control (Table 3).

As for germination index which measures uniformity of germination, all the priming treatments were better than the control. The highest level of uniformity was from CaCl₂1 followed by PEG1. They were 1.91 and 6.47% respectively better than the control (Table 3). The lowest number of days to 50% germination was achieved with the use of kinetin 2 followed by PEG1 while the highest number of days was taken by the control. So, all the priming treatments were better than the control for number of days to 50% germination (Table 2).

Effect of seed priming on root fresh mass, root length, root to shoot ratio and succulence index of rice seedlings

All the priming treatments did not improve root fresh mass, root length, root to shoot ratio and succulence index above the control. CaCl₂ 1 and the control were the same for root-shoot ratio. The lowest root mass was from kinetin1, the shortest plant root was produced by PEG 2, the smallest root to shoot ratio was from PEG1 while the lowest succulence index was from kinetin1 (Tables 4 and 5).

Effect of seed priming on root dry mass, shoot length, shoot fresh and dry masses of rice seedlings

Seed priming improved root dry mass, shoot fresh and dry masses as well as shoot

length above the control. The peak dry matter production in root was favoured by PEG 1 while the least was from kinetin1. CaCl₂1

produced the heaviest fresh shoot while PEG2 had the peak dry matter production in the same organ (Table 4). PEG1 enhanced shoot height more than any other treatment. So, the tallest plant was from this treatment while the shortest plant was from CaCl₂ 1 (Tables 5).

Effect of seed priming on root volume of rice seedlings and electrical conductivity of seed leachates

All the seed priming treatments did not increase root volume above the control except PEG1. The highest root volume (8.85 cm³) was from PEG1 while the remaining treatments were lower than the control. The lowest root volume was from kinetin 1 (Table 6). With the exception of CaCl₂ 1 and CaCl₂ 2, all the priming treatments used improved cell wall stability (through lowering of cell leachate) above the control. The best impact was from PEG2 priming which had the least value of electrical conductivity (0.00 μs/cm) while CaCl₂2 had the least impact in cell wall stability maintenance by having the highest electrical conductivity value (0.27 μs/cm). The less the leachate, the more the stability and vice versa (Table 6).

Table 6: Effect of seed priming on root volume of rice seedlings and electrical conductivity of seed leachates

Treatment	Root volume (cm ³)	Electrical conductivity of seed leachate (μs/cm) at 25°C
CaCl ₂ 1	5.32c	0.23b
CaCl ₂ 2	8.12a	0.27a
PEG 1	8.85a	0.10d
PEG 2	5.35c	0.00f
Kinetin1	5.17c	0.10d
Kinetin2	7.59b	0.07e
Control	8.43a	0.13c

Means with the same letter(s) in the same column are not significantly different from one another.

DISCUSSION

Better performance of the control above the priming treatments applied in germination percentage is a show case of occurrence of occasional chances that may occur in biological experiments where the control will perform better than applied treatment(s). Contrary to our result, Ashraf and Rauf (2001) reported that final germination percentage, fresh and dry masses of corn seed were significantly increased by seed priming treatments. Seedling vigour index (SVI) also followed the pattern of germination percentage because germination percentage is a component of the formula for calculating SVI. The implication is that all the priming treatments used in this research were below or at the same level with the control in performance. Despite our results, Ruan et al. (2002) found that higher seedling vigour level resulted from seed priming treatments. This was because SVI is a function of germination percentage as explained earlier. This does not mean that the control should be given priority in selection because factors like seed viability, plant species, type of priming media, media concentration, priming duration, temperature, oxygen, seed vigour and priming storage condition are factors influencing priming effectiveness (Mubshar et al., 2006).

Better uniformity of germination (germination index) conferred by our priming treatments could be as a result of completion of pre-germination metabolic activities which ensured rapid radicle protrusion through multiplication of radicle cells soon after sowing by accelerating the imbibition process (Basra et al., 2005). Reduction in imbibition lag time and build-up of germination-enhancing metabolites (Basra et al. 2005) might also contribute to the uniformity of germination of primed seeds. In another view, synchronized emergence could have resulted from rapid development of embryo, genetic and structural repair (Arif et al., 2008) and reduction of seed bulk physiological non-uniformity through priming process (Still and Bradford, 1997). Furthermore, Rowse (1995) explained that higher emergence rate and reduced inherent physiological heterogeneity in primed seed germination were through rapid imbibition of water which enhanced seed revival. Similarly, early emergence indicated

by lower days to 50% germination primed seeds could be the result of rapid germination metabolite production (Basra et al.,

2005) along with swift synthesis of RNA, DNA and protein which indicates better genetic repair (Bray et al., 1989). Seeds treated with PEG1, PEG2 and Kinetin2 which showed rapid germination could be through promotion of oxygen uptake which led to acceleration of seed germination processes. These results confirm the findings of the earlier researchers (Anwar et al., 2012) who reported increased speed of germination as well as synchronization of seedling growth resulting from priming treatments.

Priming treatments enhanced accumulation of dry matter in the resulting seedling roots better than the control. This confers greater strength in the root for better penetration to seek soil moisture. In line with this result, Ogbuehi et al. (2013) reported that root dry mass of bambara groundnut was influenced by hydro-priming duration from vegetative to maturity stage. This also conforms to several reports on root dry mass of maize and sorghum indicating the beneficial effect of priming (Gupta et al., 2006). The succulence index which is a measure of the proportion of moisture to dry matter content of a plant was higher in the control because of the level of the moisture content in the plants. The elongation of the root was enhanced by high moisture level which is the basis of cell turgidity and elongation. This could explain why there were longer roots in the control with consequential appreciable root to shoot ratio.

It could be inferred that priming treatments enhanced the meristematic activities like mitosis (cell division) as well as enlargement of the root and shoot cells which resulted in higher accumulation of dry matter as well as elongation of the roots and shoots. For the fact that priming treatments do not act directly on the resulting seedlings, it could be said that the enhanced metabolic activities of the treated seeds led to the observed results. Miyoshi and Sato (1997) explained that longer root and shoot as well as higher dry matter production in seedlings were the results of the stimulatory effect of

priming at the early stage of germination processes through mediation in cell division. Moreover, Sadeghi et al. (2011) supported the reason advanced for higher growth and dry matter accumulation of rice seedlings as being a stimulatory effect of priming on the treated seeds and indirectly on the resulting seedlings. In addition to that, the height of plant is genetically controlled while the environment shapes its expression. So, the competition for nutrient and light especially of the resulting seedlings of primiratments led the plants to be taller. Although Basra et al.(2003) did not report significant height increase through PEG 40%(w/v) priming for 24hours, Afzal et al.(2005) found significant height increase using priming treatments. This height increase is only beneficial up to a certain degree after which it constitutes a source of harm to the plants because it makes them prone to lodging (Kareem et al., 2013) and the photosythatate that should be partitioned to the economic part will be partitioned the non-economic plant stem. The root spread predicts the ability of a plant to be in touch with the nutrient as well as water for growth and development while the root length gives plants better anchorage to the soil against lodging or other mechanical attacks. From this work, PEG1 that produced the highest root dry matter accumulation also had the best spread or root coverage which will later result to luxuriant growth of the plants through effective absorption of the required minerals and water. It is evident from this research that the longer the priming duration, the better the cell wall maintenance against leakage of cell leachates which is measured through electrical conductivity. This was what was observed in PEG and Kinetin priming with the exception of CaCl₂. The exceptional performance of PEG could be a result of its high osmotic potential which did not give room for weakening of the cell wall and result in leakage of electrolytes (high value of electrical conductivity). The action of CaCl₂ is detrimental to the stability and well-being of the treated seed cell walls. However, cell wall breakage using CaCl₂ priming could allow for better imbibition of water which in turn aids germination. It could, therefore, be said that this osmotic chemical will be a very good material for breaking dormancy which is one of the beneficial effects of seed priming.

CONCLUSION

From this experiment, priming with 40

% (w/v) PEG₆₀₀₀ for duration of 24hours improved root and shoot dry masses, shoot length and root volume. It could, therefore, be concluded that priming with 40 % (w/v) PEG₆₀₀₀ for duration of 24hours be used for improving seedling growth of MR219 rice variety.

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