

Priming Effects on Seed Germination of Tadong Upland Rice Collected in Sabah, Malaysia

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ABSTRACT Rice (*Oryza sativa* L.) is a food crop cultivated worldwide and serves as a staple food for more than half of the world's population. However, the rice production in Malaysia is still unable to reach full sufficiency level and incapable to ensure the national food security as the production levels are still low especially the rice farms in hilly areas. The current self-sufficiency level of rice in Malaysia is 72% and still lacking of 8% from the target of 80% by 2023. Seed priming is one of the techniques to enhance seed performance with respect to uniformity and rate of germination which results better yields in crops. The objective of this research was to determine the priming effects of polyethylene glycol (PEG) 6000 on seed germination of Tadong upland rice. Seed germination of Tadong upland rice was determined under different concentrations of PEG 6000. The experiment was laid out in a completely randomized design with three replications. The priming treatments were five concentrations of 0, -2, -4, -6, and -8 bars using PEG 6000. The data was analyzed using One-way ANOVA and LSD was applied to compare means. The grain size of Tadong upland rice is very long (length of 9.90 mm) and the shape is medium (length/breadth ratio of 2.77). The colour of rice grain with husk is brownish yellow while without husk is reddish black in colour. All the germination traits including germination percentage, germination index, germination rate, mean germination time, germination speed and germination energy showed significant effects among priming concentrations. The highest germination percentage (95.67%), germination index (51.26), germination rate (0.223), germination energy (93.00%) and shortest mean germination time (4.48 days) was found from priming with distilled water (0 bar) but germination speed (100.00%) was found highest when it was treated with -2 bar osmotic potential of PEG 6000. It can be concluded that the higher the PEG concentration (-8 bar), the lower the germination index (35.99), germination rate (0.206), germination speed (94.27%) and germination energy (87.67%) and the longer the mean germination time (4.83 days).

KEYWORDS: Upland rice; Priming; Polyethylene glycol 6000; Germination; Tadong.

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INTRODUCTION

Rice (*Oryza sativa* L.), is one of the most important food crops cultivated worldwide which serves as a staple food for more than half of the world's population (Sohrabi *et al.*, 2013; Dissatian *et al.*, 2018). More than 90% of the world's rice is grown and consumed in Asia (Akinbile *et al.*, 2011; Liu *et al.*, 2019). It is a vital crop which mostly depended for its food calories and protein in most Asian countries, including Malaysia (Khush, 2005; Lesk *et al.*, 2016; Fahad *et al.*, 2017). The two types of rice planting in Malaysia are wetland rice and upland rice (Sohrabi *et al.*, 2012). Wetland rice is mainly planted in irrigated paddy field with a larger scale especially in Peninsular Malaysia while upland rice cultivation is usually practiced in a smaller scale mostly by rural communities living particularly in Sabah and Sarawak (Hanafi *et al.*, 2009; Sohrabi *et al.*, 2012). Unlike wetland rice that is planted on irrigated field, cultivation of upland rice is usually on dry areas which mostly relies on rainfed irrigation.

Upland rice varieties have not been commercialized due to their low grain yields with an average yield ranges from 0.46 to 1.1 tonnes per hectare which was attributed to the poor management of the farmers (Sohrabi *et al.*, 2012). Certain upland rice varieties have desirable characteristics such as fragrance, colours, sizes and shapes which considered by farmers for planting purposes (Hanafi *et al.*, 2009; Ahmad *et al.*, 2015; Tuhina-Khatun *et al.*, 2016). Most of the rural communities living especially in Sabah and Sarawak practice upland rice cultivation. However, the rice production in

rained lands commonly encounters drought or inconsistent rainfall. This prompts a rising focus on rice production in the rained upland environments.

Globally, water deficit is one of the major constraints which limiting the crop production especially during the seed germination stage that ultimately results in a decline or even complete inhibition of seedling emergence and stand establishment (Kaya *et al.*, 2006; Yan, 2015). Seed germination is a crucial process that influences crop yield and quality. However, the seed germination and seedling establishment were inhibited due to the reduction of water potential, which in turn decrease the water uptake by the seeds under drought stress (Farooq *et al.*, 2009). In consequence, it is necessary to alleviate the adverse effects of drought stress for achieving good crop yields (Ashraf & Rauf, 2001; Lipiec *et al.*, 2013). Among various strategies adopted to improve plant drought tolerance, seed priming is one of the techniques that is easily applied, low-cost and effective which can be used to alleviate the depressive effects of drought stress (Ashraf & Foolad, 2005).

Seed priming is a common practice which consists of soaking seeds in a given solution followed by dehydration prior to sowing in order to enhance seed performance with respect to rate and uniformity of germination which results better yields (Ashraf & Foolad, 2005; Farooq *et al.*, 2006; de Lospinay *et al.*, 2010). For instance, seed priming with PEG and water increased drought stress tolerance in seeds of rice cultivars at the germination stage (Sun *et al.*, 2010). The efficiency of differential exogenous agents, PEG, in increasing the tolerance against drought stresses has not been comparatively investigated in Tadong upland rice. Therefore, the present study was aimed to determine the potential of osmopriming with PEG 6000 on seed germination of Tadong upland rice.

METHODOLOGY

Materials

The study was conducted at the Laboratory of Plant Physiology, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. The Tadong upland rice grains used in this study were collected from Ranau, Sabah, Malaysia. The rice grains were kept in ziplock bag and stored at the cool room prior to study.

Methods

(A) Physical Measurements

Grain Size and Shape

The length and width of ten grains were measured using an electronic digital calliper with accuracy of 0.01 mm. Data obtained were interpreted based on the scale described by Graham (2002).

Thousand Grains Weight

One thousand rice grains were counted and selected randomly. The thousand grains weight was weighed using an analytical balance (AND Weighing GF-3000, Interscience Sdn. Bhd.).

Grain Colour

The colour of Tadong rice grains was measured using color reader (CR-10, Konica Minolta Sensing, INC. Japan). Measurement was based on the L*C*h and L*a*b* colour system. The colour of the rice grains was determined visually using a colour chart provided by Munsell Soil Color Book.

Moisture Content

The moisture content of rice was determined using the oven method described by the standard methods of analysis of the AOAC (1984). The moisture content of rice was calculated after dried in oven at 85°C for three days. The moisture content of rice was calculated using equation (1):

$$\text{Moisture content (\%)} = (\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight} \times 100\% \quad (1)$$

Treatments

In this study, five different concentrations of polyethylene glycol (PEG) 6000 (0, -2, -4, -6 and -8 bar) were used for priming of upland rice seeds (Sun *et al.*, 2010; Lum *et al.*, 2014). Distilled water was used as a control (0 bar) and osmotic potentials (-2, -4, -6 and -8 bar) were prepared by adding PEG 6000 to distilled water according to the equation (2) of Michel and Kaufmann (1973) and Lv *et al.* (2013):

$$\text{OP} = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T \quad (2)$$

where OP is osmotic potential, c is concentration of PEG 6000 in g/kg water, and T is temperature in °C.

The PEG solution with desired osmotic potentials (-2, -4, -6 and -8 bar) were prepared by dissolving 11.97, 17.84, 22.37 and 26.20 g PEG in 100 mL distilled water, respectively.

(B) Germination Tests

The seed samples were taken in a random manner and primed with different concentrations of PEG 6000 for 48 hours at 25°C. Seeds that float to the surface of water was discarded. After priming treatment, the treated upland rice seeds were washed thoroughly with distilled water and dried at 25°C for 24 hours. A hundred seeds were arranged on a wet paper towel in a germination box with three replications for each treatment. The wet paper towel was moistened at least once a day using distilled water depending on the moisture of the paper towel inside the germination box. The germination boxes were stored at room temperature (24±2°C) and were covered to prevent the loss of moisture by evaporation under laboratory condition. The upland rice seeds were allowed to grow into seedlings for seven days.

Germination was observed every day according to recommendations by International Seed Testing Association (ISTA, 1993) and the number of germinated seeds were recorded for seven days, after there is no further germination occurred. The number of germinated seeds were counted and expressed as germination percentage (GP) as per the formula (3) by Scott *et al.* (1984):

$$\text{GP (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100\% \quad (3)$$

The number of germinating seeds each day were counted and expressed as germination index (GI) by using the formula (4) as suggested by the Association of Official Seed Analysis (AOSA, 1983):

$$\text{GI} = \sum \left(\frac{n}{d} \right) \quad (4)$$

where n is the number of germinating seeds and d is the respective days of germination.

The number of germinated seeds, number of germinated seeds on respective growth day and number of total germinated seed were recorded and expressed as germination rate (GR) by using the formula (5) (Ellis & Robert, 1981):

$$GR = \frac{\sum N}{\sum (n \times g)} \quad (5)$$

where N is the number of germinated seeds, n is the number of germinated seeds on growth day, and g is the number of total germinated seeds.

Mean Germination Time (MGT) is the measure of time taken for seeds to germinate, which is also a speed index as quicker germination corresponds to lower values of MGT. MGT of each replicate was computed by using the daily counts of germinated seeds according to the equation (6) (Dezfuli *et al.*, 2008):

$$MGT = \frac{\sum Dn}{\sum n} \quad (6)$$

where n is the number of seeds newly germinated on day D, D is the number of days counted from the beginning of germination, and n is the number of germinated seeds (final count).

The germination speed (GS) was calculated using the formula (7) of Krishnaswamy & Seshu (1990):

$$GS (\%) = \frac{\text{Number of seeds germinated at 72 h}}{\text{Number of seeds germinated at 168 h}} \times 100\% \quad (7)$$

Germination energy (GE) was computed as percentage of seeds germinated at 72 hours as per the formula (8) (Bam *et al.*, 2006):

$$GE (\%) = \frac{\text{Number of seeds germinated at 72 h}}{\text{Total number of seeds}} \times 100\% \quad (8)$$

Statistical Analysis

All the data were subjected to One-way Analysis of Variance (ANOVA) by using the Statistical Analysis Software (SAS) Version 9.4 (SAS Institute Incorporation, 2002) software. Least Significant Difference (LSD) test at 0.05 level of probability was used to compare between means when ANOVA showed significant treatment effects of this study. The correlations between variables were determined using Pearson's correlation coefficients.

RESULT

(A) Physical Measurements

Grain appearance is considered as an important characteristic for understanding the physical properties of rice (Deepa *et al.*, 2008; Lum, 2017; Custodio *et al.*, 2019). Tadong upland rice with husk is brownish yellow in colour while without husk is reddish black in colour (Figure 1). The length, width, length-breadth (L/B) ratio, thousand grains weight, moisture content, colour (L*C*h) with husk and without husk and colour (L*a*b*) with husk and without husk of the rice obtained (Table 1).



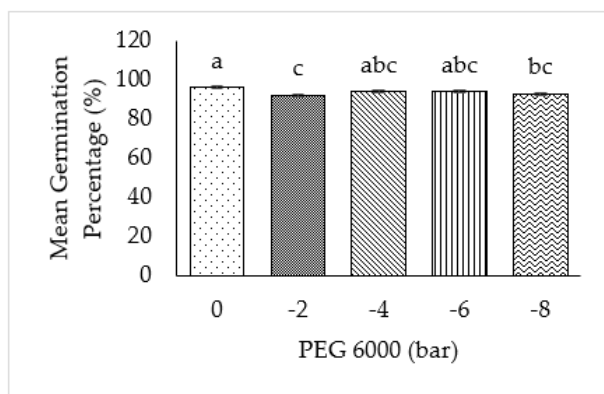
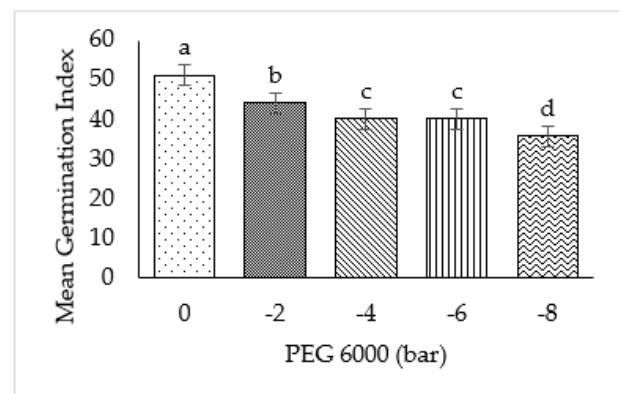
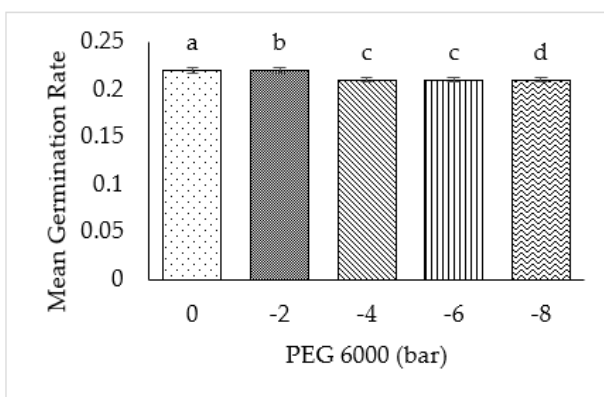
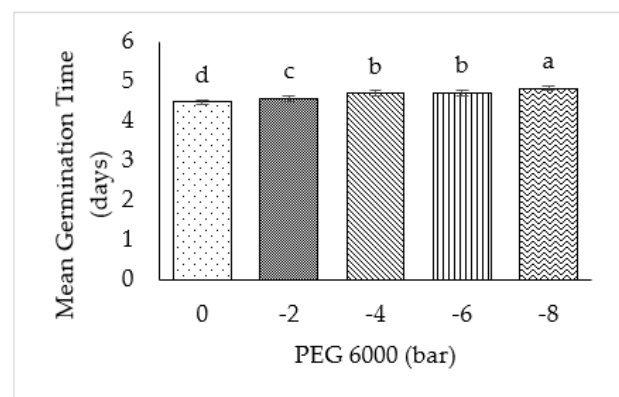
Figure 1. Tadong upland rice grains (A) with husk and (B) without husk.

Table 1. Physical characteristics of Tadong upland rice.

Physical characteristics	
Length	9.90 mm (very long)
Width	3.57 mm
Length/Breadth ratio (L/B ratio)	2.77 (medium)
Thousand grains weight	37.66 g
Moisture content	12.11%
Colour (L*C*h) with husk	L* = 49.9; C* = 72.6; h = 121.0
Colour (L*a*b*) with husk	L* = 49.9; a* = -35.3; b* = +62.1
Colour (L*C*h) without husk	L* = 47.3; C* = 65.4; h: 126.9
Colour (L*a*b*) without husk	L* = 47.3; a* = -44.2; b* = +51.2

(B) Germination Tests

The results indicated that all the germination tests were significantly affected by the concentration of PEG 6000 (Figure 2 to 7). Priming with 0 bar of PEG 6000 showed the highest mean germination percentage (Figure 2), highest mean germination index (Figure 3), highest mean germination rate (Figure 4), shortest mean germination time (Figure 5) and highest mean germination energy (Figure 7) while priming with -2 bar of PEG 6000 showed the highest mean germination speed (Figure 6).

**Figure 2.** Effects of different concentrations of PEG 6000 on mean germination percentage of Tadong upland rice**Figure 3.** Effects of different concentrations of PEG 6000 on mean germination index of Tadong upland rice**Figure 4.** Effects of different concentrations of PEG 6000 on mean germination rate of Tadong upland rice**Figure 5.** Effects of different concentrations of PEG 6000 on mean of mean germination time of Tadong upland rice

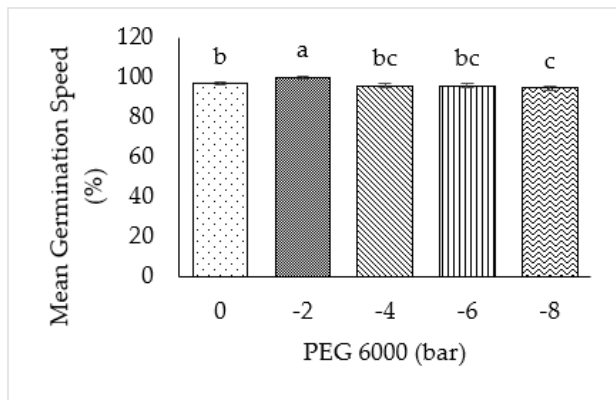


Figure 6. Effects of different concentrations of PEG 6000 on mean germination speed of Tadong upland rice

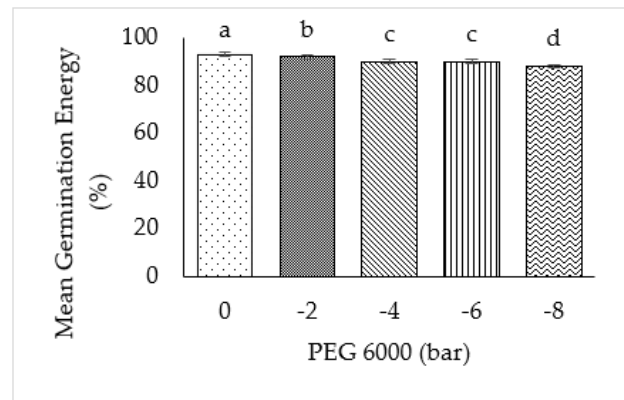


Figure 7. Effects of different concentrations of PEG 6000 on mean germination energy of Tadong upland rice

Germination Percentage

The concentration of PEG 6000 exhibited significant influence on germination percentage in this study. Figure 2 shows the germination percentage ranged from 91.67 to 95.67% for the five concentrations of PEG 6000. The highest and the lowest germination percentages were recorded with T1 (control) and T2 with 95.67% and 91.67% respectively (Figure 2). Significant differences resulted in the germination percentage between the concentrations of PEG 6000 except for T3 and T4 ($p < 0.05$). Germination percentage of T2 was reduced 4.36% compared to T1 (control treatment). Among the treatments, T3 and T4 can achieve the germination percentage of 94.00%, we can still consider these two treatments are good treatments since the germination percentage of the control treatment is 95.67%.

Germination Index

Results indicated the germination index of the five concentrations of PEG 6000 ranged from 35.99 to 51.26 (Figure 3). There were significant differences ($p < 0.01$) in germination index between the five concentrations of PEG 6000. T1 showed the highest germination index with 51.26 followed by T2 (44.33), T3 (40.28) and T4 (40.36) while T5 showed the lowest germination index of 35.99. Germination index of T2 was decreased 15.61% compared to T1 (control treatment).

Germination Rate

Germination rate for the five concentrations of PEG 6000 ranged from 0.21 to 0.22, with T1 and T2 having germination rate of 0.22 while T3, T4 and T5 having the same germination rate of 0.21 (Figure 4). The reduction in germination rate was proportional to the increasing concentration of PEG 6000. Significant differences resulted in the germination rate between the concentrations of PEG 6000 except for T3 and T4 ($p < 0.05$). Germination percentage of T2 was reduced 1.67% compared to T1 (control treatment).

Mean Germination Time

Mean germination time of the five concentrations of PEG 6000 ranged from 4.48 to 4.83 days (Figure 5). The delayed in mean germination time was proportional to the increasing concentration of PEG 6000. T5 had the longest mean germination time with 4.83 days followed by T4 (4.71 days), T3 (4.70 days) and T2 (4.56 days) while T1 had the shortest mean germination time of 4.48 days. Significant differences resulted in the mean germination time between the concentrations of PEG 6000 except for T3 and T4 ($p < 0.05$). Mean germination time of T2 was increased 1.64% (0.08 day) compared to T1 (control treatment).

Germination Speed

Significant differences resulted in the germination speed between the five concentrations of PEG 6000 except for T3 and T4 ($p < 0.05$). T2 showed the highest germination speed with 100.00% whereas the other samples ranged between 94.27% to 97.21% (Figure 6). T5 showed the lowest germination speed with 94.27%. Germination speed of T2 was increased 2.79% compared to T1 (control treatment).

Germination Energy

Results showed that germination energy had significant differences among the five concentrations of PEG 6000 ($p < 0.05$). Figure 7 shows the germination energy ranged from 87.67 to 93.00% for the five concentrations of PEG 6000. T1 had the highest germination energy with 93.00% followed by T2 (91.67%), T3 and T4 (89.67%) and T5 had the lowest germination energy of 87.67%. Germination energy of T2 was decreased 1.45% compared to T1 (control treatment).

Table 2. Correlation matrix between germination traits.

Parameters	Germination percentage	Germination index	Germination rate	Mean germination time	Germination speed	Germination energy
Germination percentage	-					
Germination index	0.41209	-				
Germination rate	0.18586	0.96549**	-			
Mean germination time	-0.17819	-0.96159**	-0.99974**	-		
Germination speed	-0.48564	0.55110*	0.73585**	-0.74134**	-	
Germination energy	0.31264	0.94677**	0.95592**	-0.95527**	0.67834**	-

*significant ($p < 0.05$) **highly significant ($p < 0.01$)

The result of the correlation analysis under priming with different concentrations of PEG 6000 showed that germination index, germination rate, mean germination time, germination speed and germination energy parameters had significant correlation (Table 2). Data showed that there was positive and significant correlation among germination index and germination rate ($r = 0.96549$, $p < 0.01$), germination speed ($r = 0.55110$, $p < 0.05$) and germination energy ($r = 0.94677$, $p < 0.01$) respectively while negatively correlated with the mean germination time ($r = -0.96159$, $p < 0.01$). Germination rate showed a strong positive correlation with germination speed ($r = 0.73585$, $p < 0.01$) and germination energy ($r = 0.95592$, $p < 0.01$). In contrast, the germination rate and mean germination time were negatively correlated in this study ($r = -0.99974$, $p < 0.01$). Moreover, there were negative and significant correlation among mean germination time and germination speed ($r = -0.74134$, $p < 0.01$) and germination energy ($r = -0.95527$, $p < 0.01$). In addition, the germination speed showed positive correlation with germination energy ($r = 0.67834$, $p < 0.01$).

DISCUSSION

Seed germination is normally the most critical stage in seedling establishment and to determine the successfulness of crop production (Almansouri *et al.* 2001; Shanjani *et al.*, 2014). In this study, the aim was to assess the ability of PEG priming to induce seed germination performance of Tadong upland rice originated from Ranau, Malaysia. Drought is an important factor that negatively affects

plant growth. PEG 6000 was used to induce drought stress as it can modify the osmotic potential of nutrient solution cultures (Lagerwerff *et al.*, 1961; Wu *et al.*, 2019).

The results of this study are consistent with those reported by Safarnejad (2008), Yagmur & Kaydan (2008) and Shitole & Dhumal (2012) where increasing concentration of PEG 6000 caused reduction in seed germination percentage. This may be due to the reduction in water potential gradient between the seeds and their surrounding media which lower the water uptake by the seeds resulting in decreases of germination (Dodd & Donovan, 1999; Afzali *et al.*, 2006; Shahriari *et al.*, 2014). The seeds apparently develop an osmotically enforced inhibition by drought stress where this may be an adaptive strategy to prevent germination occur under stressful environment for ensuring proper establishment of seedlings. When the PEG 6000 concentrations increased, some toxic ions in seeds may undergo germination process, along with restriction of water absorption, these effects may lead to alteration of cell metabolism, reduction in germination percentage and germination speed (Dodig *et al.*, 2008; Barbieri *et al.*, 2019).

Water stress induced by PEG not only caused reduction in seed germination, it also delayed the seed germination time (Kaydan & Yagmur, 2008). In contrast to all the other parameters, mean germination time was increased by increasing the PEG 6000 concentrations. Similar results were also reported by Farooq *et al.* (2006); Khodarahmpour *et al.* (2014) and Abiri *et al.* (2016) where the reduction in germination by increasing the PEG concentrations was possibly attributed to high seed nutrient imbalance, toxic ions, and reduced soluble osmotic potential. Reduction in germination at higher level of water stress may be due to the water deficit in the seeds below the threshold, which may lead to degradation and inactivation of essential hydrolytic enzymes (Wilson, 1971; Pratap & Sharma, 2010; Al-Jbawi *et al.*, 2020).

The decrease in seed germination may be due to the less availability of free water to the seeds during the early hours of imbibition, hence leaving the hydrolytic enzymes inactive (Hadas, 1976; Stout *et al.*, 1980; Chutia & Borah, 2012). As the PEG 6000 concentration increased, the germination rates decreased. Similar results were observed by Hamidi & Safarnejad (2010), Mouradi *et al.* (2016) and Wu *et al.* (2019). Drought stress induced by PEG 6000 decreased germination rate and this reduction might due to the slower decomposition or transmission of the endosperm materials into plantlets. The strongest inhibition occurred at the highest PEG 6000 concentration (-8 bar) for all the parameters.

CONCLUSION

Data obtained in this experiment demonstrated that different osmotic potentials of PEG 6000 have significant effect on the germination percentage, germination index, germination rate, mean germination time, germination speed and germination energy. Priming with distilled water (0 bar) showed highest values for all the parameters except for germination speed whereas Tadong upland rice primed with -8 bar of PEG 6000 showed lowest values for all the parameters except for germination percentage. Priming with -2 bar of PEG 6000 produced lowest germination percentage (91.67%) but highest germination speed (100.00%) among all the treatments. It can be concluded that high concentrations of PEG 6000 significantly decreased all the studied traits.

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