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Comparison Between Hydro- and Oslo-Priming to Determine Period need for Priming Indicator and its Effect on Germination Percentage of Aerobic Rice Cultivars (Oryza sativa L.)

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COMPARISON BETWEEN HYDRO- AND OSMO-PRIMING TO DETERMINE PERIOD NEEDED FOR PRIMING INDICATOR AND ITS EFFECT ON GERMINATION PERCENTAGE OF AEROBIC RICE CULTIVARS (*Oryza sativa* L.)

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ABSTRACT

This study compared hydro and osmo-priming to determine period needed for seed priming indicator to emerge and its effect against water stress on germination percentage of some aerobic rice cultivars (*Oryza sativa* L.). Experiments were arranged in completely randomized design using three aerobic rice cultivars and PEG with four replications. Rice varieties (Inpago-8, IR64 and Situ Bagendit) were combined with four different PEG concentrations. Drought stress was simulated by different PEG solutions and with distilled water as control. Results indicated differentiations between hydro and on priming indicator time, while varietal difference was not significant. Fastest recorded time was obtained between 30-36 hours under hydro priming, PEG 100 g L^{-1} (63-69 hours) and 83-93 hours in PEG 200 g L^{-1} solution. Thus showing increased solution concentration led to prolonged priming indicator time. The highest germination percentage was obtained in Situ Bagendit treated with PEG 200 mg L^{-1} (90.25%) and the lowest obtained in Inpago-8 (75.75%) under control. It is better to use osmo-priming (PEG 200 g L^{-1}) for teaching aerobic rice seed against drought stress. More research is needed to confirm benefits of seed treatment with PEG to cultivated crops under water stress, drought and salinity conditions.

Keywords: aerobic rice; germination; hydro and osmo-priming; priming indicator time

INTRODUCTION

Rice (*Oryza sativa*) belong to the Graminae family with others cereal crops such as wheat, sorghum, rye, maize and oats. It is the second most important cereal crops in the world after wheat. It is the staple food of nearly 50% of the world's population and especially in Asia, where about 92% of the total world's rice is cultivated, harvested and consumed (McLean, Dawe, Hardy, & Hettel, 2002). However, rice is also the single largest user of freshwater among cereal crops as it mostly cultivated under submerged muddy soils. Asia's irrigated rice fields consume more than 40% of the world freshwater used for agriculture. Like other crops, rice is subjected to many biotic and a biotic stresses, which effects plant growth and reduce yield and yield stability. The abiotic stress include drought, water deficit, salinity, heat, water logged soils, acidic soils, zinc deficient soils and toxic soils. These stresses can pose serious problem in rice farming, especially in the less-optimum growing environment. Lack of moisture places plants under stress, and significantly reduce crop yield. It is estimated that 30% of the world area experienced water stress where 70% of the agricultural output is affected by drought (Bouman, 2001).

Recently scientists are focusing on technologies to save water and cultivate crops under salinity, water stress and drought such as water management, breeding and genetically modify crops. However, studies on seed treatments by soaking (seed priming) were the easiest and cheapest applicable method. Seed priming is a term used for soaking seeds in water (hydro priming) or osmotic solutions (osmo-priming or sometimes referred as osmo-conditioning) before sowing and cultivating to increase and accelerate germination and emergence, which leads to greater water use efficiency and higher yields in

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crops lifecycle. In other word, seed priming is a technology in which seed are treated before germination by soaking at water potential that allow imbibitions, but prevent seed germination shown by inhibited radicle protrusion.

Water stress, drought and salinity are known to inhibit the germination and growth of most crop species. Reportedly, priming seed carried out on a number of crops has improved and increased germination behaviors, seedling establishment and in some, improved vegetative growth and increased yield (Ahmad, Anwar, & Ullah, 1998; Ashraf & Rauf, 2001; Harris, Joshi, Khan, Gothkar, & Sodhi, 1999; Ghiyasi, Seyahjani, jbakhsh, Amirnia, & Salehzadeh, 2008). Osmo-priming has increased the rate of germination and uniformity of seedling emergence especially under sub-optimal condition like water stress, drought and salinity (Ashraf & Rauf, 2001; Khalil, Mexal, & Murray, 200 Priming seed using polyethylene glycol (PEG) resulted in accelerated germination behavior. Earlier reports have shown that seed priming was influenced by many complex interactions of factors and conditions such as plant species, water potentiality of priming agent, soaking duration in priming agent, variation of temperature, seed vigor and dehydration, and storage conditions of the primed seed (Hussain, Farooq, Basra, & Ahmad, 2006). Recent works reported a very comprehensive study on priming where seeds were treated by soaking in different priming agents and treatments such as abscisic acid (ABA) (hormonal priming), calcium chloride ($CaCl_2$) (chemo-priming), and water hydro-priming), showing high performance rate of germination behaviors as compared to non-primed seeds (Ghiyasi, Seya ni, Tajbakhsh, Amirnia, & Salehzadeh, 2008; Srivastava et al., 2010).

The technology of seed priming or pre-sowing hardening seed treatment of a number of cereals (wheat, rice, rye, maize and oats) reported to increase drought tolerance, vegetative and reproductive performance, yield output and yield quality, total and productive tillers, germination rate (up to 10-15%). It also improved minerals and nutrition uptake, winter survivability (for winter crops), heat stress, frost and moisture injuries. Scientists predicted that in some wet season irrigated rice areas such as in northern China (2.5 million ha), Pakistan (2.1 million ha) and North and Central India (8.4 million ha) will be struck by drought, salinity and extreme water shortage by 2025 (ong & Bouman, 2003).

The declining availability and increasing costs of water threaten the traditional irrigated rice cultivation. Moreover, climate change resulting in fluctuation and lack of rainfall is a major production limitation in rain-fed areas where many poor rice farmers live (Tuong & Bouman, 2003). Therefore, researchers have been looking for ways to decrease and saving water use in rice production and increase water use efficiency. Hence, it proposed that aerobic rice and seed priming and combination of other technologies could be used to face the problem of water scarcity in the future (Bouman et al., 2002; Lanceras, Laza & Atlin, 2002).

The duration of soaking the seed as little as 5-10 hours can lessen the time to emergence by 10 hours (LWMP, 1992), which may be imperative in enabling seedling roots to grow way down to below a rapidly drying or crusting soil surface. For most crops seed treatment by soaking seed for 12 hours is adequate, but up to 24 hours are needed in case of rice and maize.

There are different ways for understanding seed priming practices between soaking seed in agents for some certain period or until just before embryo and radicle protrusion. The objective of this research is to investigate the comparison between hydro and osmo-priming to determine the period needed for embryo protrusion as priming indicator and its stress on germination percentage of three aerobic rice cultivars.

MATERIALS AND METHODS

Location and Description of Experimental Layout

Experiments were conducted in laboratory and greenhouse of Faculty of Agriculture, Department of Agronomy, Hasanuddin University (UNHAS), Makassar, Indonesia from 15 March to 15 June 2015. Rice seed of three varieties (Inpago-8, IR64 and Situ Bagendit) were obtained from Indonesian Center for Rice Research (ICCR), Sukamandi, West Java, Indonesia.

Moisture content of the seed was measured by grinding the seeds and drying at 130°C for 4 hours (ISTA, 08) and calculated on a fresh weight basis. The seeds were surface sterilized with 5% NaOCl (sodium hypochlorite) for 5 minutes to control and treated against fungal infestation. Seeds were primed in 3 levels of PEG *i.e.*, 0, 100, and 200g PEG per liter distilled water which Osmotic potential were equivalent to 0, -0.2, and 0.5 MPa, respectively (Khan A., Khalil, Khan A. Z., Marwat, & Afzal, 2008), except for the control treatment (un-primed).

Soaking Period Test

The seed soaking in distilled water and different PEG solution which were PEG solution was 100 g L^{-1} and 200 g L^{-1}. The period of soaking were observed every hour until primed seed indicator and the number of hours recorded. Figure 1 showed an image about priming indicator (A) with emerged radicle and no emergence in unprimed seed (B).

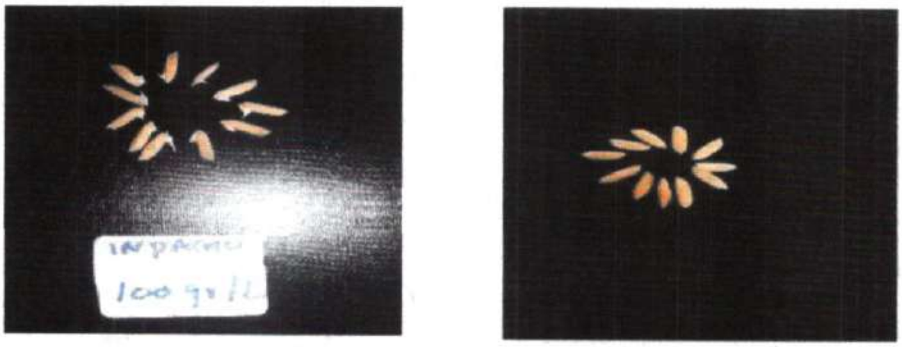
Germination and Stress Test

Experiment was carried out using two-factor (three aerobic rice cultivars and PEG) with four replications, which were arranged in factorial system design and conducted with completely randomized design. The factor was varieties which were Inpago-8 (V1), IR64 a susceptible aerobic rice cultivars (V2) and Situ Bagendit normal aerobic rice cultivars (V3). The three varieties were combined with three level of PEG concentration were 0 (P1), 100 (P2) and 200 (P3) g L^{-1} plus control (P0=untreated seed). Combinations of treatments equals to 12. Total number of experimental units were multiplied by three varieties, four soaking treatments and four replications [illegible] × 4P × 4 replications = 48). After treatments, seeds were washed three times with distilled water and dried to original weight condition. Hundred seeds from each of the treatments were placed on Whatman No. 2 filter paper in Petri dishes (90 mm diameter), wetted with 10 ml of distilled water, PEG solution 100 g L^{-1} and PEG solution 200 g L^{-1} for 1st, 2nd and 3rd experiments, respectively. Seed was incubated at room temperature under normal light conditions. Germination percentage was determined and calculated daily and continued until a fixed state, 7 days after sowing as final germination behaviors (ISTA, 2008). Data were analyzed using ANOVA. To determine the best treatment further tests using Duncan's multiple range test (DMRT) were employed.

RESULTS AND DISCUSSION

Effect of Hydro and Osmo-priming on Priming Indicator Time

Observations recorded that there were differentiation between hydro and osmo-priming and varieties on priming indicator time. The fastest one recorded by V1P1 after 30 hours and the latest V3P1 after 36 hours under hydro-priming conditions. When primed with 100 g L^{-1} solution the fastest after 63 hours recorded for V1P2 and the latest was V3P2 about 69 hours. Finally, under osmo-priming with 200 g L^{-1} solution the fastest after 83 hours recorded for V1P3 and the latest was V3P3 about 93 hours (Figure 2). Thus, meaning an increased PEG solution concentration led to prolonged priming indicator time and it was slightly different among varieties.



A: Primed seed with priming indicator

B: Non-primed seed

Figure 1. Difference between primed and unprimed seed. (A) Primed seeds with emerged priming indicator (the radicle ≤ 1 mm. (B). Unprimed seed.

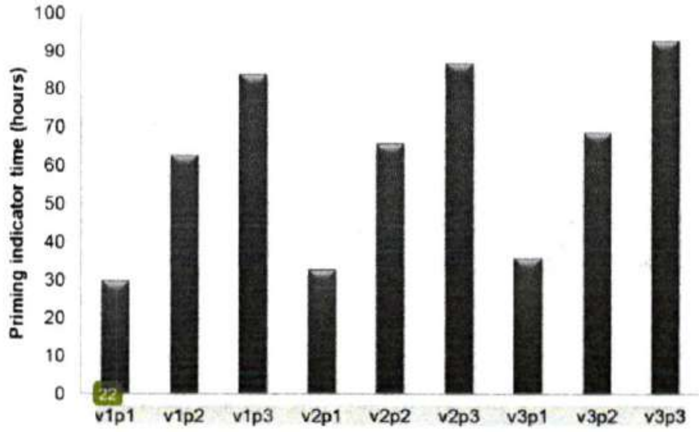


Figure 2. Priming indicator time (hours) of some aerobic rice varieties: Inpago-8 (V1), IR64 (V2) and Situ Bagendit (V3), under hydro-priming (P1) and osmo-priming (P2: 100 g L^{-1} solution and P3: 200 g L^{-1} solution)

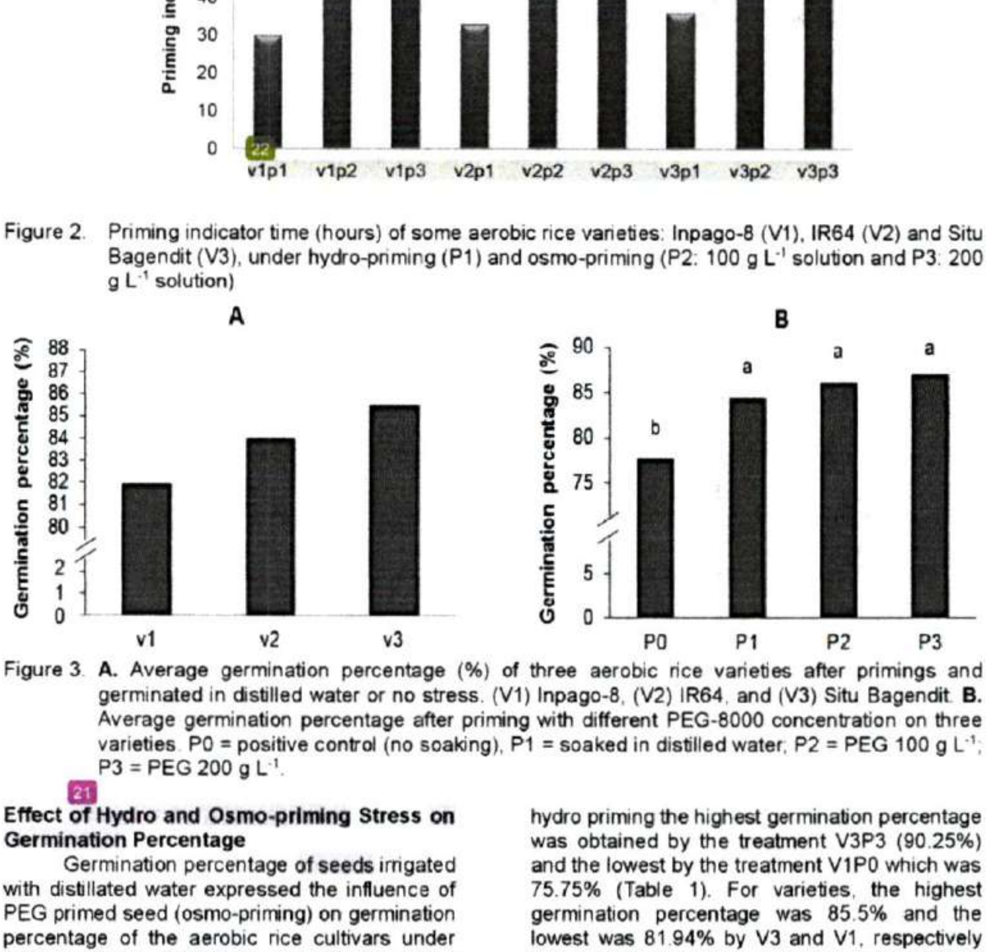


Figure 3. **A.** Average germination percentage (%) of three aerobic rice varieties after primings and germinated in distilled water or no stress. (V1) Inpago-8, (V2) IR64, and (V3) Situ Bagendit. **B.** Average germination percentage after priming with different PEG-8000 concentration on three varieties. P0 = positive control (no soaking), P1 = soaked in distilled water; P2 = PEG 100 g L^{-1}; P3 = PEG 200 g L^{-1}.

Effect of Hydro and Osmo-priming Stress on Germination Percentage

Germination percentage of seeds irrigated with distillated water expressed the influence of PEG primed seed (osmo-priming) on germination percentage of the aerobic rice cultivars under field conditions. PEG had no significance effect between varieties (V) and interaction (varieties (V) × seed priming (P)) but there was a very high significance between seed priming (P). Among hydro priming the highest germination percentage was obtained by the treatment V3P3 (90.25%) and the lowest by the treatment V1P0 which was 75.75% (Table 1). For varieties, the highest germination percentage was 85.5% and the lowest was 81.94% by V3 and V1, respectively (Figure 3A). Between seed priming the highest germination percentage 87% by P3 and the lowest by 77.67% by P0 (Figure 3B).

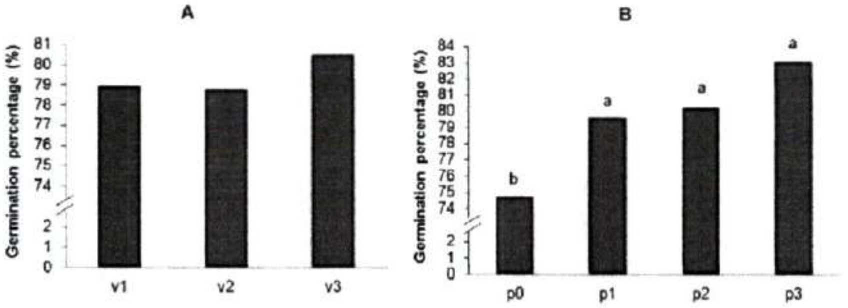


Figure 4: **A.** Average germination percentage (%) of three aerobic rice varieties: Inpago-8 (V1), IR64 (V2) and Situ Bagendit (V3) after priming treatment with hydro and osmo-priming and grown under stress irrigated with PEG solution (100 g L^{-1}). **B.** Average germination percentage (%) after priming with different PEG-8000 concentration on three varieties. P0= positive control (no soaking), P1= soaked in distilled water; P2= PEG 100 g L^{-1}; P3= PEG 200 g L^{-1}. Further test by DMRT α =0.05.

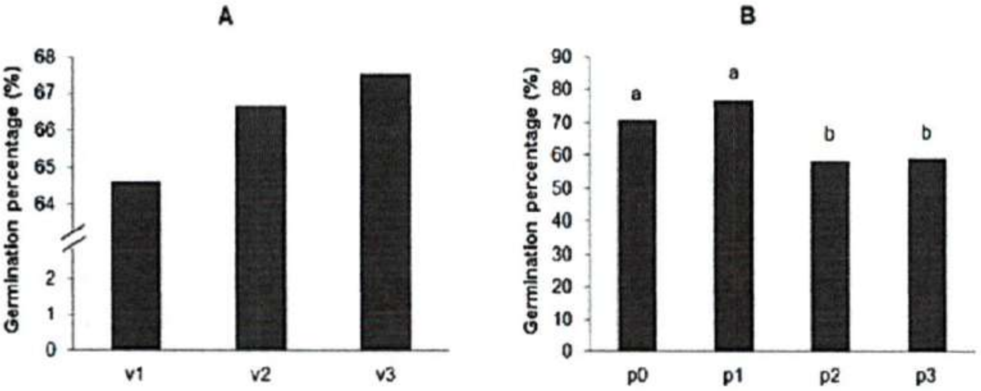


Figure 5. **A.** Average germination percentage (%) of three aerobic rice varieties Inpago-8 (V1), IR64 (V2) and Situ Bagendit (V3) after priming treatment with hydro and osmo-priming and grown under stress irrigated with PEG solution (200 g L^{-1}). **B.** Average germination percentage (%) after priming with different PEG-8000 concentration on three varieties. P0= positive control (no soaking), P1= soaked in distilled water; P2= PEG 100 g L^{-1}; P3= PEG 200 g L^{-1}. Further test by DMRT α =0.05.

Among results in germination percentage under PEG 100 g L^{-1} stress presented in Figure 4 and Table 1, PEG had no significance between varieties (V) but there was a significance between seed priming (P) and interaction (varieties (V) × seed priming (P)). The highest germination percentage was obtained by the treatments V3P3, V3P2 and V2P3 (all 84.5%) and the lowest by the treatment V1P0 which was 73.5% (Table 1). For varieties the highest germination percentage recorded by V3 (80.56%) and the lowest 78.81% recorded by V2 (Figure 4A). Seed priming itself the highest germination percentage in P3 (83.08%) and the lowest in P0 (74.75%), (Figure 4B).

Germination test moistened with PEG solution 200 g L^{-1} stress after each priming treatments had no significance between varieties (V) but there was a very high significance between seed priming (P) and a significant difference between interaction (varieties (V) × seed priming (P)). The highest germination percentage was obtained by the treatment V1P1 (78%) and the lowest by the treatment V2P2 (54.25%) (Table1). Among varieties the highest and lowest germination percentage was in V3 and V1 which was 67.56% and 64.63%, respectively (Figure 5A). Between seed priming the highest output of germination percentage was in P1 (76.75%) and the lowest was found in P2 (58.58%) (Figure 5B).

Results showed that aerobic rice seeds under hydro-priming condition reached faster to priming indicator time (hour) than osmo-priming. Furthermore, osmo-priming at PEG 100 g L^{-1} solution required lesser to priming indicator time compared to PEG 200 g L^{-1}, it meant that increased concentration of priming solution has led to delay in priming indicator emergence. This may be due to factors influencing seed water imbibitions such as difficulties of water imbibitions which were confirmed by many researchers who recorded that water imbibitions and germination enhancement depend upon temperature, period of soaking, water potential, and other a biotic factors conditions (Hardegree, 1996; Parera & Cantliffe, 1992). Although some seeds have taken longer duration to reach to priming indicator time, low water potential or increasing priming temperature treatments can have adverse effects on subsequent germination response or due to oxygen shortage.

Ghiyasi, Myandoab, Tajbakhsh, Salehzadeh, & Meshkat (2008) found reduced oxygen solubility in solutions of PEG 4000, 6000 and 8000 by nearly 20%. Availability of oxygen is thought to be important in the maintenance of metabolic functions necessary for dormancy release and prevention of secondary dormancy induced by oxygen deficiency (Hardegree, 1996; Parera & Cantliffe, 1992). Ghiyasi, Myandoab, Tajbakhsh, Salehzadeh, & Meshkat (2008) reported seed osmo-priming of maize (*Zea mays* L.) with polyethylene glycol 8000 (PEG 8000) with osmotic potential equivalent to -0.5 MPa had increased emergence, grain and biological yields compared with other control. It was reported that seed priming had meaningful effect on acceleration of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in water scarcity and salinity condition (Kaya, Okçu, Atak, Çikili, & Kolsarici, 2006).

Table 1. Germination percentage (%) of three aerobic rice varieties Inpago-8 (V1), IR64 (V2) and Situ Bagendit (V3) irrigated with distilled water, PEG solution (100 g L^{-1} and 200 g L^{-1}) after priming

Treatments	Stress		
	Hydropriming (distillated water)	Osmo-stress	
		PEG solution 100 g L^{-1}	PEG solution 200 g L^{-1}
	Germination percentage (%)		
V1P0 (control)	75.75	73.50^{b}	68.75^{abc}
V1P1 (hydro priming)	82.50	81.25^{ab}	78.00^{a}
V1P2 (osmopriming, 100 g L^{-1})	84.75	80.75^{ab}	57.25^{cde}
V1P3 (osmopriming, 200 g L^{-1})	84.75	80.25^{ab}	54.50^{e}
V2P0 (control)	80.00	75.25^{b}	68.25^{abc}
V2P1 (hydro priming)	84.75	80.00^{ab}	77.50^{a}
V2P2 (osmopriming, 100 g L^{-1})	85.25	75.5^{b}	54.25^{e}
V2P3 (osmopriming, 200 g L^{-1})	86.00	84.50^{a}	66.75^{abcd}
V3P0 (control)	77.25	75.50^{b}	75.25^{ab}
V3P1 (hydro priming)	86.00	77.75^{ab}	74.75^{ab}
V3P2 (osmopriming, 100 g L^{-1})	88.50	84.50^{a}	64.25^{bcde}
V3P3 (osmoprimimig, 200 g L^{-1})	90.25	84.5^{a}	56.00^{de}
SE±	2.55	2.37	3.74

Remarks: Figures with different letters reported, differ significantly at DMRT α =0.05.

Basra, Zia, Mehmood, Afzal, & Khaliq (2002) reported the improvement in germination, emergence and number of vigorous seedling by using seed priming techniques. In fact, priming could stimulate a range of metabolic activities and biochemical changes in the seed required for initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation. Previous researches reported some or all process that precedes the germination was caused by priming and remained persisting following the re-desiccation of the seed (Asgedom & Becker, 2001). Thus upon sowing, primed seed will rapidly imbibe and reach the seed optimum metabolism rate, resulting in higher germination percentage and a reduction in the original physiological heterogeneity in germination (Asgedom & Becker, 2001). Other reports showed that seed priming resulted anti-oxidant increment as ascorbate and glutathione in seed. These enzymes increased germination speed via reduction of lipid proxidation activity especially in oil crops seed with high oil contents. In 2006, Kaya, Okçu, Atak, Çikili, & Kolsarici, affirmed that priming resulted in more germination speed especially in saline stress, drought stress and low temperatures in sorghum, sunflower and melon.

As a comparison between hydro and osmo-priming, our results showed that osmo-priming has increased germination percentage more than hydro-priming when the germination process is under stress which was simulated by PEG concentrations. Similar condition was corroborated by Basra, Zia, Mehmood, Afzal, & Khaliq (2002). Osmo-priming is a successful practice for improving seed germination performance under water stress, drought and salt stress. These results are reinforced by the previous study on improved germination by osmo-priming in wheat (Basra, Zia, Mehmood, Afzal, & Khaliq, 2002; Srivastava et al., 2010). In addition, the increase in priming indicator emergence with priming might be due to initiating metabolic events in primed seed which was corroborated by Elkheir et al. (2016) mentioned that soaking rice seed in the water and PEG solutions 100 g L^{-1} and 200 g L^{-1} until priming indicator will lead to accelerated and improved germination behaviors shown by parameters observed such as: germination percentage, Germination Index (GI), days of 50% germination, seedling fresh and dry weight (mg), seedling shoot fresh and dry weight (mg), root fresh dry and weight (mg), shoot/root ratio, seedling length (cm), seedling root length (cm), shoot length (cm) and seed vigor index under laboratory and greenhouse condition.

Among the effect of stress of PEG on germination percentage, the results revealed that high stress as result of high concentration of PEG solution led to decreased in germination that may be according to high concentration or osmotic potential which could be due to solute leakage during priming (Hegarty, 1978) or water might have come out of the primed seeds into PEG solution, most possible by osmosis, thereby, arresting the germination process (Murungu, Nyamugafata, Chiduza, Clark, & Whalley, 2005). Osmo-priming has increased the rate and uniformity of germination and seedling emergence, especially under sub-optimal conditions such as salinity and drought (Ghiyasi, Seyahjani, Tajbakhsh, Amirnia, & Salehzadeh, 2008; Khalil et al., 2001). Results of this study has shown osmo-priming with PEG affected germination and seedling growth. Comparison of means figures of parameters observed indicated final germination behaviors percentage, Mean Germination Time (MGT), time to 50% germination, root and shoot length, mean emergence time, Germination Index, root/shoot ratio and seedling fresh and dry weight were significantly affected by PEG treatments. Furthermore, the results indicated that PEG was a successful practice for improving seed germination performance under droughts and salt stress. Result of this study is supported by earlier researches on improved germination by PEG in cereal crops like wheat (Basra, Zia, Mehmood, Afzal, & Khaliq, 2002).

CONCLUSION

PEG play famous role to increase the germination percentage of the aerobic rice varieties especially Inpago-8, IR64 and Situ-bagendit. Furthermore, under hydro and osmo-priming seed of aerobic rice varieties showed different performance and it is better to use (PEG 200 g L^{-1}) for teaching aerobic rice seed against water stress. Otherwise, increased PEG concentration from 0 to 200 g L^{-1} stress will lead to decreased germination percentage.

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