ACTA SCIENTIFIC AGRICULTURE (ISSN: 2581-365X)

Volume 2 Issue 11 November 2018

Research Article

Hydro Priming Stimulates Seedling Growth and Establishment of Malaysian *Indica* rice (MR219) Under Drought Stress

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Received: August 14, 2018; **Published:** October 04, 2018

Abstract

Drought stress severely effects on seed germination and seedling establishment as critical stages in crops lifetime. Seed hydro priming is useful process for improving crops tolerance to drought stress. The present investigation was designed to evaluate the effect of hydro priming on adaptation strategies of Malaysian *Indica* rice (MR219) under drought conditions. Rice aseptic seeds were soaked at 20°C for 8h distilled water. Primed and non-primed seeds were subjected to polyethylene glycol (0, - 0.4, - 0.8 and -1.2) MPa treatments. Results showed that germination percentage, germination index, the fresh and dry weight, shoots and roots lengths decreased with increasing polyethylene glycol concentrations. We observed that polyethylene glycol tolerance of primed seeds is higher than non-primed seeds at all polyethylene glycol levels. Mean germination time and relative polyethylene glycol injury are increased in non-primed seeds as compare to primed seeds under drought stress. Proline content positively correlated increased with the increasing polyethylene glycol concentrations. The results indicated that hydro priming of Malaysian *Indica* rice (MR219) seeds is associated with the accumulation of proline and modulating the activity of ascorbate peroxidase and catalase under drought stress. This study suggests the hydro priming as an effective technique on rice seeds to withstand under drought condition which could be a step forward to commercialization.

Keywords: Oryza sativa; Germination; Proline; Enzyme Activity

Introduction

The most devastating abiotic stress in the plants is drought stress. It negatively affected on crop growth, biomass accumulation and the world food security [1]. Rapid world population growth makes water problem more complicated [2]. According to climate models prediction, drought is expected to threat more than 50% of arable lands by 2050. Nowadays, about 20% of the world's cultivated area is actually irrigated, contributing to 40% of total food production [3]. World Resource Institute ranked drought stress in 167 countries all over the world in 2040. Base on this report climate change makes 33 countries, mostly in Middle East, face severe drought stress [4].

Water shortage in soil caused reduction of cell division, leaf size, stem elongation and root proliferation. Furthermore, lack of water effects on availability of soil nutrients such as nitrate, SO_4, Ca, Mg, and Si [5]. Plants developed adaptation mechanisms to overcome the oxidative stress. Accumulation of organic solutes such as proline and total soluble sugar stabilize cellular and protect membranes structure. Meanwhile, stress condition caused production of Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals. Plants scavenged of reactive oxygen species by non-enzymatic and enzymatic antioxidant defenses systems [6]. However, severe oxidative stress leads to changing physiological parameters, decrease the varied number of plants per unit area and decreased seed yield [7].

Rice (*Oryza sativa* L.) is an important and essential food item that feed more than 50% of the world population. In Asia, rice provides 40 - 70% of the total food calories consumed [8]. Rice is sensitive to drought stress with limited adaption strategies [9]. Drought stress effected 23 million h of rice field in South and Southeast Asia [10]. In recent years, extensive research is being done for developing various adaption strategies to improve drought tolerance crops. Priming or pre-sowing treatment as most feasible and low-cost strategy improves the seed germination during drought stress. Seed priming stimulate wide range of physiological characters, biochemical cascades, cellular and molecular changes [11]. A plenty of research had been done to improve drought-tolerant crops by using hydro priming [12,13]. Seeds soaked in distilled water which permitted them to absorb sufficient water to reach a pre-germinative stage without radicle emergence [14]. However, no research has done to analyse effect of hydro priming of MR219 under drought stress until to date. The purpose of this study was to evaluate the impact of hydro priming on germination and seedling growth of Malaysian *Indica* rice under drought stress. It was hypothesized that hydro priming positively effects on subsequent differentiation of MR219 seeds by physiological and biochemical cascades under drought stress.

Material and Methods

Plant material: The seeds of MR219 rice variety provided by Malaysian Agriculture Research and Development Institute (MARDI) Serdang, Selangor, Malaysia.

Seed sterilization and hydro priming: The selected mature rice seeds were dehulled manually. All seeds were then surface sterilized by immersed in 70% ethyl alcohol for 30 seconds. Subsequently, the ethanol was discarded and seeds were washed once with distilled water and, then 40% sodium hypochlorite was added and the seeds were stirred along with this solution for 20 minutes. The sterilized seeds were rinsed five times with sterile double-distilled water. Six hundred seeds were soaked in 10.0 mL of distilled water at 20°C for 8h respectively. The treated seeds were air-dried at 20°C for 48h back to their initial moisture content.

PEG priming and experimental design

Primed and non-primed seeds were germinated in petri dishes on top of double-layered of Whatman filter papers No.1. To make solutions with the osmotic potentials (Ψs) consisting of the control (without PEG), -0.4, -0.8 and -1.2 MPa treatments, polyethylene glycol (PEG 6000) was dissolved in distilled water based on Michel and Kaufmann [15] formula. Three replicates with fifty seeds per replicate were used for each treatment. The petri dishes were put in a germinator at 25 ± 2°C and irrigated with distilled water. Seeds were considered to have germinated upon the emergence of radicles (≥ 2 mm). After 7 days, the growth parameters were measured by International Seed Testing Association (ISTA) standard method.

Measurement of seedlings-related parameters

Germination percentage (GP) which is as estimation of the viability of a population of seed for early seedling growth under PEG treatment in laboratory condition was assessed according to method of Kandil., *et al* [16].

$$GP = \frac{\text{Number of germination seeds}}{\text{Total number of seed sown}} \times 100$$

Mean germination time (MGT) represented the mean time a seed requires to initiate and end germination. It is evaluated by the following formula [16]:

$$MGT = \frac{(\sum dx)}{(\sum x)}$$

Where x in the number of seeds which germinated on the day 'd', and 'd' is the number of days counted from the beginning of germination.

Germination index (GI) gives an indication of the percentage of seeds germination per day of the experiment period. The germination index was calculated as describe by Dezfuli., *et al*. [17] with the following formula.

$$GI = \frac{\text{Number of germination seeds}}{\text{Day of first count}} \times 100 + \ldots + \frac{\text{Number of germination seeds}}{\text{Days of last count}}$$

Relative PEG injury rate was the measurement of cumulative effect of PEG treatment at different concentration on germinating percentage. It was calculated based on following method [18].

$$\text{Relative PEG injury Rate} = \frac{\text{GP in control-GP in PEG treated seeds}}{\text{GP in control}}$$

Lastly, the PEG tolerance rate was calculated by using the standard formula proposed by Kaymakanova (2009).

$$\text{PEG tolerance rate} = \frac{\text{Seedling dry weight of treated}}{\text{Seedling dry weight in control}}$$

Assaying for compatible solutes

Proline was extracted and determined by the method of Bates., *et al* [19]. The absorbance of proline-ninhydrin product was recorded at 520 nm and expressed as µg/g. Total soluble sugar (TSS) was determined according to Watanabe., *et al.* [20] and expressed as mg/g.

Assaying for antioxidant enzyme

A 2g of seedlings were ground in liquid nitrogen and the powders were extracted with 10 ml 50 mmol/l phosphate buffer (pH 7.0), followed by centrifuged at 14,000g for 15 minutes at 4°C. The supernatant was collected to measure the antioxidant enzyme activities and soluble protein content. Total soluble protein was measured according to Bradford [21] using BSA as a standard. The activities of enzymes were calculated by the following formula:

Enzyme activity = $[(A_2 - A_1) / (T_2 - T_1)]$ / mg protein = change of absorbance / per mg protein per min.

Measurement of catalase activity (hydrogen peroxide)

Catalase activity was measured using the method of Aebi [22]. The assay mixture contained 20 µl extract, 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), and 400 µl 15 mM H_2O_2. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm (ε =36 mM^{-1} cm^{-1}). Catalase activity was expressed in unit mg^{-1} fresh weight.

Measurement of ascorbate peroxidase activity

Ascorbate peroxidase activity was assayed using the method reported by Jebara., *et al* [23]. The reaction mixture (1.0 ml) consisted of 15 µl extract, 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and 0.1 mM H_2O_2. The activity was at 290 nm for 1 min (ε= 2.8 mM^{-1} cm^{-1}) APX activity was expressed per min^{-1} mg^{-1} fresh weight.

Statistical analyses

All data obtained for germination and early seedling growth were organized, analysed and interpreted by using one- way ANOVA of SPSS Statistic Windows version 22 ($p \leq 0.05$). Duncun test was carried out to access the significant difference between PEG treatments. Tables were used to show the distribution of the seeds response to different treatments.

Material and Methods

Effects of PEG on seedlings-related parameters

Seedlings-related parameters (germination percentage and germination index) of primed and non-primed significantly ($p \leq 0.05$) decreased with increasing of PEG concentrations (Table 1).

	Treatment	Germination percentage (%)	Mean Germination Time (day)	Germination Index
Primed	Control	92 ± 0.5^{a}	4.58 ± 0.09^{b}	1.12 ± 0.02^{a}
	0.04	84 ± 0.04^{b}	6.61 ± 0.42^{a,b}	0.73 ± 0.7^{b}
	0.08	40 ± 0.02^{c}	6.48 ± 0.34^{a,b}	0.61 ± 2.8^{c}
	1.2	10 ± 0.02^{d}	10.0 ± 2.5^{a}	0.28 ± 1.6^{c}
Non-primed	Control	92.0 ± 0.5^{a}	6.28 ± 0.28^{b}	0.99 ± 0.03^{a}
	0.04	77.0 ± 0.2^{b}	7.22 ± 0.72^{a,b}	0.44 ± 0.02^{b}
	0.08	39.6 ± 0.2^{c}	8.7 ± 0.02^{a,b}	0.28 ± 0.05^{c}
	1.2	10.0 ± 0.5^{c}	12.33 ± 0.02^{a}	0.25 ± 0.05^{c}

Table 1: Effect of PEG on germination percentage (%), germination index, and mean germination time of primed and non-primed seeds.

Data represent average values of three replicates and letters indicate significant ($p \leq 0.05$) differences among treatments. Mean ± S.E.

Primed seeds showed higher germination percentage and germination index compare with non-primed seeds under PEG treatments. Germination index indicated negatively correlated with mean germination time ($r = 0.489$, $p < 0.05$) (Table 5). Mean germination time in non-primed seeds was higher than primed seeds (Table 1). According to results mean germination time significantly negatively correlated with the germination ($r = 0.622$, $p < 0.01$) (Table 5).

Primed and non-primed seeds showed that PEG tolerance percentage significantly ($p \leq 0.05$) reduced under PEG treatments. The results showed that hydro priming affected positively on PEG tolerance percentage compare with non-primed seeds (Table 2). The results indicated that PEG tolerance significantly positively correlated with the germination percentage ($r = 0.929$, $p < 0.01$) and germination index ($r = 0.528$, $p < 0.01$) and negatively correlated with mean germination time ($r = 0.764$, $p < 0.01$) and relative PEG injury rate ($r = 0.936$, $p < 0.01$) (Table 5).

Treatments	Primed		Non-primed	
	Relative PEG Injury Rate (%)	PEG tolerance (%)	Relative PEG Injury Rate (%)	PEG tolerance (%)
Control	0.0 ± 0.0^{d}	100.0 ± 0.0^{a}	$0.0. \pm 0.0^{d}$	100.0 ± 0.01^{a}
0.04	0.1 ± 0.05^{c}	86.0 ± 0.5^{b}	0.22 ± 0.11^{c}	75.01 ± 0.06^{b}
0.08	0.43 ± 0.04^{b}	66 ± 0.4^{c}	0.43 ± 0.57^{b}	45.3 ± 0.04^{c}
1.2	0.79 ± 0.33^{a}	49.6 ± 0.33^{d}	0.9 ± 0.16^{a}	23.5 ± 0.33^{d}

Table 2: Effect of PEG relative PEG injury, and PEG tolerance of primed and non-primed seeds.

Data represent average values of three replicates and letters indicate significant ($p \leq 0.05$) differences among treatments. Mean ± S.E.

According to the results, primed and non-primed seeds significantly ($p \leq 0.05$) increased relative PEG injury rate during drought stress (Table 2). Seeds treated by hydro priming showed less relative PEG injury rate compare with non-primed seeds. Relative PEG injury negatively correlated with the germination percentage ($r = 0.978$, $p < 0.01$) and germination index ($r = 0.415$, $p < 0.05$) and positively correlated with mean germination time ($r = 0.685$, $p < 0.01$) (Table 5).

Primed and non-primed seeds indicated that fresh and dry weight significantly ($p \leq 0.05$) reduced at high concentrations of PEG (Table 3). Hydro primed seeds indicated a positive influence on fresh and dry weight compare with non-primed seeds under drought stress. The results indicated that fresh weight negatively correlated with mean germination time ($r = 0.472$, $p < 0.05$) and PEG injury rate ($r= 0.691$, $p < 0.01$) and positively correlated with the germination percentage ($r = 0.847$, $p < 0.01$) and PEG tolerance ($r = 0.802$, $p < 0.01$). Dry weight negatively correlated with mean germination time ($r = 0.457$, $p < 0.05$) and relative PEG injury rate ($r = 0.451$, $p < 0.05$) and positively correlated with the germination percentage ($r = 0.439$, $p < 0.05$) and PEG tolerance ($r = 0.505$, $p < 0.05$) (Table 5).

	Treatment	Fresh Weight (g)	Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
Primed	Control	0.05 ± 0.02^{a}	0.08 ± 0.01^{a}	1.9 ± 0.03^{a}	0.5 ± 0.06^{a}
	0.04	0.03 ± 0.7^{b}	0.005 ± 0.01^{b}	1.6 ± 0.02^{b}	0.4 ± 0.07^{b}
	0.08	0.01 ± 2.8^{c}	0.005 ± 0.01^{b}	1.4 ± 0.03^{c}	0.3 ± 0.06^{c}
	1.2	0.01 ± 1.6^{c}	0.005 ± 0.03^{b}	1.2 ± 0.03^{d}	0.2 ± 0.03^{d}
Non-primed	Control	0.08 ± 0.08^{a}	0.005 ± 0.01^{a}	1.3 ± 0.02^{a}	0.4 ± 0.01^{a}
	0.04	0.05 ± 0.07^{b}	$0.002 \pm 0.01^{a,b}$	1.01 ± 0.02^{b}	$0.2 \pm 0.05^{a, b}$
	0.08	0.02 ± 0.08^{c}	0.001 ± 0.01^{b}	0.7 ± 0.01^{c}	0.1 ± 0.08^{b}
	1.2	0.01 ± 0.03^{d}	0.0007 ± 0.03^{b}	0.3 ± 0.03^{d}	0.008 ± 0.07^{c}

Table 3: Effects of PEG on fresh weight, dry weight, shoot and root length of primed and non-primed seeds.

Data represent average values of three replicates and letters indicate significant ($p \leq 0.05$) differences among treatments. Mean ± S.E.

According to the results, shoot and root length significantly ($p \leq 0.05$) reduced at high concentrations of PEG (Table 3). Primed seedlings showed higher shoot and root length compare with non-primed seedlings. Results indicated that shoot length negatively correlated with mean germination time ($r = 0.779$, $p < 0.01$) and relative PEG injury rate ($r = 0.705$, $p < 0.01$) and positively correlated with germination ($r = 0.655$, $p < 0.01$), germination index ($r = 0.45$, $p < 0.01$), PEG tolerant ($r = 0.85$, $p < 0.01$) and dry weight ($r = 0.627$, $p < 0.01$) (Table 5).

The result showed that proline content in primed and non-primed seedling significantly ($p \leq 0.05$) increased with the increasing of PEG (Table 4). Our result showed that proline content significantly negatively correlated with the germination ($r = 0.755$, $p < 0.01$), PEG tolerant ($r = 0.582$, $p < 0.01$) and fresh weight ($r = 0.838$, $p < 0.01$) and positively correlated relative PEG injury rate ($r = 0.716$, $p < 0.01$) and sugar ($r = 0.952$, $p < 0.01$) (Table 5).

	Primed				Non-primed			
Treatments	**Proline (µg/g)**	**Total soluble sugar (mg/g)**	**APX (unit mg^{-1} FW)**	**CAT (unit mg^{-1} FW)**	**Proline (µg/g)**	**Total soluble sugar (mg/g)**	**APX (unit mg^{-1} FW)**	**CAT (unit mg^{-1} FW)**
Control	0.04 ± 0.02^{c}	0.65 ± 0.03^{d}	$3.33 \pm 0.25^{b,c}$	9.0 ± 0.49^{c}	0.04 ± 0.33^{d}	0.59 ± 0.02^{c}	$2.32 \pm 0.26^{b,c}$	7.47 ± 0.62^{d}
0.04	0.3 ± 0.48^{c}	0.84 ± 0.2^{c}	$4.2 \pm 0.25^{a,b}$	15.2 ± 0.49^{b}	0.21 ± 0.17^{c}	0.63 ± 0.008^{c}	$3.3 \pm 0.26^{a,c}$	12.43 ± 0.8^{b}
0.08	0.6 ± 0.02^{b}	0.97 ± 0.04^{b}	4.9 ± 0.25^{a}	16.96 ± 0.49^{a}	0.3 ± 0.21^{b}	0.74 ± 0.01^{b}	4.2 ± 0.27^{a}	15.5 ± 0.2^{a}
1.2	0.7 ± 0.29^{a}	1.07 ± 0.01^{a}	3.03 ± 0.25^{c}	10.08 ± 0.49^{c}	0.4 ± 0.33^{a}	0.83 ± 0.02^{a}	1.96 ± 0.25^{c}	9.36 ± 0.21^{c}

Table 4: Effects of PEG on compatible solutes and antioxidant activity of primed and non-primed seeds.

Data represent average values of three replicates and letters indicate significant ($p \leq 0.05$) differences among treatments. Mean ± S.E.

Total soluble sugar in primed and non-primed seedling significantly ($p \leq 0.05$) increased with increasing of PEG concentrations (Table 4). Result indicated that total soluble sugar significantly negatively correlated with the germination ($r = 0.738$, $p < 0.01$), PEG tolerant ($r = 0.524$, $p < 0.01$) and fresh weight ($r = 0.829$, $p < 0.01$) and positively correlated relative PEG injury rate ($r = 0.691$, $p < 0.01$) (Table 5).

	GP	Meantime	Gindex	Relative	PEGt	FW	DW	Shoot	Root	TSS	Proline	APX	CAT
GP	1	-.622**	.327	-.978**	.929**	.847**	.439*	.665**	.855**	-.738**	-.755**	.103	-.112
Meantime		1	-.489*	.685**	-.764**	-.472*	-.457*	-.779**	-.786**	.199	.291	-.215	-.03
Gindex			1	-.415*	.528**	.428*	.136	.45*	.506*	-.122	-.114	-.106	-.93
Relative				1	-.936**	-.812**	-.451*	-.705**	-.864**	.691**	.716**	-.219	.027
PEGt					1	.802**	.505*	.85**	.969**	-.524**	-.582**	.13	-.168
FW						1	.294	.394	.655**	-.829**	-.838**	-.293	-.495*
DW							1	.627**	.596**	-.296	-.463*	-.01	-.33
Shoot								1	.941**	-.072	-.183	.349	.022
Root									1	-.355	-.435*	.21	-.107
TSS										1	.952**	.253	.345
Proline											1	.321	.456*
APX												1	.781**
CAT													1

Table 5: Pearson correlation coefficient between seedlings-related parameters, compatible solutes and enzyme activities.

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

In both primed and non-primed seeds APX and CAT activity peaked at -0.8 that significantly ($p \leq 0.05$) higher than other concentrations of PEG. APX activity at - 0.8 MPa of PEG was significantly ($p \leq 0.05$) higher than control (1.33 unit mg^{-1} FW). The result showed that APX activity significantly ($p \leq 0.05$) reduced at - 1.2 MPa of PEG treatment. Similarly, CAT activity significantly ($p \leq 0.05$) increased after exposure to a PEG treatment. Activity of CAT peaked at - 0.8 MPa (15.96 unit mg^{-1} FW). The result showed that CAT activity significantly ($p \leq 0.05$) reduced at -1.2 MPa of PEG treatment. According to table 5 there is no correlation for APX activity with any of the parameters studied. CAT activity negatively correlated with the fresh weight ($r = 0.459$, $p < 0.05$) and positively correlated with the proline ($r = 0.456$, $p < 0.05$) and APX activity ($r = 0.781$, $p < 0.01$) (Table 5).

Discussion

The results obtained by current research showed that seed germination and seedling growth highly affected by the increasing drought stress. Hydro priming improved germination characteristics and seedling growth through modulation in activity of antioxidant and accumulation of proline content and total soluble sugar. Drought stress limited water uptake by seeds and delay or inhibit germination [24]. Reduction capacity of water uptake by seeds cause accumulates ions, thereby, seeds require more time to absorb sufficient of water [25]. According to the results, hydro primed seeds indicated higher germination percentage and germination index and lower mean germination time than non-primed seeds under drought treatments. Pre-sowing treatment improves the pre-germination metabolic processes

through imbibe sufficient water and makes the seed ready for radicle emergence [14]. Previous results confirmed that water uptake and then germination traits are improved by priming treatment under drought stress in plant species [26,27]. Water uptake directly effects on cell division and elongation within growing seedlings and then shoot and root system development [28]. Our results indicated that shoot and root length of primed seeds were higher than non-primed seeds under drought stress. Moreover, drought stress suppressed the vital nutrients absorption (N, P and K) which could severally affect seedlings-related parameters [29]. Increasing PEG concentrations caused increasing the relative PEG rate injury as a useful tool to adaption plants under drought stress [30]. Similar results in other plants reported by Ibrahim [14], Nawaz., *et al*. [31] and Dezfuli., *et al*. [17] that hydro priming stimulated germination traits and thereby priming seeds showed high PEG tolerance and less relative PEG injury rate compare to non-priming seeds.

Plants adaptation mechanism to abiotic stress is associated with osmotic adjustment (OA). Osmotic adjustment is accumulation of organic solutes such as proline and total soluble sugar to balance cell water potential and protect proteins during stress [32]. Meanwhile, one of hydro priming strategies is accumulation of organic solutes and increasing activity of antioxidants. We observed general trend of proline content and total soluble sugar in primed and non-primed seeds with increasing of PEG concentrations. Drought stress also causes the production of ROS in chloroplast and mitochondria which leads to damage sub-cellular component [33]. ROS scavenging system decreased the harmful effects of ROS by two main mechanisms; non-enzymatic and enzymatic detoxification [34]. A non-enzymatic scavenging system involves phenolic compounds and lipid peroxidation. Enzymatic scavenging system includes catalase (CAT) and ascorbate peroxidase (APX) which detoxified hydrogen peroxide [35]. Severe drought stress caused accumulation of hydrogen peroxide which decrease the activity of APX and CAT [36]. Same results reported by Krasensky and Jonak [37], Umezawa., *et al*. [38] and Keting Chen and Arora [39] that primed seeds enhanced the proline content, total soluble sugar, APX and CAT activity to control ROS production compare to non-primed seeds.

Conclusion

The result of growth performance, accumulation of organic solutes and enzymes activity determined markedly improving germination traits and seedling growth of primed seeds under drought stress. Hydro priming as a simple, cheap, and effective method could improve drought tolerance of MR219 seeds at early seedling growth stage. To supporting the result that obtained in this research the field performance of MR219 seeds need to be evaluated.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

The authors would like to acknowledge to Universiti Putra Malaysia for the research grants, PUTRA GRANT- IPS- 9446700 for financial support.

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