TROPICAL AGRICULTURIST, VOL.166 (1), 2018 DOI: https://doi.org/10.4038/ta.v166i1.108

PHYSIOLOGICAL RESPONSE OF PRIMED RICE SEEDS TO SUBMERGENCE AT SEED GERMINATION AND SEEDLING GROWTH

H.M.S.HERATH[1], A.M. BALTAZAR[3], F.D. ENTILA[2], E.S. ELLA[2], P. STA.CRUZ[3], A.M. ISMAIL[2] AND D E. JOHNSON[2]

[1] *Rice Research Station, Ambalantota, Sri Lanka*

[2] *International Rice Research Institute, Los Baños, Laguna, Philippines*

[3] *University of the Philippines Los Baños, Collage, Laguna, 4031, Philippines*

ABSTRACT

Submergence plays an important role in management of weeds in the early stage of the rice, but it hinders the crop establishment in Direct-Seeded Rice (DSR). Seed priming techniques are employed to achieve efficient crop establishment in DSR despite submergence. However, performance of primed rice seeds under submergence has not been well studied. Then, the influence of seed hydro-priming duration (0, 24, 36 and 48 hours) on seed germination, seedling growth of rice and related physiological factors under different submerged depth (0, 1 and 5 cm) was studied using the rice variety IR64. Seed germination and seedling vigor, growth and survival were assessed at 5, 7, 10, 14 and 21 Days After Sowing (DAS). Lipid peroxidation was assayed in dry seeds and amylase activity and soluble sugar concentration were assayed in germinating seeds at 4 DAS. Seed priming treatments improved the seed germination by 79%, seedling vigor and survival by more than two fold under submerged conditions. Overall, the 36-hours appeared to be the best hydro-priming duration for rice. Priming significantly reduced the Malondialdehyde (MDA) content of dry seeds and MDA content correlated negatively (r=-0.86) with seedling survival under submergence. Priming increased the total amylase activity by 18-fold and alpha amylase by 6-fold under submerged conditions at 4 DAS. Further, priming significantly improves the soluble sugar concentration of germinating seeds at 4 DAS under flooding. Seed priming improved the physiological traits associated with flooding during germination such as, germination, growth, vigor, survival and carbohydrates metabolism. Therefore, IR64 rice genotype with seed priming for 24, 36 and 48 hours could be used to improve the germination, seedling vigor, emergence and survival under flooding conditions.

Key words: Direct seeded, Rice, Seed germination, Seedling growth, Seed hydro-priming, Submergence

INTRODUCTION

Direct Seeding Rice (DSR) establishment method is popular among the farmers in various parts of Asia due to reduced labor requirement by 50% compared to transplanting (Pandey and Velasco, 2002). Although the DSR is popular, it accompanies by several disadvantages such as, poor seedling establishment and high weed pressure (De Datta, 1986; Moody, 1982). Poor land preparation and levelling accompanied by standing water in the field lead to poor crop establishment in Wet Direct-Seeded Rice (WDSR). Absence of standing water at the initial stage ensures the good crop establishment and encourages the emergence and growth of weeds in DSR (Balasubramanian and Hill, 2002). In DSR system, poor seedling establishment is a major constraint due to germinating seeds is subjected to various source of stresses such as flooding. Therefore, physiological traits such as good seed germination, effective seedling emergence and seedling vigor are important to achieve successful crop establishment under flooded conditions (Mori *et al*., 2012). One strategy to avoid conflict among crop stand establishment and early water management in WDSR is to use of flooding tolerant rice cultivars. Aside from the crop improvement through breeding, another possible agronomic strategy to achieve good crop establishment under flooded condition is the use of seed priming technique.

Seed priming is a pre-treatment technique of seeds in which seeds are partially hydrated to a point where germination related metabolic processes begin but radical emergence does not occur (Farooq *et al*., 2006a). Different seed priming techniques such as hydro-priming (Farooq *et al*., 2006a), on farm priming (Harris *et al*., 2001) and hardening (Farooq *et al*., 2004) have been employing to enhance the establishment of different crops. However, hydro-priming is a simple, economically viable and environmentally friendly technique that can perform easily to enhance the crop performance during establishment. Hydro-priming; soaking seeds in tap water at room temperature for a particular time period and re-drying back to original moisture content is a seed priming technique (Farooq *et al*., 2006 a ;2006 b). Seed priming has long been established as an effective method for ensuring rapid and uniform seed germination in cereals and vegetables sown in aerated soils (Lee *et al*., 1998; Farooq *et al*., 2006b). On-farm seed priming (overnight seed soaking, surface drying and sowing) improved establishment and early vigor of upland rice, maize and chickpea (Harris *et al*., 2001). However, the effect of rice seed priming on the physiological performance at seed germination and seedling stage under the flooded condition has not been well characterized. Therefore, the present study aimed to determine the importance of different seed hydro-priming durations on seed germination and seedling growth, and survival and the related physiological factors of IR64 rice variety under flooded conditions.

MATERIALS AND METHODS

Greenhouse experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Philippines. The rice variety IR64 was used in the experiment. Initial seed moisture content was determined in each seed batch before hydro-priming and seed moisture content was found to be 11± 0.3 %. For hydro-priming, 200 g of seeds from each batch were soaked in tap water at room temperature (26 °C) in the dark for 24, 36 and 48-hours followed by drying to initial moisture content under shade. Dried primed seeds were stored in a refrigerator at 5°C until used.

The experiment was a two factor factorial experiment where factors being hydro-priming duration and submergence depth. Treatments consisted of three levels of submergence depths including, 0 (no submergence), 1, and 5-cm and four levels of hydro priming durations; 0-hours (unprimed) 24-hours, 36-hours and 48-hours, So that the experiment had 12 treatment combination. Experiment was conducted in the green house where open-top submergence chamber (292 cm x 98.5 cm) where no submergence with unprimed treatments was the control. Hundred and twenty eight seeds of each treatment were sown at 1- cm soil depth in plastic root trainers each consisted of 32 cells filled with sterilized soil. The different submergence levels were continuously maintained until 21 Dys After Sowing (DAS). The experiments were conducted using a split-plot arrangement where submergence depth was assigned into main plots and priming duration was assigned into sub-plots within a randomized complete block design with three replications. Lipid peroxidation was determined in dry seeds before sowing, amylase activity and soluble sugar concentrations were determined at 4 DAS.

Germination, growth and seedling survival

Samples harvested at 5, 7, 10, 14 and 21 days were used for the growth measurements. Seedling emergence (seedling above the soil surface) was recorded continuously up to 21 DAS. Seed germination (coleoptiles length higher than 2 mm) count was recorded at 5 DAS. Final seedling emergence count and survival percentage were recorded at 21 days after sowing. Seedling vigor was recorded at 5, 7, 10, 14 and 21 DAS based on seedling length. Seedling vigor was calculated using the following equation as described by Abdul-Baki and Anderson (1973); Vigor index = (Germination % x Seedling length (Root + Shoot))/100. Experiment was repeated at least twice each with three replications.

Determination of lipid peroxidation

Lipid peroxidation was determined by measuring Malondialdehyde (MDA) formation using the thiobarbuturic acid method described by Hodges *et al*.(1999). Malondialdehyde equivalents were calculated using equations given in Hodges *et al*.(1999).

Amylase activity assay

Total amylase and α-amylase activity were measured using the method of Bernfeld (1955). The total protein concentration was determined using the Bradford method (Bradford, 1976), with brovine serum albumin (BSA) as a protein standard. One unit of amylase activity is defined as µmoles maltose produced per minute and activity was expressed in units per milligrams protein.

Soluble sugar analysis

Soluble sugars were determined from the samples before sowing and samples harvested at 4 DAS using the method of Fales (1951). Soluble sugar in the sample was calculated based on the standard curve of glucose. Soluble sugar concentration was expressed as percentage by weight of glucose per unit dry weight of the sample.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using STAR for Windows version 2.1 (IRRI, 2014). Differences among treatments were compared using least significant differences (LSD). Relationships between different attributes were determined using linear regression.

RESULTS AND DISCUSSION

Seed germination and seedling growth, vigour and survival

Primed and unprimed IR64 rice seed had similar seed germination percentages at 5 DAS under no submerged conditions, but seed germination decreased substantially under submergence, with a greater reduction in unprimed treatments (Table 1). However, Priming increased seed germination under submerged conditions (1 and 5cm) where on the average germination was 79%. All the priming treatments gave greater germination percentage than that of unprimed treatment under 5 cm submerged depth. However, 36-hours priming duration was found to be the best at all levels of submergence depth and it was the comparable shorter priming duration with high performance. This is in agreement with Farooq *et al*. (2006a) who reported the, highest final seed germination when rice seed hydro-primed for 36 hours and 48 hours. Dezfuli *et al*. (2008) reported that, maize seeds showed highest germination with hydro-priming for 36-hours followed by 24-hours and 48-hours compared to unprimed seeds. Under no submerged condition, primed as well as unprimed treatments had longer seedling length, but under submerged condition primed treatments had significantly higher seedling length compared to unprimed treatment at 5 DAS (Table 1). Submergence decreased seedling lengths of both primed and unprimed treatments, but reduction was lower in primed treatments compared to unprimed treatments. However, seedling height was comparatively than 10-fold longer under submerged condition.

Under no submerged condition both primed and unprimed IR64 rice seedling had comparatively higher seedling vigor, but primed treatments had significantly higher seedling vigour than that of unprimed treatment at 5 DAS (Table 1). Submergence decreased seedling vigour, but primed treatments had greater seedling vigor under submergence than that of unprimed treatments. Primed treatment of 36-hours gave greater seedling vigor under the 5 cm submerged depth.

Generally, seedling vigor increased over time in both no submergence and submergence conditions, but significant vigor increase was observed in primed treatments over time compared to that of unprimed treatment only under submergence condition (Figure 1). More than 2-fold vigor increase was observed in primed treatments when compared to unprimed treatment under submerge conditions at 21 DAS (Figure1B and C). This is in agreement with the results of Farooq *et al*. (2006a; 2006b) who was reported that, priming improved the performance and seedling vigor of DSR. Among the seed priming treatments, hydro-priming for 36-hours gave greater seedling vigor under flooding. This is also in agreement with the results of, Farooq *et al*., 2006c, that rice seed priming for 12-hours, 24-hours, 36-hours and 48-hours increased the seedling vigor while priming for 60-hours was detrimental to the vigor of seeds.

Priming significantly enhanced the seedling emergence of rice under submerged conditions and more than 2-fold emergence increase was observed under submerge conditions when compared to that of no submergence, suggesting priming can improve the tolerance of IR64 for submergence (Figure 2). Greater increase in emergence was observed in 36-hours primed treatment. Farooq *et al*., 2006 reported that, highest emergence percentage of rice was observed when seeds were hydro-primed for 48-hours followed by 36-hours and 24-hours priming than the unprimed control.

Table 1. Germination percentage, seedling length, and vigor index (VI) of IR64 as affected by hydro-priming treatments under different flooding depths at 5 days after sowing.

Flooding depth (cm)	Hydro-priming duration (hours)	Germination (%)	Seedling length (mm)	Vigor index (vi)
0 cm	24h-priming	97.22 ± 02.78	80.89 ± 00.73	135.88 ± 14.02
	36h-priming	100.00 ± 00.00	89.97 ± 02.48	179.56 ± 04.66
	48h-priming	100.00 ± 00.00	77.69 ± 01.42	164.31 ± 08.91
	Unprimed	94.44 ± 02.78	63.39 ± 06.66	110.39 ± 06.97
1 cm	24h-priming	72.22 ± 11.11	4.86 ± 01.26	3.79 ± 01.31
	36h-priming	83.33 ± 04.81	6.97 ± 01.07	5.71 ± 00.60
	48h-priming	83.33 ± 12.73	6.78 ± 02.00	6.16 ± 02.31
	Unprimed	29.44 ± 02.78	0.44 ± 00.28	0.10 ± 00.07
5 cm	24h-priming	77.78 ± 05.56	4.33 ± 01.13	3.43 ± 01.04
	36h-priming	88.89 ± 05.56	6.25 ± 01.53	5.68 ± 01.65
	48h-priming	75.00 ± 08.33	3.61 ± 00.86	2.85 ± 00.87
	Unprimed	25.00 ± 04.81	0.44 ± 00.17	0.13 ± 00.07
Flooding depth		* *	* * *	* * *
Hydro-priming duration		* * *	* * *	* * *
Flooding depth* Hydro-priming duration		* * *	* *	* * *
LSD (0.05)		18.6	5.91	15.98

*Data were presented as means ± standard errors. P ≤ 0.05 (significant); * * P ≤ 0.01(highly significant); *** P ≤ 0.001(extremely significant)*

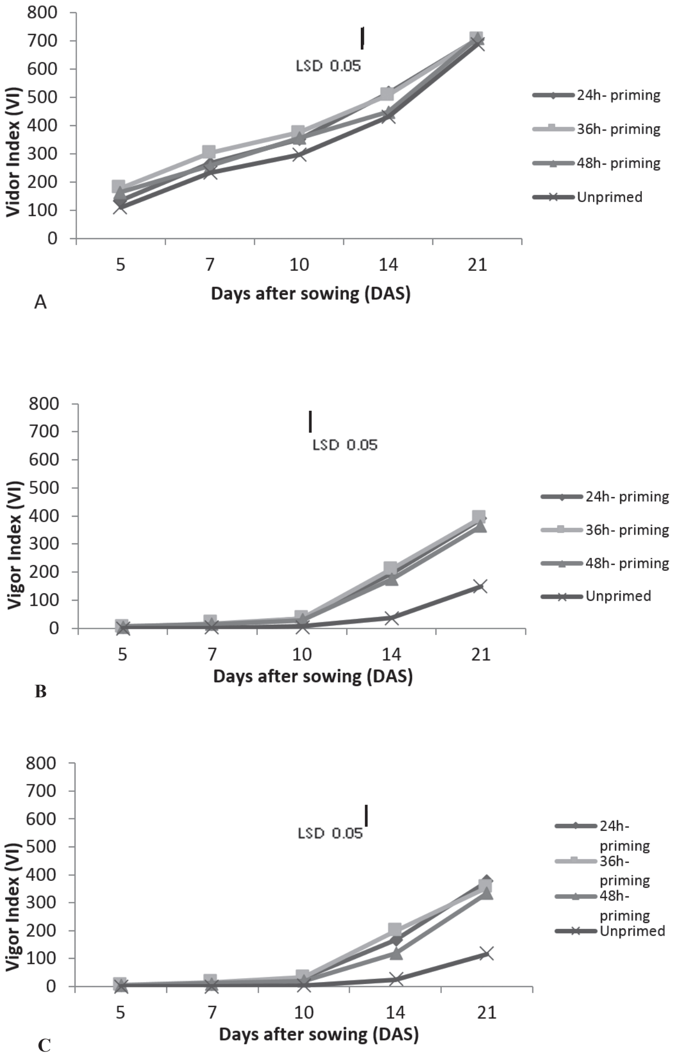

Figure 1. Change in vigor index of IR64 as affected by hydro-priming treatments under different submergence depth at different days after sowing. A=0 cm , B=1 cm and C=5 cm. Vertical bar indicates LSD $_{0.05}$.

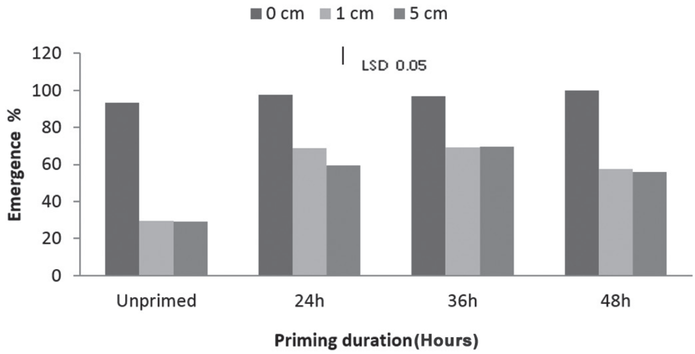

Figure 2. Emergence percentage of IR64 as affected by hydro-priming treatments under different submergence depths at 21 DAS. Vertical bar indicates LSD $_{0.05}$.

Primed treatments had higher seedling survival under submergence at 21 DAS compared to unprimed treatments (Figure 3). Greater seedling survival was achieved when seed was primed for 36-hours, suggesting optimum priming duration to achieve maximum seedling survival. Higher seedling survival of primed treatments might be due to increase in germination, seedling vigor and amylase activity. These results further supported by the significant correlation between seedling survival and seedling vigor ($r = 0.85$) under 5 cm submergence depth at 21 DAS (Figure 4). These results are in agreement with previous results reported by Ella *et al*., (2010). Results suggested that, the importance of employing priming treatments to improve the submergence tolerant characteristics of rice.

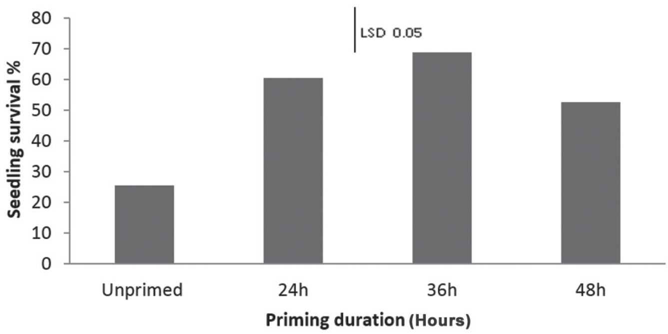

Figure 3. Seedling survival percentage of IR64 as affected by hydro-priming treatments when submerged 5 cm depth at 21 DAS. The data represent the mean values of three replications per treatment and vertical bar indicates LSD $_{0.05}$.

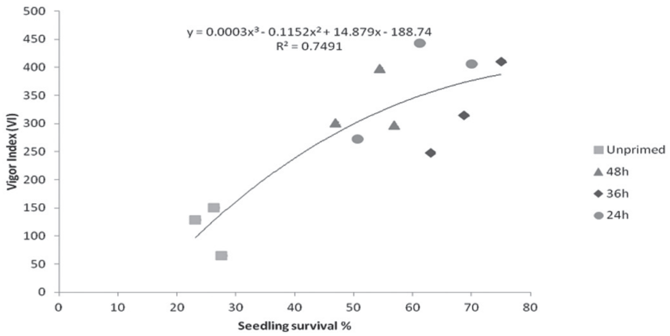

Figure 4. Correlation between percentage seedling survival and vigor index (VI) of IR64 as affected by hydro-priming treatments when submerged to 5 cm at 21 DAS.

Lipid peroxidation, amylase activity and soluble sugar concentration

Priming significantly decreased the Malondialdehyde (MDA) content of rice dry seeds before sowing (Figure 5), suggesting that seed priming induce some protective mechanisms thereby reducing the lipid peroxidation in cellular membrane. Ella *et al*., (2011) reported that priming reduced the lipid peroxidation through induction of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT). Lipid peroxidation is the free- radical that induces injury to cellular damage. The reduced MDA content of primed treatments might be associated with the increased survival of seedling under submergence. Further, these results in supported by the significant negative correlation between seedling survival at 21 DAS and MDA content of dry seeds before sowing (r = -0.86) as shown in Figure 6. This positive effect of reducing lipid peroxidation is one of the advantages of seed priming.

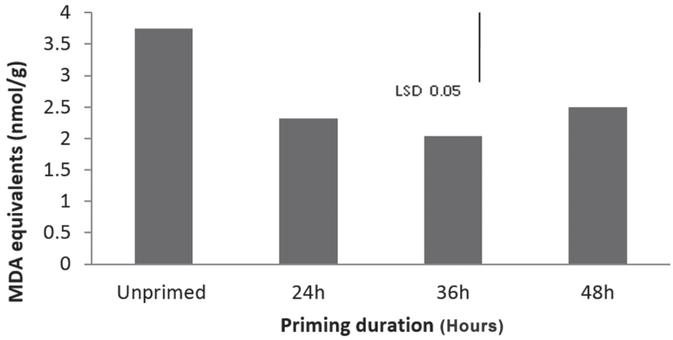

Figure 5. MDA content as affected by hydro-priming treatments of seeds before sowing. The data represent the mean values of the three replications per treatment and vertical bar indicates LSD $_{0.05}$.

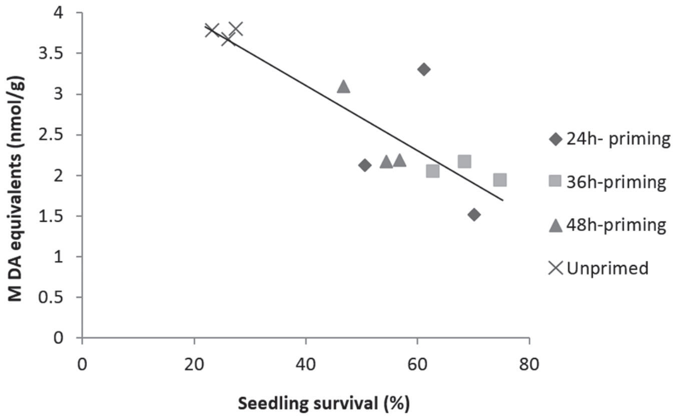

Figure 6. Correlation between MDA content before sowing and seeding survival of IR64 as affected by hydro-priming treatments when flooded to 5 cm at 21 DAS.

Under no submergence conditions, primed and unprimed treatments had higher total amylase activity at 4 DAS, but total amylase activity significantly decreased under submergence (Figure 7). Primed treatments had significantly higher total amylase activity compared to unprimed treatments under submergence (maximum increase was about 11 fold on the average), suggesting priming accelerated the starch degradation process through the induction of amylases activities. Amylase activity plays a key role in degradation of starch to soluble sugar in the germination process (Perata *et al*., 1998). Total amylase activity in seeds germinating under submergence correlated positively with germination, seedling length and seedling vigor at 5 DAS (Table. 2).

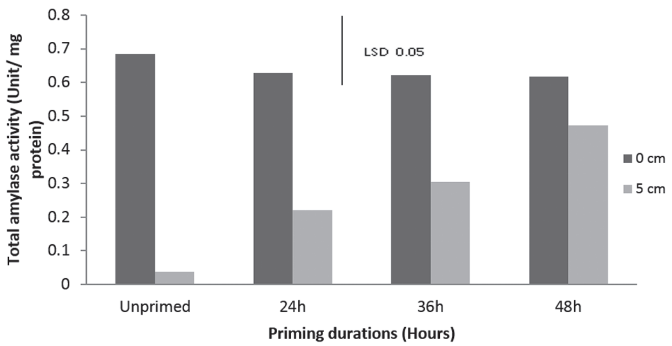

Figure 7. Total amylase activity of IR64 as affected by hydro-priming treatments when submergence to 0 cm and 5 cm depth at 4 DAS. Vertical bar represents the $LSD_{.05.}$

Table 2. Correlation coefficients for associations among total amylase activity, seed germination, seedling length, and seedling vigor.

	Total amylase activity	Germination percentage	Seedling length
Total amylase activity	-		
Seed germination percentage	0.62**	-	
Seedling length	0.78***	0.67***	-
Seedling vigor	0.75***	0.65***	0.98***

****, **, significant at $p \leq 0.001$ and $p \leq 0.01$*

Alpha amylase activity increased upon priming in IR64 rice genotype under submergence at 5 cm depth at DAS (Figure 8). There was 5, 4 and 8-fold increase in α-amylase activity in 24-hours, 36-hours, and 48-hours primed treatment compared to unprimed treatment under submergence at 4 DAS. This is agreement with the results of Mori *et al*., (2012) who reported that, α-amylase activity of the primed rice seeds was 4-fold higher than that of unprimed rice seeds under anoxia. Previous researchers reported that, α-amylase is the key enzyme involve in degradation of carbohydrates particularly under low oxygen conditions (Ismail *et al*., 2009 ; Ella *et al*., 2011). Results suggested that, employing priming in rice seeds positively induced the amylases activities and which improved the tolerant characteristics of IR64 to submergence during germination and early growth.

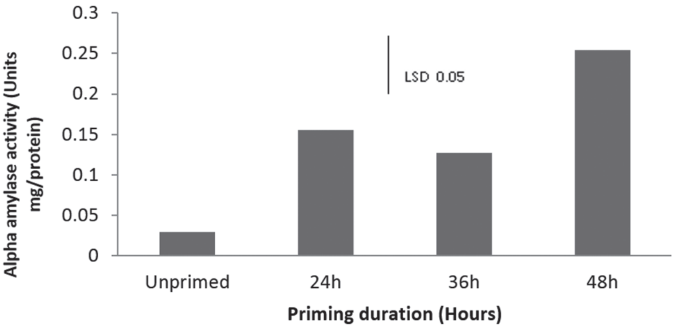

Figure 8. Alpha amylase activity of IR64 as affected by hydro-priming treatments when submerged to 5 cm depth at 4 DAS. Vertical bars represent the $LSD_{.05.}$

No submergence condition, germinating both primed and unprimed seeds of IR64 had high soluble sugar concentration at 4 DAS (Figure 9), but decreased substantially under flooding (Figure 10). However, reduction of soluble sugar concentration under submergence was lower in primed treatments compared to that of unprimed treatments. Soluble sugar concentration was significantly higher in primed rice seeds compared to unprimed seeds under submergence, indicating that priming accelerates the carbohydrate metabolism. These results confirm the findings of Ella *et al*., (2011) that, primed rice seeds increased the soluble sugar concentration than the unprimed seeds under submergence during germination. This could be probably due to the higher induction of amylase activity of primed rice seeds under submergence, that accelerate the degradation of starch into soluble sugars. These results are consistence with the results of Mori *et al*., (2012) that, primed rice seeds increase the alpha-amylase activity and soluble sugar concentration over time. This result suggested that, the primed rice seeds can supply soluble sugars comparatively faster by degrading starch into sugar under submergence conditions.

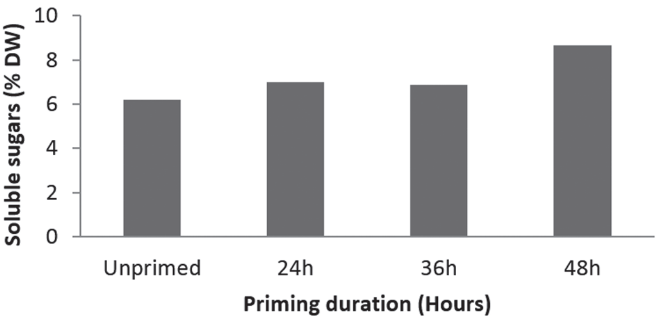

Figure 9. Soluble sugar concentration of IR64 as affected by hydro-priming treatments under no submergence condition at 4 DAS.

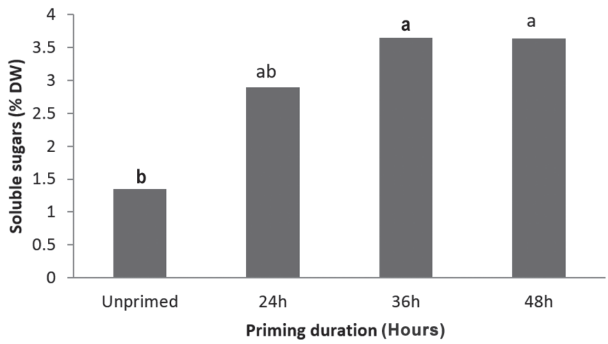

Figure 10. Soluble sugar concentration of IR64 as affected by hydro-priming treatments when submerged to 5 cm depth at 4 DAS. Different letters indicate significant differences at (*p*< 0.05) according to LSD$_{.05.}$

CONCLUSIONS

Present study highlighted the importance of employing rice seed hydro-priming to ensure high seed germination and good seedling growth, vigor and survival under submerged condition and high amylase activity and reduced lipid peroxidation in dry seeds. IR64 hydro-priming for 24-hours and 36-hours and 48-hours significantly improved seed germination and seedling vigor and survival under submergence. Almost 11-fold increase in total amylase and 6-fold increase in alpha-amylase activity of primed treatment contributed to improve submergence tolerance of IR64.

REFERENCES

Abdul-baki, A.A. and J.D. Anderson. 1973. Relationship between decarboxylation of glutamic acid and vigor in soybean seed. Crop Science 13(2): 227-232.

Balasubramanian, V., and J.E. Hill. 2002. Direct seeding of rice in Asia: Emerging issues and strategic research needs for the 21st century. In Direct Seeding: Research Strategies and Opportunities, Eds. S. Pandey, M. Mortimer, L. Wade, T.P. Tuong, K. Lopez and B. Hardy. pp. 15-39. International Rice Research Institute, Los Ban˜os, Philippines.

Bernfeld, P. 1955. Amylases, alpha and Beta. Methods in Enzymology 1: 149-152.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72(1): 248-254.

De Datta, S.K. 1986. Technology development and the spread of direct- seeded rice in Southeast Asia. Experimental Agriculture 22: 417-426.

Dezfuli, P.M., F. Sharif-zadeh and M. Janmohammadi. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L.). ARPN Journal of Agricultural and Biological Science 3(3): 22-25.

Ella, E.S, M.L. Dionisio-Sese and A.M. Ismail. 2010. Proper management improves seedling survival and growth during early flooding in contrasting rice genotypes. Crop science, 50(5), 1997-2008.

Ella, E.S., M.L. Dionisio-Sese and A.M. Ismail. 2011. Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. Annals of Botany plants 2011: plr007. https://doi.org/10.1093/aobpla/plr007

Fales, F.W. 1951. The assimilation and degradation of carbohydrates by yeast cells. Journal of Biological Chemistry 193: 113-124.

Farooq, M., S.M.A. Basra, I. Afzal and A. Khaliq 2006c. Optimization of hydropriming techniques for rice seed invigoration. Seed Science and Technology 34(2): 507-512.

Farooq, M, S.M.A. Basra, R. Tabassum, I. Afzal 2006b. Enhancing the performance of direct seeded fine rice by seed priming. Plant production Science 9: 446- 456.

Farooq, M., S.M.A. Basra, A. Wahid 2006a. Priming of field- sown rice seed enhances germination, seedling establishment, allometry and yield. Plant growth Regulator 49: 285- 294.

Farooq, M., S.M.A. Basra, H.A. Karim, I. Afzal. 2004. Optimization of seed hardening techniques for rice seed invigoration. Emirates Journal of Food and Agriculture 16(2): 48-57.

Harris, D., B.S. Raghuwanshi, J.S. Gangwar, S.C. Singh, K.D. Joshi, A. Rashid, P.A. Hillington. 2001. Participatory evaluation by farmers of ‘on farm’ seed priming in wheat in India, Nepal and Pakistan. Experimental Agricsulture 37: 403 415.

Hodges, D.M., J.M. DeLong, C.F. Forney, R.K. Prange. 1999. Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207: 604–611.

IRRI 2014, International Rice Research Institute. IRRISTAR for Windows Version 2.0.1. Los Banõs, Philippines.

Ismail, A.M., E.S. Ella, G.V. Vergara and D.J. Mackill. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). Annals of Botany 103: 197-209.

Lee, S.S., J.H. Kim, S.B. Hong, S.H. Yun. 1998. Effect of humidification and hardening treatment on seed germination of rice. Korean Journal of Crop Science 43:157-160.

Moody, K., S.K. De Datta. 1982. Integration of weed control practices for rice tropical Asia. In Weeds Control in Small Farms, Eds. M. Soerjani, D.E. Barnes and T.O. Robson. pp. 37-47. Biotrop Special Publication 15. Bogor (Indonesia): BIOTROP.

Mori, S., H. Fujimoto, S. Watanabl, G. Ishioka, A. Okaba, M. Kamei and M. Yamauchi. 2012. Physiological performance of iron- coated primed rice seeds under submerged conditions and the stimulation of coleoptile elongation in primed rice seeds under anoxia. Soil science and plant nutrition 58: 469-478.

Pandey, S. and L. Velasco. 2002. Economics of direct seeding in Asia: patterns of adoption and research priorities. In: Direct Seeding: Research Strategies and Opportunities, Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities, Eds. S. Pandey, M. Mortimer, L. Wade, T.P. Tuong, K. Lopez, B. Hardy. pp. 3-14. International Rice Research Institute.

Perata, P., E. Loreti, L. Guglielminetti, A. Alpi. 1998. Carbohydrate metabolism and anoxia tolerance in cereal grains. Acta botanica neerlandica 47:269-283.