

AGRIVITA Journal of Agricultural Science

Potential of Wood Vinegar for Enhancing Seed Germination of Three Upland Rice Varieties by Suppressing Malondialdehyde Production

Atchima Dissatian¹, Jirawat Sanitchon¹, Paweena Pongdontri², Nuntawoot Jongrungklang¹) and Darunee Jothityangkoon^{1*}

¹⁾Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

²⁾ Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

ABSTRACT

Keywords: Biochemical changes Drought *Oryza sativa* Plant derived smoke Seed priming

Article History: Received: March 9, 2017 Accepted: April 27, 2018

*) Corresponding author: E-mail: darcho@kku.ac.th Upland rice usually gives poor germination under rainfed upland environement. To improve the seed germination, seed priming technique was assessed in this study. Seed of three upland rice (Oryza sativa L.) varieties (Leum Pua, ULR038 and Sakon Nakhon1, SKN) were primed with three different seed priming agents; distilled water, CaCl₂ and 300-fold diluted wood vinegar. Compared to untreated dry seeds, wood vinegar improved field emergence and improved drought tolerance of the rice seeds better than other priming agents, which 50 % for Leum Pua, 20 % for ULR038 and 16% for SKN, when watering was delayed for 7 days. All three priming agents increased the field emergence percentage by similar amounts when watering was delayed for 14 days. To understand how priming helped improvement of germination, antioxidation mechanism and sugar metabolism were examined. Level of malondialdehyde was markedly reduced in all three rice varieties in response to priming, which were associated with increased activity of antioxidant enzymes, guaiacol peroxidase and ascorbate peroxidase. Wood vinegar also accelerated amylase activity in ULR038 and SKN, but not the sugar content. The results suggest that wood vinegar is a potent priming agent for achieving rapid and uniform seed germination in upland rice.

INTRODUCTION

Rice is the most important crop for one-third of the world's population. However, rice production in the rainfed lowlands regularly encounters drought and/or erratic rainfall. This leads to an increased focus on rice production in the rainfed upland environments. In the upland rice growing environments, due to the erratic nature of early wet-season rain, it is a common practice to plant the seed into dry seedbeds, where it can remain for 2-3 weeks before sufficient rainfall for germination. However, this can result in the poor and uneven establishment and low final yields (Rehman, Basra, Farooq, Ahmed, & Afzal, 2011).

There are technologies that assist in reducing the risks to yield in the rainfed environments,

including seed priming (Ashraf & Foolad, 2005). The most common practice in seed priming of lowland rice is pre-germination, in which the seeds are hydrated until radicle emergence is achieved (McDonald, 2000). However, pregermination might be an inappropriate technique for upland rice production because of unpredictable rainfall. Another type of seed priming is where the seeds are imbibed to allow early events of germination to initiate and then stop before radicle protrusion, followed by drying back to original seed moisture content (McDonald, 2000). In this type of priming, seed imbibition does not enter phase 3 of germination process, but stimulates early events only at phases 1 and 2. This would prompt upland rice seed germinability. Even seed establishment

ISSN: 0126-0537 Accredited by DIKTI Decree No: 60/E/KPT/2016

Cite this as: Dissatian, A., Sanitchon, J., Pongdontri, P., Jongrungklang, N., & Jothityangkoon, D. (2018). Potential of wood vinegar for enhancing seed germination of three upland rice varieties by suppressing malondialdehyde production. *AGRIVITA Journal of Agricultural Science, 40*(2), 371–380. http://doi.org/10.17503/agrivita.v40i2.1332

can be achieved when the rain falls. This is believed to be a response to important biochemicals for seed germination, which are released and resulted in a uniform start to germination.

The early events of germination include expressions of genes involved in many metabolic processes, such as sugar mobilization, cell expansion, etc., which can occur during imbibition phases 1 and 2 by water or hydro priming. In fact, water imbibition can be controlled by osmopriming and halopriming using polyethyleneglycol (PEG) solution and organic salt solution as priming agents, respectively. In addition, seed can be mixed with water and insoluble matrix particles, such as vermiculite, agrolite and soil in matrix priming or matripriming. Not only water is controlled during imbibition, but some chemicals in priming solutions are also able to signal extra-biochemical mechanisms, such as an anti-oxidative pathway. This enables crop uniformity and performance in many crops under both favorable and unfavorable conditions. includina biotic arowina and abiotic stresses (Chen & Arora, 2013).

Various methods of seed priming have been introduced to improve seed germinability and yield. Primed seed achieves higher percentage of germination, together with the rapid and uniform emergence of seedlings in many crops, including rice (Abdallah, Musa, Mustafa, Sjahril, & Riadi, 2016). However, certain priming methods can achieve an improvement of certain types of seeds. This is believed to reflect certain changes in seed chemical composition during storage and during priming. For example, changes of lipids to malondialdehyde (MDA) have been recorded, as well as changes in proteins, nucleic acid and starch during storage (Zhou, Robards, Helliwell, & Blanchard, 2002). Although the molecular basis of seed priming remains unclear, it has been suggested that it involves a DNA repair mechanism and gene expression for syntheses of new RNAs and proteins (Varier, Vari, & Dadlani, 2010). Evidence in spinach has shown that osmoprimed seeds have increased levels of antioxidant enzymes and stress proteins, which are associated with improving biotic and abiotic tolerance (Keting Chen, Fessehaie, & Arora, 2012). In addition to the molecular basis, seed priming promotes germination related activities, such as respiration and endosperm weakening; thereby they assist the transition of seed quiescence to the germination state (Chen & Arora, 2013). Energy

metabolism (e.g., the utilization of seed storage products), is also enhanced by seed priming. Starch degradation is detected by an increase in amylase activity, including an increase in soluble sugars in primed rice seeds (Ella, Dionisio-Sese, & Ismail, 2011).

Wood vinegar or pyroligneous acid is made from plant-derived smoke which is condensed under the pyrolysis process and contains ketones, phenols, organic acids, ester, benzene and its derivatives, aldehydes, alcohols, and sugar derivatives (Zhai et al., 2015). Variation in the chemical composition reflects different wood types and pyrolysis temperature (Nakai, Kartal, Hata, & Imamura, 2007). Wood vinegar has been widely utilized in agriculture in Asia, especially where charcoal is a source of energy for household cooking. It has been reported to be a stimulant for seed germination and seedling development in several plants, including rice (Kulkarni, Sparg, Light, & Van Staden, 2006). Wood vinegar, therefore, has been of interest as a potential priming agent for upland rice, in comparison to distilled water, and CaCl, solution.

To verify which priming methods are most suitable for upland rice seeds for drought stress, and what molecular mechanisms are involved, this study undertook a comparison of three different priming methods, distilled water priming, CaCl₂ priming and wood vinegar priming, on germination improvement, drought tolerance and some biochemical changes, in the seed of three upland rice varieties.

MATERIALS AND METHODS

Seed and Wood Vinegar Sources

Purple glutinous upland rice seeds, Leum Pua and ULR038, were obtained from the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. White non-glutinous rice, SKN1, was purchased from the Chumphae Rice Research Center, Khon Kaen, Thailand. All seeds were produced in 2012 and used for experiment conducted in March-November in 2013. Wood vinegar was obtained from Energy Ashram, Appropriate Technology Association, Thailand. Its major chemical components are acetic acid (30.39 %), propanoic acid (6.08 %), phenol (3.75 %), phenol, 2-methoxy- (12.31 %), and thiirane, methyl- (26.96 %) and low amounts of plant nutrients including N (0.03 %), P (0.10 %), K (0.01 %) and Ca (0.01 %), as reported by Mungkunkamchao, Kesmala, Pimratch, Toomsan, &

Jothityangkoon (2013).

Priming Methods

Seeds were soaked in distilled water, 0.15 M $CaCl_2$ or 300-fold diluted wood vinegar solution at 25°C for 48 h, rinsed with distilled water and airdried at room temperature. The comparative treatments were:

Dry seed	= unprimed seed as the control
Distilled water	= primed seed with distilled water
CaCl ₂	= primed seed with CaCl ₂
Wood vinegar	= primed seed with wood vinegar

Germination Test

Four replicates of 50 seeds of each treatment were germinated between moistened double layered rolled germination papers and incubated in a chamber (25 °C, 12 h of fluorescent light, 120 μ E m⁻² s⁻²). Germination was evaluated on Day 5 and Day 14 and normal seedling and abnormal seedling were considered according to ISTA (2004). Germination was expressed as a percentage of normal seedlings.

Drought Tolerance at Seeding Stage

Twenty five seeds were sown into 17-cmdiameter plastic pots containing 1 kg soil. Four water regime treatments were applied on the day of seeding (T1), 7 days-after-seeding (DAS) (T2),14 DAS (T3) and 21 DAS (T4). Each pot was re-watered every two days to retain the moisture content at field capacity using the amount of water as determined by Doorenbos & Pruitt (1977). Field emergence was recorded on Day 5 and Day 14 after the water regimes commenced. The pots were arranged in completely randomized designed (CRD) with three replications. The study was conducted under greenhouse conditions in the period February - April 2013.

Malondialdehyde (MDA), Activity of Enzymes and Sugar Assays

MDA was extracted from 1 g seed using 0.1 M potassium phosphate buffer (pH 7.0) containing 1 % (w/v) polyvinylpyrolidone (PVP) and reacted with thiobarbituric acid as described by Demiral & Türkan (2005). MDA was calculated by the molar extinction coefficient of 155 mM⁻¹ cm⁻¹ at A₅₃₂ and expressed as MDA per gram fresh weight (nmol MDA per g FW).

Crude enzyme was extracted from 1 g seed by 10 mL 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA and 1 % (w/v) PVP. Protein concentration was determined by Bradford (1976) using bovine serum albumin (BSA) as the standard.

Catalase and guaiacol peroxidase were assayed according to Chance & Maehly (1955). Catalase activity (CAT) was expressed as mmol of decomposed H₂O₂ per minute per mg protein. Guaiacol peroxidase activity (GPX) was expressed ${\scriptstyle \Delta A_{_{415}}}$ per minute per mg protein. Ascorbate as peroxidase activity (APX) was assayed as described by Nakano & Asada (1987) and expressed as µmol reduced ascorbate per minute per mg protein. Superoxide dismutase activity (SOD) was assayed according to Beauchamp & Fridovich (1971) and expressed as the percentage of inhibition of nitroblue tetrazolium (NBT) reduction per minute by SOD, in the xanthinexanthine oxidase system. All assays were performed in 200 µL reactions. Absorbances were read using a microplate reader SpectraMax M5 (USA).

Crude amylase and soluble and reducing sugar were extracted using 0.1 M sodium citrate (pH 5.0). The amylase activity accorded to Miller (1959) and expressed as mmol glucose per minute per g FW. Seed soluble and reducing sugars were analyzed by the Anthrone method (Srivong et al., 2015). The sugar content was expressed as mmol soluble sugar per g FW.

Data Analysis

Data from the pot experiment was presented after being transformed by arcsine square root before ANOVA analysis. Means comparisons were done using LSD at p < 0.05. The experiments in laboratory were performed in triplicate and expressed as mean \pm SD. Statistical analysis between the groups involved one-way ANOVA using SPSS version 17.0. The difference was considered at p < 0.05 using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Effect of Seed Priming on Seed Germination

The first count on day 5 after germination suggested that the three priming agents, distilled CaCl and wood vinegar, water, stimulated germinability of the tested upland rice seeds, Leum Pua, ULR038 and SKN, which corresponded to the final count on day 14 (Fig.1). The priming agents; i.e. distilled water, CaCl, and wood vinegar insignificantly increased germination of Leum Pua seeds at 4, 5, 7 % (p > 0.05), respectively, while dry seeds had not yet germinated at the first count. However, final count on day 14 of Leum Pua dry

seeds was at 39.5 ± 5.3 % germination, which was still significantly less than the primed seeds. Distilled water and CaCl, priming gave relatively similar germination percentages of Leum Pua (59.5 ± 1.6 % and 60 ± 3.4 %, respectively), while wood vinegar significantly increased germination percentage at the final count (71.5 ± 7.7 %). ULR038 seeds also improved their germinability similar to Leum Pua seeds in response to all priming agents. ULR038 seeds primed with distilled water and CaCl, were not significantly different in their germination percentages at the first count (being 3 % and 6 %, respectively), in parallel to their final count (42.5 ± 5.9 % and 48 \pm 3.8 %). Wood vinegar was the best priming agent for ULR038, with a significant 10 % increase in germination at the first count and 65 ± 2.0 % at the final count. SKN dry seed gave 6 % germination percentage at the first count, which was relatively similar to distilled water- and CaCl₂-primed seeds (both at 8 %, p > 0.05), but significantly less than wood vinegar primed SKN seeds (12.5 %). However, CaCl, primed SKN seed reached a relatively similar germination percentage to that of wood vinegar primed seed (56 ± 2.3 % and 61 ± 2.6 %, respectively). The results of this germination test showed that all the priming agents enhanced the germination percentages of the seed of the three tested rice varieties. Wood vinegar gave the greatest improvement in seed germination, although the highest germination percentage recorded in Leum Pua seeds, was only 71.5 %.

In the pot experiment (Table 1), late rainfall at 0, 7, 14 and 21 days after sowing was imitated in treatments T1, T2, T3 and T4 to examine the effect of priming on drought tolerance of the three tested upland rice varieties. The field emergence percentage of seed of each variety tended to decrease when water application was delayed, but some of priming methods improved the percentage emergence.

Leum Pua seeds primed with wood vinegar showed highest field emergence in treatment T1 (45 % on day 5 and 78 % on day 14). Increases of field emergence percentages of wood vinegar primed Leum Pua seeds were detected in T2, at final count, and gave best field emergence on the first count of T3 (25 %) and T4 (5 %). Distilled water and CaCl, primed Leum Pua seeds also improved field emergence in the T1 treatment, but this was not significantly different from that of the dry seeds. In the T2 treatment none of priming methods improved field emergence at the first count, but they did in the final count. Primed Leum Pua seeds with CaCl, also improved their field emergence percentages at the first count of the T3 treatment (13 %), although at a lower level than wood vinegar (25 %). Field emergence percentages were dramatically reduced in the T4 treatment.



Fig. 1. The levels of: (A) first germination (day 5) and (B) final germination (day 14) in primed and unprimed seeds of three rice varieties. Data represents the means and standard deviations of three replicates. Different characters indicate statistically significant differences between treatments at p < 0.05 by LSD.

Table 1. Effect of seed priming on field emergence of upland rice when exposed to drought at the seeding stage.

Priming Treatments -	Percentage of Field emergence at 5 and 14 days after seeding when first watering was applied at different DAS									
	T1) at seeding		T2) at 7 DAS		T3) at 14 DAS		T4) at 21 DAS			
	5	14	5	14	5	14	5	14		
	Leum Pua									
Dry seed	8 b	30 b	9	30 c	7 b	30	0 c	29		
Distilled water	25 ab	47 ab	16	50 bc	15 b	49	3 ab	32		
CaCl ₂	17 b	57 ab	13	55 ab	13 ab	52	1 bc	47		
Wood Vinegar	45 a	78 a	31	81 a	25 a	64	5 a	44		
F-Test	*	*	ns	**	*	ns	*	ns		
C.V. (%)	32	21	30	16	20	20	39	21		
	<u>ULR038</u>									
Dry seed	5 b	31 ab	7 c	52 c	1 b	34 b	0 b	23 b		
Distilled water	25 a	63 a	22 b	61 b	24 a	55 ab	2 ab	38 a		
CaCl ₂	29 a	64 a	28 ab	69 a	28 a	61 a	2 ab	26 b		
Wood Vinegar	37 a	74 a	35 a	71 a	44 a	65 a	3 a	49 a		
F-Test	*	**	**	**	**	*	*	**		
C.V. (%)	30	13	11	3	31	15	37	9		
	SKN									
Dry seed	22	71	31	56 b	20	46 b	5	22		
Distilled water	27	75	33	76 a	27	64 a	10	27		
CaCl ₂	27	77	23	75 a	25	65 a	6	25		
Wood Vinegar	27	72	34	72 a	25	70 a	8	29		
F-Test	ns	ns	Ns	*	ns	**	ns	ns		
C.V. (%)	39	8	13	6	36	6	16	18		

Remarks: Dry seed = unprimed seed (control), Distilled water = primed seed with distilled water (hydropriming), $CaCl_2$ = primed seed with $CaCl_2$ (osmopriming), Wood Vinegar = primed seed with wood vinegar (wood vinegar priming). Data in percentages was transformed by arcsine square root before analysis and backed transform data are presented. Means in the same column with the same letters are not significantly different at p < 0.05 by LSD. NS = non-significant, * = significant at p < 0.05, ** = Significant at p < 0.01. DAS = Days after seeding. T1, T2, T3 and T4 are treatments that first watering was applied at 0, 7, 14, 21 days after seeding

Primed ULR038 seeds improved their first field emergence count in the T1 treatment from 5, 25, 29 and 37 % when primed with distilled water, CaCl₂ and wood vinegar, respectively. However, the final count for T1 treatment did not show a significant difference among the primed and dry seeds. Field emergence percentages of ULR038 dry and primed seeds in the T2 treatment were almost similar to those for T1 treatment, although water application was delayed for 7 days. Priming with CaCl₂ and wood vinegar gave better field emergence of ULR038 than for dry seed. Decreases in field emergence percentages of the dry seeds were detected in the T3 and T4 treatments; however, all primed seeds improved their field emergence percentages.

None of the priming agents improved SKN field emergence percentage in the T1 and T4 treatments, for which the final count of field emergence percentages showed a significant decrease from 71-77 % to 22 – 29 %. However,

all primed SKN seeds showed an improvement in their final count of field emergence percentages, the improvement was relatively similar from 56 % to 72-76 % in T2 treatment, and from 46 % to 64–70 % in T3 treatment.

The results from the pot experiment suggested that seed priming could enhance drought tolerance of the tested upland rice seeds. Wood vinegar improved field emergence of Leum Pua by almost 50 % and 20 % in ULR038 when watering was delayed for 7 days. When watering was delayed for 14 days, all three priming agents increased the field emergence percentage by similar amounts. These results supported previous findings that seed priming improved rice seed germination and field emergence. Germination test of three varieties of aerobic rice seeds have shown that PEG- and hydroprimed seed could maintained germination percentages during PEG- induced drought situation (Abdallah, Musa, Mustafa, Sjahril, & Riadi, 2016).

Osmopriming with CaCl₂ and hydropriming have increased mean emergence time (MET), seed establishment indicator, of super basmati rice seeds, in addition to a better yield and quality of the direct seeded rice (Rehman, Basra, Farooq, Ahmed, & Afzal, 2011). Moreover, 30 mM proline- primed rice seeds have exhibited better morphological index, such as root fresh weight, dry weight of each part of tillering rice plants than that of unprimed seeds (Hualong et al., 2014). In this study, wood vinegar showed a significant impact in enhancing germination of the seed of the three upland rice varieties.

Effect of Priming Methods on Controlling Oxidative Stress and Sugar Metabolism

A priming with distilled water, CaCl₂ and wood vinegar improved the germination and seed establishment of all three rice varieties and was relative to the unprimed dry seed. Oxidative stress is one of the mechanisms that generally occur during seed stress. However, several priming methods have shown the ability to lower the stress level. For example, chillingoxidative stress in spinach seeds was repressed by PEG-osmopriming by inducing either CAT and SOD, or APX (Chen & Arora, 2013). KCI- and hydroprimed rice seeds decreased oxidative stress during flooding by elevating SOD and CAT activities in association with less lipid peroxidation (Ella, Dionisio-Sese, & Ismail, 2011). This also includes β-amino butyric acid (BABA) priming of seed of Vignia radiata L. that BABA alleviated seedling growth under drought and salt stresses in association with accumulation of proline, reduction of MDA and increased activities of POX, and SOD (Jisha & Puthur, 2016). In this study a number of oxidative stress indicators were examined. Malondialdehyde (MDA) content that indicates lipid peroxidation of the biological membrane is primarily determined as shown in Fig. 2. Initial MDA content in Leum Pua, ULR038 and SKN dry seeds were at 5.1, 3.0 and 6.0 nmol MDA per g FW, respectively. After priming with distilled water, CaCl₂ and wood vinegar, MDA contents were reduced in all primed seeds, with wood vinegar markedly reducing the MDA content in Leum Pua (1.9 nmol MDA per g FW, 62.7 %), ULR038 (0.8 nmol MDA per g FW, 73.3 %) and SKN (1.6 nmol MDA per g FW, 73.3 %), while distilled water and CaCl, gave relatively similar degrees of MDA content reduction (31.4 - 63.3 %).



Fig. 2. Levels of malondialdehyde in primed and unprimed seeds of three rice varieties. Data represents the means and standard deviations of three replicates. Different characters indicate statistically significant differences between treatments at p < 0.05 by LSD.



Atchima Dissatian et al.: Wood Vinegar for Enhancing Seed Germination of Upland Rice Varieties

Fig. 2. Activities of antioxidant enzymes in prime and unprimed seeds of three rice varieties. (A) CAT, (B) GPX, (C) APX, (D) SOD. Data represent the means and standard deviations of three replicates. Different characters indicate statistically significant differences between treatments at p < 0.05 by LSD.

The results suggested that seed priming improved the germination of rice seed by modulating MDA accumulation, for which wood vinegar was a better primer than priming with distilled water and/ or CaCl_a. Since MDA is a product of lipid peroxidation that is produced by oxidative stress occurred during seed storage or/and priming, the determination of antioxidant enzymes may suggest a role for priming agents in controlling oxidative stress. Three H₂O₂ destroying enzymes; catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) were assayed, as well as the superoxide (O,) eliminating enzyme, superoxide dismutase (SOD), as shown in Fig. 3 A-D, respectively. CAT was not significantly changed by any of the priming agents (Fig. 3A), while CaCl, and wood vinegar had a marked effect on GPX of ULR038 (Fig. 3B). Highest GPX levels were detected in wood vinegar primed Leum Pua and SKN, while distilled water and CaCl, did not increase GPX in Leum Pua and SKN, respectively. APX did not change in response to priming in Leum Pua, while primed ULR038 seeds had a slight increase APX, which was significantly different from dry seeds. Primed

SKN seeds had a significant increase in APX when compared to dry seeds, while wood vinegar increased APX to a higher level than distilled water and CaCl (Fig. 3C). SOD was also significantly increased in wood vinegar primed Leum Pua and SKN, but not in ULR038. Distilled water and CaCl, priming did not change SOD in all tested seeds (Fig. 3D). The information collated on MDA content and antioxidant enzyme activities suggested that a marked reduction of MDA could be associated with the GPX and APX, which was stimulated by wood vinegar. This could be indicated by a significant reduction of MDA which occurred after priming, with the lowest MDA content being detected in wood vinegar primed seeds, in comparison with the dry seeds. Moreover, the phenol contained in diluted eucalyptus wood vinegar used in this study might be involved in antioxidative role of the primed seeds. However, it should be noted that the dry and primed seeds can possibly have different antioxidant process mechanisms. A previous study on KNO, primed tomato showed the induction of CAT (Lara, Lira, Rodrigues, Rakocevic, & Alvarenga, 2014), while GPX was induced in the rice seeds in this study.



Fig. 2. Levels of malondialdehyde in primed and unprimed seeds of three rice varieties. Data represents the means and standard deviations of three replicates. Different characters indicate statistically significant differences between treatments at p < 0.05 by LSD.

Sugar metabolism was also determined by assaying soluble sugar (Fig. 4A) and amylase activity, and starch hydrolyzing enzymes (Fig. 4B). Soluble sugar (glucose and sucrose) are prerequisites for seed germination. However, a relative decrease in sugar content in distilled water and wood vinegar primed Leum Pua seeds were detected after priming. None of the dry and primed seeds of ULR038 and SKN showed any changes in soluble sugar content (Fig. 4B). Amylase activity of Leum Pua seed was also not altered by the priming agent. Increases in amylase activity were detected in wood vinegar primed ULR038 and SKN seeds (Fig. 4B). This suggests that soluble sugar was maintained for some reasons, while increases of amylase activity were likely to be associated with the production of energy resources for seed germination. The findings in this study are different from those reported for CaCl₂-, salicylic acid-, and ascorbic acid-primed French marigold seed (Mukhtar et al., 2013), which responded to a significant increase in α-amylase activity.

CONCLUSION

In this study, 300-fold diluted wood vinegar showed its significance as a potential priming agent for seed of the three upland rice varieties, Leum Pua, ULR038 and SKN, with the primed seeds being protected from oxidative stress while being supplied with more glucose during germination. Distilled water and CaCl, also showed their potential as priming agents, but to a lesser degree. All three priming agents improved percentage germination, with reduced MDA at 31.4 - 73.3 %, which wood vinegar gave markedly MDA reduction at 62.7 for Leum Pua seed and 73.3 % for ULR038 and SKN seeds. The reductions of MDA were associated with the increases in some antioxidant enzyme activities of GPX, APX and SOD, but not CAT. Amylase was unlikely to be involved in sugar metabolism during priming, while free sugar was maintained. Based on the results of the study, it is suggested that wood vinegar has the potential for use as a priming agent for the germination of upland rice seed by promoting rapid and more uniform seed germination during periods of erratic rainfall, especially at the seeding stage.

ACKNOWLEDGEMENT

This research was supported by the Higher Education Research Promotion and National Research University Project of Thailand, and the Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of

Khon Kaen University. Acknowledgements are made to the Thailand Research Fund (TRF) (Project code: IRG5780003), Khon Kaen University (KKU) and the Faculty of Agriculture KKU, for providing financial support for manuscript preparation activities. The Department of Biochemistry of the Faculty of Science and Salt-tolerant Rice Research Group of Khon Kaen University, are also sincerely acknowledged for analyzing antioxidant enzymes. Thanks also to Dr. John Schiller, Honorary Senior Fellow, Faculty of Science, School of Agriculture and Food Sciences, University of Queensland, Australia, for his valuable comments on the manuscript.

REFERENCES

- Abdallah, E. H., Musa, Y., Mustafa, M., Sjahril, R., & Riadi, M. (2016). 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.). *AGRIVITA Journal of Agricultural Science*, *38*(3), 222–230. http://doi.org/10.17503/agrivita. v38i3.886
- Aebi, H. (1984). [13] Catalase in vitro. Methods in *Enzymology*, *105*(C), 121–126. http://doi. org/10.1016/S0076-6879(84)05016-3
- Ashraf, M., & Foolad, M. R. (2005). Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in Agronomy, 88, 223–271. http://doi.org/10.1016/ S0065-2113(05)88006-X
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276–287. http://doi. org/10.1016/0003-2697(71)90370-8
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Analytical Biochemistry*, 72, 248–254. http://doi. org/10.1016/0003-2697(76)90527-3
- Chance, B., & Maehly, A. C. (1955). [136] Assay of catalases and peroxidases. *Methods in Enzymology*, 2(C), 764–775. http://doi.org/10.1016/S0076-6879(55)02300-8
- Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. *Environmental and Experimental Botany*, 94, 33–45. http://doi. org/10.1016/j.envexpbot.2012.03.005

- Chen, K., Fessehaie, A., & Arora, R. (2012). Dehydrin metabolism is altered during seed osmopriming and subsequent germination under chilling and desiccation in *Spinacia oleracea* L. cv. Bloomsdale: Possible role in stress tolerance. *Plant Science*, 183, 27–36. http://doi. org/10.1016/j.plantsci.2011.11.002
- Demiral, T., & Türkan, I. (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany*, *53*(3), 247–257. http://doi. org/10.1016/j.envexpbot.2004.03.017
- Doorenbos, J., & Pruitt, W. O. (1977). Guidelines for predicting crop water requirements. FAO *Irrigation and Drainage Paper* (Vol. 24). Retrieved from http://www.fao.org/3/a-f2430e.pdf
- Ella, E. S., Dionisio-Sese, M. L., & Ismail, A. M. (2011). Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. *AoB PLANTS*, *11*(1), plr007. http://doi.org/10.1093/aobpla/plr007
- Hua-long, L., Han-jing, S., Jing-guo, W., Yang, L., Detang, Z., & Hong-wei, Z. (2014). Effect of seed soaking with exogenous proline on seed germination of rice under salt stress. *Journal* of Northeast Agricultural University (English Edition), 21(3), 1–6. http://doi.org/10.1016/ S1006-8104(14)60062-3
- ISTA. (2004). International rules for seed testing. Zurich, CH: International Seed Testing Association.
- Jisha, K. C., & Puthur, J. T. (2016). Seed priming with beta-amino butyric acid improves abiotic stress tolerance in rice seedlings. *Rice Science*, 23(5), 242–254. http://doi.org/10.1016/j. rsci.2016.08.002
- Kulkarni, M. G., Sparg, S. G., Light, M. E., & Van Staden, J. (2006). Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *Journal of Agronomy and Crop Science*, 192(5), 395–398. http://doi.org/10.1111/j.1439-037X.2006.00213.x
- Lara, T. S., Lira, J. M. S., Rodrigues, A. C., Rakocevic, M., & Alvarenga, A. A. (2014). Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. *Journal of Agricultural Science*, 6(2), 72–80. http://doi.org/10.5539/jas.v6n2p72

- McDonald, M. B. (2000). 9. Seed priming. In M. Black & J. D. Bewley (Eds.), Seed technology and its biological basis (pp. 287-325). Sheffield, UK: Sheffield Academic Press & Boca Raton, USA: CRC Press LLC.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428. http://doi. org/10.1021/ac60147a030
- Mukhtar, K., Afzal, I., Qasim, M., Maqsood, S., Basra, A., & Shahid, M. (2013). Does priming promote germination and early stand establishment of French marigold (*Tagetes patula* L.) seeds by inducing physiological and biochemical changes? Acta *Scientiarum Polonorum Hortorum Cultus*, *12*(3), 13–21. Retrieved from http://wydawnictwo.up.lublin.pl/acta/hortorum_ cultus/2013/ streszczenia2013_3/02 Mukhtar Qasim Hort 12_3_2013.pdf
- Mungkunkamchao, T., Kesmala, T., Pimratch, S., Toomsan, B., & Jothityangkoon, D. (2013). Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (*Solanum lycopersicum* L.). *Scientia Horticulturae*, *154*, 66– 72. http://doi.org/10.1016/j.scienta.2013.02.020
- Nakai, T., Kartal, S. N., Hata, T., & Imamura, Y. (2007). Chemical characterization of pyrolysis liquids of wood-based composites and evaluation of their bio-efficiency. *Building and Environment*, 42(3), 1236–1241. http://doi.org/10.1016/j. buildenv.2005.11.022
- Nakano, Y., & Asada, K. (1987). Purification of ascorbate peroxidase in spinach chloroplasts; Its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant and Cell Physiology*, 28(1), 131–140. http:// doi.org/10.1093/oxfordjournals.pcp.a077268

- Rehman, H., Basra, S. M. A., Farooq, M., Ahmed, N., & Afzal, I. (2011). Seed priming with CaCl2 improves the stand establishment, yield and quality attributes in direct seeded rice (*Oryza sativa*). *International Journal of Agriculture and Biology*, *13*(5), 786–790. Retrieved from https://www. researchgate.net/publication/245536192_Seed_ Priming_with_CaCl_2_Improves_the_Stand_ Establishment_Yield_and_Quality_Attributes_ in_Direct_Seeded_Rice_Oryza_sativa
- Srivong, T., Zhu, Y. J., Pongdontri, P., Pliansinchai, U., Sakuanrungsirikul, S., Borthakur, D., ... Kosittrakun, M. (2015). Optimization of callus induction and plant regeneration in sugarcane (Saccharum spp.) for a study of sucrose accumulation in relation to soluble acid invertase expression. Chiang Mai Journal of Science, 42(4), 797-805. Retrieved from https://www.researchgate. net/publication/284901333_Optimization_of_ Callus_Induction_and_Plant_Regeneration_ in Sugarcane Saccharum_spp_for_a_Study_ of_Sucrose_Accumulation_in_Relation_to_ Soluble Acid Invertase Expression
- Varier, A., Vari, A. K., & Dadlani, M. (2010). The subcellular basis of seed priming. *Current Science*, *99*(4), 450–456. http://doi.org/10.2307/24109568
- Zhai, M., Shi, G., Wang, Y., Mao, G., Wang, D., & Wang, Z. (2015). Chemical compositions and biological activities of pyroligneous acids from walnut shell. *BioResources*, 10(1), 1715–1729. Retrieved from http://ojs.cnr.ncsu.edu/index.php/ BioRes/article/view/BioRes_10_1_1715_Zhai_ Chemical_Compositions_Walnut_Shell
- Zhou, Z., Robards, K., Helliwell, S., & Blanchard, C. (2002). Ageing of stored rice: Changes in chemical and physical attributes. *Journal of Cereal Science*, *35*(1), 65–78. http://doi.org/10.1006/ jcrs.2001.0418