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The Seed Water Sorption Isotherm and Antioxidant-Defensive Mechanisms of *Hordeum vulgare* L. Primed Seeds

Pinpinatt Junhaeng[1], Pitipong Thobunluepop[1*], Wanchai Chanprasert[1], Damrongvudhi Onwimol[1], Sutkhet Nakasathien[1], Elke Pawelzik[2]

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
[2]Division of Quality of Plant Products, Department of Crop Sciences, Faculty of Agricultural Sciences, Georg-August-Universität Göttingen, Göttingen, Germany
Email: *fagrppt@ku.ac.th

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Abstract

The key successful for seed priming technique was the management of seed imbibition process. This study was aimed to indicate the seed moisture sorption characteristics of *Hordeum vulgare* L. cv. Samerng 2 in relation to the seed priming technique and its effect on the seed qualities. The experiment was designed by Completely Randomized Design (CRD) with four replications. Seed hydro-priming technique was done under 25 degree Celsius, and 40% - 60% RH. Barley seed moisture content was determined every hour after imbibition for 24 hours. Then, primed barley seed qualities were evaluated at 6, 8, 10, 12, 14, and 16 hours of hydrotime. The results revealed that barley seeds were imbibed rapidly from 10.55% to 37.69% seed moisture content during 0 - 9 hours of imbibition. The constantly seed moisture content was continued until 17 hours of imbibition. Radicle was protruded with 42.74% seed moisture content. Seed moisture sorption characteristic equation was $Y = 0.0055x^3 - 0.267x^2 + 4.5931x + 13.348$. Coefficient of determination (R^2) was 0.916. Primed seed germination was not affected by hydrotime. Primed seeds were improved in speed of germination and seedling growth rate. The best primed barley seed vigorous was noted at 14 - 16 hours of hydrotime. In addition, catalase activity (CAT) and superoxide dismutase activity (SOD) were not significantly different for non-primed and 14 - 16 hours primed seeds. However, lipoxygenase activity (LOX) was increased and malondialdehyde (MDA) content was decreased for 14 - 16 hours primed seeds. Subsequently, 14 - 16 hours primed barley seeds can maintain high seed germination and vigor for 8 - 12 months under 5 degree Celsius. Thus, it could be concluded that hydro-priming for 14 - 16 hours enhances barley seed quality

by reducing germination time, induced defenses mechanism, and could be stored for 8 - 12 months.

Keywords

Seed Moisture Sorption Characteristic, Barley Seed, Hydro-Priming, Seed Germination, Speed of Germination, Storability, Defenses Mechanism

1. Introduction

Barley (*Hordeum vulgare* L.) seeds were used as raw material of malt production for food and beverage industry. The fast and uniformity of barley seed germination was expected for malt production standard. The requirement of malt industry was high seed qualities which could be germinated well with high seedling vigor. Those seeds were able to ensure uniform of germination, which was able to increase malt productivity. However, low seed germination, low seed vigor especially microorganisms contamination was commonly found in Thai's grown barley seed [1] [2] [3]. Those reports were recognized that barley seed germination was found lower than 75% and contaminated with more than 70% of pathogen. This problem might cause 30% - 40% of losing in malting production efficiency.

Many techniques were used to improve barley seed germination and vigor. Seed priming was one of the most effective techniques that would improve germination and crop establishment of several crops such as barley under phosphorus and zinc deficiency conditions [4], wheat [5] [6], safflower under salt stress [7], and *Aeluropus Macrostachys* [8]. The technique was controlled seed imbibition process to allow metabolic activation occurring and prevented completion of germination. The seeds would be brought in the stoppable but irreversible germination [9]. Bewley *et al.* [10] have published that several factors govern the movement of water into the seed, but particularly important was the water relations between seed and ambient environment.

A mature dry seed imbibition characteristic was triphasic. A rapid initial uptake of water was called "Imbibition Phase" or Phase I, then, followed by a plateau phase which was called "Activation Phase" or Phase II. Further, the increasing of water uptake occurred after germination completed as the embryonic axis elongated. This phase was called "Growth Phase" or Phase III [10]. Water is well known as the most important physiological factor for seed germination. The measurement of water status or water available for germinating seed was necessary to describe the status of water in seeds. Water potential (Ψ) is a measure of the energy status of water per unit volume relative to pure water as reference. It measures the availability of water to participate in chemical and physiological mechanisms. It is generally expressed as pressure units, megaPascal (MPa), by −1 MPa = −10 bar [10]. Pure water has the highest potential, which assigned a zero value. Dry seeds usually had water potentials (Ψ) between −350 MPa and

−50 MPa. Water molecules flow through seeds pores from high water potential sites to low water potential sites to reach equilibrium water potential within seed environments over time [10]. Therefore, the huge different between Ψ in dry seed tissue and ambient Ψ resulted in rapid water influx from outside to dry sites during imbibition (Phase I) [11]. The total water potential is the sum of three components as showed in Equation (1).

$$\Psi_{cell} = \Psi_s + \Psi_p + \Psi_m \quad (1)$$

where, Ψ_s = solute or osmotic potential

Ψ_p = turgor potential

Ψ_m = matric potential

The water potential of seeds in seed priming technique was controlled by controlling either matric potential or osmotic potential of seed environment. The events suddenly dry seed was brought into contact with water, there was a very large of water potential for the rapid movement of water into the seed. Water was entered through the micropyle [12]. Water would diffuse into seed rapidly in response to the low matric potential of the dry seed. Then, after seeds start to take up water, there was often leakage of solutes such as sugar, organic acids, ions, amino acids, and proteins into the surrounding medium. The phospholipid components in cell wall membrane were achieved from the gel phase during maturation drying to the normal hydrated liquid-crystalline state [13]. Then, in a short time of rehydration, the membranes return to their more stable configuration at which time solute leakage was curtailed. Then, seed was entered to plateau phase (Phase II) [12]. The breakdown of cell walls and converting food reserves to mobile substances (Modification process), was occurred continually until the radicle protruded (Phase III).

Furthermore, primed seed qualities were needed to evaluate. Several researchers reported on primed seed quality. Bradford and Bewley [14] reported that primed seeds were increased in speed of germination, improved germination uniformity, improved seedling establishment, reduced time to fifty percentage germination, and pronoun with low germination seed lot. However, primed seeds were reduced in seed storability and needed low storage temperature. Zheng *et al.* [15] reported that canola primed seed had improved germination and reduced germination time. Sharma *et al.* [16] also reported that priming okra cv. *Hisar Unnat* seeds by 12 hours of hydrotime had affected on increasing germination rate, seedling vigor, mean emergence time, and yield. Kazem *et al.* [17] reported that primed seeds of pinto bean (*Phaseolus vulgaris* L.) cv. Talash, COS_{16}, and Khomain, by 7 and 14 hours of hydrotime resulted in lower mean emergence time and higher seedling emergence percentage when compared with non-primed seeds. Naceur [18] studied on the effects of hydro-priming on wheat (*Triticum durum*) and barley (*Hordeum vulgare*) seeds. The results found that the seeds adsorbed water rapidly till 9.5 hours of hydrotime at 25˚C and 35˚C in the dark. The results of primed seed by 5.5 - 6.5 hours (late of Phase I) of hydrotime found that speed of radicle emergence, coleoptile and side roots emergence

and seedling fresh weight were improved more than non-primed seeds, especially under 25˚C. Additionally, Ibrahim *et al.* [19] reported on upland rice (*Oryza sativa* L. cv. NERICA 2) seeds that had significantly affect ($p < 0.05$) of hydro-priming on plant growth parameters. The successful was hydro-priming technique with 12 hours of hydrotime. The upland rice was greater in seedling establishment and growth performance in Sokoto, under Nigeria climatic conditions.

However, it has been no report in the effects of priming technique on barley seed (*Hordeum vulgare* L.) qualities which applied for malt industry, including barley seed moisture sorption characteristics as well as seed qualities. Thus, this study was aimed to indicate the seed moisture sorption characteristics of *Hordeum vulgare* L. cv. Samerng 2 in relation to the seed priming technique and its effect on the seed qualities.

2. Materials and Methods

Seed materials:

Barley seeds (*Hordeum vulgare* L. cv. Samerng 2) were cultivated on November 2012 to February 2013 in the area of Samerng Rice Research Center, Chiangmai, Thailand. The seeds were 10.55% of initial seed moisture content and 91% of germination. Seeds were processed and graded before used in this experiment.

The experimental design:

The experiment was conducted in Complete Randomized design (CRD) with four replications. The experimental treatment was imbibition time which was conducted in 6 levels; 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, and 16 hours. Barley seeds were primed at room temperature (25˚C). An aeration pump was added during the experiment to provide oxygen. Then, the barley seeds were dried in a fan-forced oven at 32˚C for 48 - 72 hours to obtain required seed moisture content of 9% - 10%. Finally, primed seed was sampling for seed qualities test.

Barley seed moisture sorption characteristics:

Barley seeds were primed in distilled water under room temperature (25˚C). An aeration pump was used throughout 24 hours of the experiment. Primed seed was sampling to measure seed moisture content every hour by hot air oven method [20].

The primed seed moisture content values were plotted versus imbibition time. The curve and equation was fitted by best-of-fit method [21]. The coefficient of determination (R^2) was represented the best fit by high value. The type of curve that could be fitted was often suggested by theoretical considerations [10] [21].

Seed quality evaluation:

Seed moisture content, (*smc*)

Seed moisture content was measured by the hot air-oven method [20]. 5 g of seed were dried by a hot air oven at 130˚C for 2 hours. Then, the samples were

transferred to desiccator for cooling down. The samples were then weighed and finally the percentage of seed moisture content was calculated and expressed based on a wet basis (wb) as following Equation (2):

$$\%\text{seed moisture content} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100 \quad (2)$$

Seed germination percentage (*Germ*)

The percentage of seed germination was determined by between papers (BP) method, according to the standard germination test [20]. 100 seeds were placed between papers for four replications. Then, seeds were incubated in a germination chamber at 20˚C for 3 days as final count according to malt quality criteria. The results were calculated as the average of four replicates of 100 seeds, and expressed as a percentage of normal seedlings.

Seed germination index (*GI*)

The seed germination percentage was done following ISTA [20]. The number of germinated seeds was counted every day for three days and the germination index was calculated following Equation (3);

$$\text{Germination index}\left(\text{GI}\right) = \frac{n1}{1} + \frac{n2}{2} + \frac{n3}{3} \quad (3)$$

where $n1$, $n2$, $n3$: were the number of seed germinated on day 1 to day 3 and 1, 2, 3: were the number of days.

Seedling growth rate (*SGR*)

Standard seed germination test was incubated in dark condition for 7 days. Then, endosperm was removed from germinated seeds. Finally, shoot and root were taken for dry weight in hot air oven for 80 degree Celsius for 24 hours. Seedling growth rate was calculated according to ISTA [20] as following Equation (4);

$$\text{Seedling growth rate}\left(\text{SGR}\right) = \frac{\text{shoot and root dry weight}}{\text{number of germinated seed}} \quad (4)$$

Mean emergence time (*MET*)

Mean emergence time was done by following Demir *et al.* [22]. Standard germination was done by following ISTA [20]. Then, number of germinated seeds were counted every day and the mean emergence time (MET) was calculated as following Equation (5);

$$\text{MET} = \frac{\sum n \cdot D}{\sum n} \quad (5)$$

where n = number of seeds newly germinated at time D;

D = days from the beginning of the germination test;

Σn = final germination.

Time to fifty percentage germination (T_{50})

400 seeds of four replications were sampling and placed between papers. The number of germinated seeds were counted before fifty percentage germination

and every day for 7 days after sowing. T_{50} was calculated according to Coolbear *et al.* [23] as Equation (6):

$$T_{50} = t_i + \left[\left[\left\{\left(N+1\right)/2\right\} - n_i\right] \Big/ \left(n_j - n_i\right)\right] \times \left(t_j - t_i\right) \quad (6)$$

where, t_i = time before fifty percentage germination

n_i = number of germinated seeds at t_i

N = number of total germinated seed

t_j = time next to t_i

n_j = number of germinated seeds at t_j

The electrical conductivity test (*Cond*)

The electrical conductivity test was used to determine seed membrane integrity by measuring the seed leakage. This test followed procedures described by ISTA [20]. First, 100 seeds were weighted and soaked into de-ionized water. Then, they were placed at 25˚C for 24 hours. Finally, the solution was determined for electrical conductivity by the conductivity meter (Sartorius, model PP-20). The electrical conductivity unit was μS cm^{-1} g^{-1}).

Seed biochemical evaluation:

The best selections of imbibition time were evaluated for seed biochemical properties including protein content, carbohydrate content, lipid content, catalase activity, *α*-amylase activity, soluble sugars content, superoxide dismutase activity (SOD), total free fatty acid (TFA), malonaldehyde (MDA), and lipoxygenase activity (LOX).

Determination of protein content

Elemental analyses of total nitrogen and carbon (and sulphur) were performed to provide carbonate and organic carbon. The total nitrogen, carbon, and sulphur were determined using a CHNS/O Analyser, model LECO CHNS-932 and VTF-900. Freeze-dried barley seeds were ground (UDY, Cyclone Sample Mill No.2). The samples were weighed 125 mg and mixed with an oxidizer (vanadium pentoxide [V_2O_5]) in a tin capsule, which was then combusted in a reactor at 1000˚C. The sample and container melt, and the tin promote a violent reaction in a temporarily enriched oxygen atmosphere. The combustion products CO_2, SO_2, and NO_2 were carried by a constant flow of carrier gas (helium) that passes through a glass column packed with an oxidation catalyst of tungsten trioxide (WO_3) and a copper reducer, both kept at 1000˚C. At this temperature, the nitrogen oxide was reduced to N_2. The N_2, CO_2, and SO_2 were then transported by the helium to, and separated by, a 2-m-long packed column and quantified with a TCD (set at 290˚C).

The chromatographic responses were calibrated against pre-analysed standards, and the CHNS elemental contents were reported in weight percent. 628 series software was used for running the equipment, storing the data, and for postrun analysis.

The protein content of the sample was obtained from the multiplying the nitrogen determined by 6.25 as Equation (7):

$$\text{Total protein content}\left(\text{g } 100\text{ g}^{-1}\text{ DM}\right) = \text{Total nitrogen content} \times 6.25 \quad (7)$$

Determination of carbohydrate content

The carbohydrate content of the samples was measured and modified according to Åman *et al.* [24]. 30 mg of barley starch were dissolved in 15 mL of 80% ethanol in order to rupture granules. The screw cap tubes were placed in a boiling water bath in order to dissolve and extract low molecular weight carbohydrates. Then, cooling to room temperature. Then, the samples were centrifuged at 830 rpm per 10 min and the pellet formed was washed twice with 15 mL of 80% ethanol. Finally, the test tubes were emptied carefully and inverted for 5 minutes. Samples were incubated with thermostable α-amylase (50 μL in 25 mL acetate buffer) in a boiling water bath for 30 minutes. Tubes were shaken three times during incubation and observed regarding adhering material, lumps and tight capping. Then, samples were cooled to a temperature of 40˚C before a total degradation to glucose units was assured by treatment with amyloglucosidase (100 μL in 0.1 M acetate buffer, 140 U/mL) in a shaking water bath at 60˚C overnight. Samples were cooled to room temperature and centrifuged at 830 rpm for 10 minutes. The supernatant was diluted in water to 1:25 and 1.00 mL aliquots were mixed with 3.00 mL GOPOD and placed in a 50˚C water bath for 20 min where after absorbance was measured at 510 nm using spectrophotometer. The glucose concentration was determined from a standard curve and the carbohydrate content was calculated according to the equation following;

$$\%\text{carbohydrate}\left(\text{DM}\right) = \left(\left[\text{glucose}\right]\left(\text{mg/mL}\right) \times 25.15 \times 0.9 \times 25\right) \Big/ \text{sample weight}\left(\text{DM, mg}\right)$$

where, 25.15 = total volume of solution in mL

0.9 = the factor for conversion of free glucose to a glucopyranosyl unit of starch

25 = the dilution factor

Determination of total lipid and free fatty acid (FFA) content

According to Lam and Proctor [25], the gravimetric method was used to determine the total lipid content. Lipid content was extracted by vortexing 10 g of barley sample with 4 ml of isopropanol for 5 min. Then, added 5 ml of isopropanol into the sample and vortexes for 5 min. After that the extract was centrifuged at 2500 rpm for 10 min. The weight of extracted lipids was determined after evaporating the solvent on an electric hot plate at 40˚C.

According to Walde and Nastruzzi [26], FFA of the sample was determined by preparing an assay solution, which contained 0.375 ml of solution A (0.1 M tris/HCl; pH 9.0), 0.125 ml of solution B (2 mM phenol red in 0.1 M tris/HCl; pH 9.0), and 0.5 ml of solution C (50 mM Bis (2-ethylhexyl) sodium sulfosuccinate in isooctane). Then, 30 μL of isopropanol extract was mixed with 1 ml of assay solution in a 1-cm wide cuvette and was shaken for one minute before measuring absorbance at 560 nm. FFA of each extract was obtained from a calibration curve. The calibration curve was prepared by dissolving oleic acid in

isopropanol to produce oleic acid solution of 0.001% to 0.02% (w/w).

Determination of catalase activity

According to Aebi [27], purified catalase was incubated with methanol and hydrogen peroxide in 250 mM KH_2PO^{4-}NaOH buffer, pH7.0. By the standard procedure, 50 µl buffer, 50 µl 100% (w/v) methanol, giving a final concentration of 5.9 M, and 10 µl 0.27% (w/v) hydrogen peroxide, giving a final concentration of 4.2 mM, were mixed in small polystyrene test tubes. The enzymatic reaction was initiated by addition of 100 µl of a catalase-containing sample. For evaluation of methodological details, the sample consisted of 0.125 - 2.0 µg/ml purified catalase from barley milled, with an activity of 2500 units/mg, dissolved in 25 mM KH_2PO^{4-}NaOH buffer, pH 7.0. Pure buffer was used as blanks. The reaction mixture was incubated with continuous shaking for exactly 20 min at room temperature (25˚C). The enzymatic reaction was terminated by addition of 50 µl 7.8 M potassium hydroxide solution to each tube. Immediately thereafter the tubes were supplied with 100 µl each of 34.2 mM Purpald in 480 mM hydrochloric acid, and a second incubation with continuous shaking was performed for 10 min at 20˚C. To obtain a colored compound, the product of the reaction between formaldehyde and Purpald was oxidized by adding 50 µl 65.2 mM potassium periodate in 470 mM potassium hydroxide to each tube. Any particulate material in the tubes was pedimented by centrifugation at 9500 g for 10 min, and using glass standard microcuvettes of 2-mm width and 10-mm light path, the absorbance of the clear liquid was measure at 550 nm.

Determination of α-amylase activity

The *α*-amylase activity of an enzyme extract was determined at 30˚C by kinetic measurement of its reaction with a standard limit dextrin substrate made from Lintner starch [28]. Grind the barley seeds sample to pass through a 0.8 mm sieve, and then blend thoroughly.

Preparation of enzyme extract: 5 g of barley flour with 100 ml calcium chloride solution for 1 hr at 30˚C. Mix sample and liquid thoroughly in the mixing flask by shaking 10 times. After mixing, immediately flasks was immersed in a water bath at 30˚C for 15, 30, and 45 min, then takes out each sample from the water bath, and turn upside down exactly 10 times, and reimmerse. 60 min after extraction the sample was taken out immediately without shaking and decant into centrifuge tube. Centifuge the suspension for 10 min at 2000 - 3000 rpm without let the sample to stand in the mixing tube. The Whatman no. 42 filter paper was used.

Adjustment of colorimeter: mix 2 ml calcium chloride solution with 10 ml dilutes iodine solution. Dilute with 40 ml distilled water and attempter to 20˚C. With this mixture adjust the extinction at 20˚C to the value 0 on the colorimeter at 575 nm.

Substrate control: mix 1 part of limit dextrin substrate with 3 parts of 0.2% calcium chloride solution. Mix 2 ml of this solution with 10 ml dilutes iodine solution and dilute with 40 ml distilled water. The extinction reading at 20˚C

should be 0.55 - 0.60.

Determination of *α*-amylase activity: attempter the standard substrate to 30˚C. Transfer 15 ml of the clear enzyme extract into a test tube and place in 30˚C baths. After 5 - 10 min, when the extract has attained a temperature of 30˚C, added 5 ml of the standard substrate, using a rapid-flow pipet, and shake the mixture vigorously. Simultaneously with pipetting of the substrate into the extract, start a stop watch. At intervals of 5 or 10 min take the following actions. (a) Pipet 2 ml volume of this solution into 10 ml iodine solution prediluted with 40 ml distilled water, (b) attempter to 20˚C, (c) read the extinction on the colorimeter.

The method of calculation and formulas used: *α*-amylase activity (*A*) was expressed as a function of *α*-amylase concentration and of the velocity constant for the hydrolytic degradation of limit dextrin. Calculated the activity using equation:

$$A = \frac{500k}{c/f}$$

where, A = *α*-amylase activity

500 = factor to obtain more practicable values

k = velocity constant for hydrolytic degradation of limit dextrin

c = concentration of enzyme extract: normally $c = 5$

f = dilution factor

Determination of sugar content

Sugar content was determined by iodine titration of excess copper of Luff-Schoorl method [29]. Sample solution preparation: 5 g of ground sample was accurately weighed to nearest 1 mg and transferred to a 250 ml-volumetric flask, which contained 200 ml distilled water. Five millilitres of Carrez solution I, (21.795 g of zinc acetate dehydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) and 3 ml of glacial acetic acid were dissolved and made up to 100 ml with distilled water) and 5 ml of Carrez solution II, (10.76 g of potassium hexacyanoferrate II trihydrate ($K_4[Fe(CN)_6]{\cdot}3H_2O$) in water and make up to 100 ml with water), were mixed after each addition and made up to 250 ml with water and mix well.

Luff-Schoorl reagent preparation: citric acid solution (50 g of citric acid dehydrate ($C_6H_8O_72H_2O$) in 50 ml of water) was added to sodium carbonate solution (143.78 g of anhydrous sodium carbonate in 300 ml of warm water and allow to cool) in a one-liter volumetric flask with gentle swirling. Then, copper II sulphate pentahydrate solution (25 g of $CuSO_4{\cdot}5H_2O$ in 100 ml of water) was added and made up to 1000 ml with water. The solution was allowed to stand overnight and the filtered.

Twenty-five milliliters of Luff-Schoorl reagent and 25 ml of sample solution were transferred into 250 conical flasks and extracted at 80˚C in ultrasonic bath (Bandelin, Germany) for 10 min. After that, the sample was immediately cooled for 5 min. Then, 10 ml of 30% (w/v) potassium iodide solution were added, and immediately followed by 25 ml of 3 M sulphuric acid adding. Titration was done

with 0.71 M sodium thiosulphate solution until the solution was almost colorless, then added a few milliliters of starch indicator (5 g of soluble starch; Sigma, St. Louis, MO, USA) slurred in 30 ml of water, boil for 3 min, allowed the mixture to cool and added water to make the 1 L solution). The titration was continued until the blue color disappears.

Finally, sugar content was calculated from the standard table by using the weight of glucose or the weight of invert sugar in percentage corresponding to the difference between the two-titration readings, expressed in milliliters of 0.71 M sodium thiosulphate. Express the results in terms of invert sugar or D-glucose as percentage of the dry matter.

Determination of amylose content

Amylose content was analyzed using the method described by Juliano *et al.* [30]. In brief, barley powder (100 g) was mixed with 1 ml of 95% ethanol and 9 ml of 1 M NaOH in a 100 ml beaker and left overnight. On the subsequent day, distilled water was added to the mixture to make up the final volume to 100 ml. A sample of 5 ml of the mixed solution was measured into a volumetric flask, 1 ml of 1 M acetic acid and 2 ml iodine solution were added into the solution and the volume made up to 100 ml with distilled water. The samples were then stirred and allowed to stand for 20 min before absorbance was measured at 620 nm with a UV-Spectrophotometer. The amylose content of the barley samples were calculated based on the method described by McGrance *et al.* [31] using a standard curve plotted from absorbance of amylose standards. Amylopectin content of the barley samples was calculated by the difference based on Juan *et al.* [32] using the following equation;

$$\text{Amylopectin}(\%) = 100\% - \text{amylose}(\%)$$

Determination of superoxide dismutase activity (*SOD*)

The method of Oberley and Spitz [33], with some modification was used to determine the activity of SOD. One millilitre of reaction mixture for the determination of SOD activity contained 800 µL of reaction mixture (0.1 mM Xanthine, 0.056 mM NBT, 1.0 mM DETAPAC, and 1 U CAT in 0.05 M phosphate buffer (pH 7.8)), 100 µL of enzyme extracted, and 100 µL of 0.1 U/ml xanthine oxidase. SOD was evaluated by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction was initiated by illuminating the reaction mixtures at 26˚C - 28˚C for 30 min and absorbances was read at 590 nm. One unit of SOD was defined as the enzyme activity that inhibited photoreaction of NBT to blue formation by 50%. SOD activity of the extracts was expressed as Δ activity mg^{-1} protein.

Determination of malonaldehyde content (*MDA*)

Barley seeds were stored at −80˚C until used for assays. Before the extraction, samples were first ground into fine powder with liquid nitrogen.

Malonaldehyde (MDA) content of seeds was measured according to Dhindsa *et al.* [34] and Shi *et al.* [35]. 0.1 g of ground seed was mixed with 1.5 ml of 10% trichloroacetic acid (TCA) and then centrifuged at 10,000×g for 15 min. Super-

natant (350 µl) was mixed with 350 µl of 0.6% (w/v) thiobarbituric acid. The mixture was heated at 95˚C for 30 min and then quickly cooled on ice for 5 min. After centrifugation at 10,000 ×g for 10 min at 4˚C. The absorbance of the reaction mixture was measured at 450, 532 and 600 nm. The MDA concentration (µM) was calculated according to the formula below:

$$[\text{MDA}] = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

where A_{532}, A_{600}, and A_{450} represent the absorbance of the mixture at 450, 532, and 600 nm, respectively.

Determination of lipoxygenase activity (*LOX*)

Enzyme extraction: 3.0 g of barley powder were homogenized with 5 ml of extraction buffer (0.05 M sodium borate buffer, pH 9.0) and shaken for 30 s. The mixture was centrifuge at 12,000 rpm at 26˚C - 28˚C for 5 mins. For LOX assay, 5 ml of 96% (v/v) ethanol were added and incubated at 26˚C - 28˚C for 15 s, then filtered. Afterwards, 3 ml of 70% (v/v) ethanol were added to 50 µL of extract solution and absorbance was read at 234 nm using 70% (v/v) ethanol for blank. LOX activity of the extracts was expressed as Δ activity mg^{-1} protein [36].

Seed storability:

The best selection of hydrotime were stored in sealed polyethylene bag under 5˚C for 1 year. The seed qualities were evaluated every 4 months of storage.

Statistical analysis

The analysis of variance and correlation was performed for data analysis and differentiated with the Least Significant Difference (LSD) test at $p < 0.05$ using the software R version 3.2.5 (2016-04-14) copyright (C) 2016 the R Foundation of Statistical computing platform: i386-w64-mingw32/i386 (32 bit).

3. Results and Discussion

1) Seed moisture sorption characteristic

The achievement of the increment primed seed quality needed to clarify the seed moisture sorption characteristic. Since, seed moisture sorption characteristic represented seed imbibition rate and radicle protrusion point. These data determined the hydrotime for seed hydro-priming technique. Seed moisture sorption characteristic was upon seed variety, seed germination and vigor, protein content, temperature, and water potential. Seed moisture sorption characteristic curve of *Hordeum vulgar*e L. cv. Samerng 2 was revealed as **Figure 1**.

10.55% wb. barley seeds were imbibed rapidly due to the large cell water potential gradient between outside water and inside seed. Bewley *et al.* [10] stated that dry seed usually had water potential between −350 MPa and −50 MPa, while distilled water had water potential 0.00 MPa. Moreover, the initial uptake of water in Phase I was a consequence of the matrix forces (Ψ_m) of cell walls and cell matrixes of seed [10]. Water would enter to the seed rapidly in response to the low matric potential of the dry seed. The rapid imbibition rate was continued to 9 hours and the seed moisture content was 37.69% wb. Then, the imbibition rate was plateau in Phase II until 17 hours after imbibed. At this point, the radicle

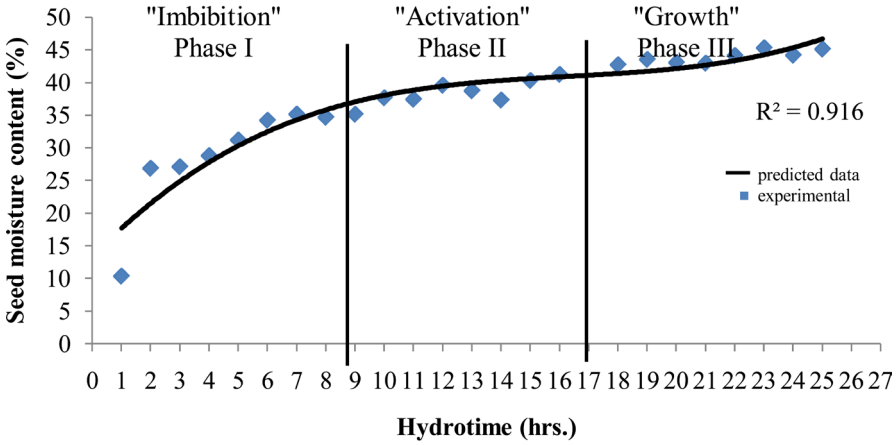

Figure 1. Seed moisture sorption characteristic of *Hordeum vulgare* L. cv. Samerng 2 in distilled water at 25˚C, 40% - 60% RH.

was protruded with the seed moisture content of 42.74% wb. Subsequently, seed moisture content was increased again due to seedling growth. Therefore, it can be indicated that the start and end point of Phase II was 9 and 17 hours after imbibition, respectively (**Figure 1**).

Figure 1 represented the seed moisture sorption characteristic of *Hordeum vulgare* L. cv. Samerng 2 in distilled water at 25˚C and 40% - 60% RH. The characteristics could be classified as triphasic characteristics. There consisted of a rapid initial uptake (Phase I or "Imbibition Phase"), followed by a plateau phase (Phase II or "Activation Phase"). A further increasing in water uptake occurred after germination was completed, as the embryonic axes elongated as Phase III "Growth Phase". In generally, the seed moisture sorption characteristic was sigmoidal based on the seed imbibition theory [10]. The results by mathematical description shown that "Samerng 2" barley seed moisture sorption characteristic was polynomial of order 3 model. The polynomial equation was $Y = 0.0055x^3 - 0.267x^2 + 4.5931x + 13.348$. The best-of-fit reveal the coefficient of determination (R^2) as 0.916. The result showed a good fitness between the experimental and predicted seed moisture content values. Similar results have been reported by many investigators for food materials and grains [37] [38] [39] [40].

2) Hydro-primed barley seed qualities

Barley seed germination percentage was not significantly different (**Table 1**). However, germination index (GI) and seedling growth rate (SGR) were significantly increased. Additionally, mean emergence time (MET) and time to fifty percentage germination (T_{50}) were significantly decreased for 14 - 16 hours hydro-primed seeds. The results indicated that hydro-priming technique affected to seed vigorous especially speed of germination and germination uniformity. These results were supported by previous reports that hydro-priming technique can advance germination or could improve germination rate [16] [41] [42]. Vitor *et al.* [43] reported that seed hydro-priming technique could reduce time to germination, resulted in rapid and uniform seedling emergence, high seed vigor, better and uniform seedling stand establishment. Ahmadi *et al.* [44] indicated

that hydro-priming technique for 12 hours of hydrotime was efficient to improve speed of germination, vigor index and seedling dry weight of wheat (*Triticum aestivum* L.). Ghassemi *et al.* [45] reported that lentil (*Lens culinaris* Medik.) seeds were improved in standard seed germination percentage and seedling emergence in filed after hydro-priming technique for 12 hours of hydrotime. Hamdollah and Kamyar [46] reported that hydro-priming technique was significantly improved germination rate, seed vigor index, and seedling dry weights of cowpea (*Vigna sinensis*). Singh [47] also reported that hydro-priming technique for 24 and 48 hours of hydrotime was significantly on *Aegle marmelos* seed germination percentage, seedling length, seedling vigor, and dry matter production more than non-primed seed. Hydro-priming technique was one of the seed priming technique which easy to operate, cheap and safe [14]. The present study showed the best hydro-primed seed vigorous for 14 - 16 hours hydrotime (**Table 1**), which was the late of Phase II.

Subsequence, non-primed and 14 - 16 hours hydro-primed seeds were evaluated in seed biochemical changing (**Tables 2-4**). The aim was deeply evaluation for how hydro-priming technique improved barley seed vigorous. The results found that cellular leakage was increased as hydrotime increasing. The EC value of primed barley seed at 16 hours was significantly highest than 14 hours and non-primed seed. Protein content and carbohydrate content were not significantly different between non-primed and 16 hours hydro-primed seeds, but 14 hours hydro-primed seeds were significantly decreased from non-primed seeds (**Table 2**). However, lipid content was significantly decreased for 14 - 16 hours hydro-primed seeds. Catalase and α-amylase were not significantly different between non-primed and 14 - 16 hours hydro-primed seeds. Soluble sugars content such as glucose, sucrose, maltose, and amylose, (**Table 3**), were significantly decreased for 14 - 16 hours hydro-primed seeds. Nevertheless, lactose was not significantly different. **Table 4** was showed, superoxide dismutase

Table 1. Mean ± standard deviation values of barley seed qualities changing after hydro-priming technique.

Hydrotime (hrs.)	smc (%)	Germ (%)	GI	SGR (mg/seedling)	MET (days)	T_{50} (hrs.)
non-primed	10.55 ± 0.05b	91 ± 0.19a	28 ± 0.48bc	3.2 ± 0.20c	1.89 ± 0.03ab	32.76 ± 0.82a
6	11.12 ± 0.06a	90 ± 0.96a	23 ± 1.35d	4.3 ± 0.12ab	2.00 ± 0.05a	36.28 ± 1.50a
8	11.05 ± 0.05a	89 ± 0.77a	26 ± 0.77cd	3.9 ± 0.13bc	1.87 ± 0.03b	34.19 ± 1.10a
10	10.79 ± 0.02ab	89 ± 0.48a	28 ± 0.40bc	4.7 ± 0.10a	1.78 ± 0.01bc	32.86 ± 0.84a
12	10.65 ± 0.03b	88 ± 0.67a	30 ± 0.27b	4.3 ± 0.02ab	1.68 ± 0.02c	30.25 ± 0.52a
14	10.54 ± 0.05b	88 ± 0.67a	35 ± 0.95a	4.7 ± 0.09a	1.44 ± 0.05d	18.36 ± 1.95b
16	10.79 ± 0.07ab	87 ± 1.25a	36 ± 1.22a	4.4 ± 0.03ab	1.41 ± 0.06d	14.78 ± 2.64b
Mean	10.79	89	30	4.3	1.73	28.50
$LSD_{0.05}$	0.62*	13.92	7.25*	0.04*	0.21*	12.03*
CV. (%)	2.13	5.79	9.10	13.54	4.67	15.64

Note the different letter(s) indicated the statistically significant difference by LSD at 5% level; CV indicated Coefficient of variation; *indicated significant differences at 5% probability level; smc = seed moisture content.

Table 2. Mean ± standard deviation value of electrical conductivity (EC), protein content, carbohydrate content, lipid content, catalase activity (CAT), and *α*-amylase activity for non-primed and 14 - 16 hours hydro-primed seeds.

Treatment	EC ($\mu S\ cm^{-1}\ g^{-1}$)	Protein (%)	Carbo. (%)	Lipid (%)	CAT (U/mg Protein)	*α*-amylase (U/mg Protein)
non-primed	68.9 ± 1.58b	10.2 ± 0.14a	79.9 ± 0.03a	5.5 ± 0.07a	118.21 ± 1.19a	15.27 ± 0.10b
14 hrs hydro-primed	74.5 ± 0.58ab	10.0 ± 0.21b	79.7 ± 0.02b	5.3 ± 0.02b	111.04 ± 0.99a	15.82 ± 0.07a
16 hrs hydro-primed	78.9 ± 1.95a	10.3 ± 0.12a	79.8 ± 0.02a	5.2 ± 0.03b	113.55 ± 2.08a	15.69 ± 0.11ab
Mean	74.1	10.6	79.8	5.3	114.26	15.59
$LSD_{0.05}$	15.5*	0.4*	0.1*	0.3*	20.19	1.14
CV. (%)	6.23	0.99	0.07	2.01	5.23	2.18

Note the different letter(s) indicated the statistically significant difference by LSD at 5% level; CV indicated Coefficient of variation; *indicated significant differences at 5% probability level.

Table 3. Mean ± standard deviation value of soluble sugars content for non-primed and 14 - 16 hours hydro-primed seeds.

Treatment	Glucose (%)	Sucrose (%)	Lactose (%)	Maltose (%)	Amylose (%)
non-primed	1.1 ± 0.04a	2.54 ± 0.11a	5.99 ± 0.13a	0.25 ± 0.01a	26.4 ± 0.48a
14 hrs hydro-primed	0.9 ± 0.04b	1.98 ± 0.07b	5.84 ± 0.11a	0.19 ± 0.01b	23.9 ± 0.28b
16 hrs hydro-primed	0.9 ± 0.05b	2.02 ± 0.11b	5.71 ± 0.11a	0.19 ± 0.01b	24.0 ± 0.25b
Mean	1.0	2.18	5.85	0.21	24.8
$LSD_{0.05}$	0.4*	0.90*	1.71	0.09*	1.9*
CV. (%)	12.27	12.25	8.65	13.38	2.32

Note the different letter(s) indicated the statistically significant difference by LSD at 5% level; CV indicated Coefficient of variation; *indicated significant differences at 5% probability level.

Table 4. Mean ± standard deviation value of superoxide dismutase activity (SOD), lipoxygenase activity (LOX), total fatty acid content (TFA), and malondialdehyde content (MDA) for non-primed and 14 - 16 hours hydro-primed seeds.

Treatment	SOD (U/mg Protein)	LOX (U/mg Protein)	TFA (mg/g DM)	MDA (μmole/g-FW)
non-primed	18.80 ± 0.16a	0.01 ± 0.18E−03b	27.11 ± 0.17c	10.68 ± 0.22a
14 hrs hydro-primed	18.97 ± 0.14a	0.02 ± 1.53E−03a	28.35 ± 0.21a	9.67 ± 0.10b
16 hrs hydro-primed	19.55 ± 0.13a	0.02 ± 1.52E−03a	27.11 ± 0.05b	9.49 ± 0.15b
Mean	19.11	0.01	27.66	9.95
$LSD_{0.05}$	2.12	0.007*	0.51*	1.36*
CV. (%)	3.29	16.92	0.55	4.07

Note the different letter(s) indicated the statistically significant difference by LSD at 5% level; CV indicated Coefficient of variation; *indicated significant differences at 5% probability level.

activity (SOD) was not significantly difference between primed and non-primed seed. Lipoxygenase activity (LOX) and total fatty acid content (TFA) were significantly increased for 14 - 16 hours hydro-primed seeds. In addition, malondialdehyde content (MDA) was significantly decreased for 14 - 16 hours hydro-primed seeds. The present study indicated that the advance starch hydrolysis was occurred. Carbohydrate was hydrolyzed to soluble sugars and might be

mobilized to growing tissue at the late of Phase II. Thus, soluble sugars were significantly decreased for 14 - 16 hours hydro-primed seeds. In addition, soluble sugars were antioxidant molecule. It played a role to eradicate hydrogen peroxide (H_2O_2) and glutathione from lipid peroxidation process. On the other hand, lipid peroxidation process was occurred by the increasing of LOX and TFA (**Table 4**). Meanwhile, MDA content was significantly decreased. Hussain *et al.* [48] stated that higher respiration rate may protect the plants against oxidative damage.

Furthermore, barley seed storability was presented in **Table 5**. Seed germination, GI, and MET can be maintain positive effect of hydro-priming technique as long as 1 year. Primed barley seed germination was 89% after 1 year storage, which was same as non-aged primed seeds. GI of 1 year primed seeds and non-aged primed seeds were the same as 30. Similarly, MET of 1 year primed seeds and non-aged primed seeds were 1.69 days and 1.73 days, respectively. However, T_{50} was significantly increased at 8 months of storage. Therefore, it could be concluded that primed barley seeds were improved germination uniformity and storability especially by the hydrotime of 14 and 16 hours.

Additionally, the correlation of barley seed qualities weight on hydrotime was showed in **Table 6**. The results clearly found that seed germination percentage and lactose content were in positive correlation. Seedling growth rate was negatively correlated with catalase activity and soluble sugars such as glucose, sucrose, and maltose. This might cause of those soluble sugars were mobilized for seedling growth. Meanwhile, speed of germination such as MET and T_{50}, were positively correlated with EC and LOX. In the other hand, speed of germination and EC were negatively correlated with amylose content. In addition, α-amylase activity was negatively correlated with MDA. The results might be explained by the high seed vigorous, high enzyme activity, and high seedling growth, could have high metabolism for seedling growth. However, high speed of germination seed could affected to high electrolyte solute leakage. This might cause of high LOX activity which occurred for eradicate the leak electrolyte solute.

The principle of seed priming technique was induce pre-germinative metabolism and then enhance germination efficiency [49]. Suddenly when seed imbibition was occurred, the chain of metabolism activities were occurred sequent. The physiological and biochemical event were occurred during imbibition process. Bewley *et al.* [10] explained that the leakage of solutes such as sugar, organic acids, ions, amino acids, and proteins were occurred suddenly after imbibition process. Cell wall rearrangement was proceeded. The aerobic respiration was occurred as well. The metabolic activities were resumed. Food reserves were mobilized. Some of the matrices, for example, protein and carbohydrate storage products in the storage organs, were later hydrolyzed to low molecular weight. These osmotically-active substrates decreased the Ψ_s of both the seed as a whole, and, when transported there, of the growing embryonic axis. A chain of events were initiated which ultimately results in the emergence of the embryo, usually

Table 5. The interaction for storage duration and hydrotime on barley seed qualities.

Storage duration (months)	Treatment	Germ (%)	GI	SGR (mg/seedling)	MET (days)	T_{50} (hrs.)
0	non-primed	88	28cde	3.25jkl	1.89abcd	33.00
	6	87	23fghi	4.37bcdefgh	2.00ab	36.25
	8	88	26efg	3.90fghijk	1.87bcd	34.00
	10	92	28cde	4.74abcdef	1.79def	33.00
	12	90	30c	4.40bcdefg	1.68efg	30.25
	14	89	35b	4.75abcdef	1.45ijk	23.02
	16	90	37ab	4.42bcdefg	1.42ijk	14.75
4	non-primed	97	27cde	4.84abcd	1.90abcd	34.00
	6	86	30cd	3.42ijk	1.64fgh	34.25
	8	90	35b	4.20defghi	1.51hij	32.75
	10	88	37ab	3.84ghijk	1.35jkl	29.25
	12	92	36ab	3.53hijk	1.48ij	29.50
	14	88	39a	3.71ghijk	1.23l	23.50
	16	90	39a	3.07kl	1.29kl	17.50
8	non-primed	87	22i	5.13ab	2.04a	36.50
	6	86	22i	5.03abcd	2.00ab	36.00
	8	90	23hi	5.47a	2.02ab	36.25
	10	84	23ghi	5.38a	1.94abcd	34.75
	12	88	22hi	5.23ab	2.00abc	35.75
	14	89	27cde	5.54a	1.80def	31.75
	16	90	27def	5.09abc	1.84cde	32.50
12	non-primed	94	26efg	2.51l	1.90abcd	34.00
	6	86	26efgh	3.92fghijk	1.83de	34.25
	8	85	26efg	3.96efghij	1.94abcd	46.25
	10	92	34b	4.78abcde	1.53ghi	30.75
	12	91	30cd	4.25cdefghi	1.71ef	33.50
	14	90	34b	4.18defghi	1.49hij	31.50
	16	86	34b	4.84abcd	1.45ij	24.00
Mean		89	29	4.35	1.71	31.53
$LSD_{0.05}$		10.12	5.70*	1.47*	0.27*	13.27
CV. (%)		4.73	8.04	14.04	6.65	17.43

Note the different letter(s) indicated the statistically significant difference by LSD at 5% level; CV indicated Coefficient of variation; *indicated significant differences at 5% probability level.

Table 6. The correlation of barley seed qualities weight on hydrotime.

	Germ	GI	SGR	MET	T50	EC	Prot	Carbo	Lipid	CAT	*α*-amylase	Gluc	Suc	Lac	Malt	Amylose	SOD	LOX	TFA
GI	ns																		
SGR	ns	ns																	
MET	ns	ns	ns																
T50	ns	ns	ns	0.999*															
EC	ns	ns	ns	0.999*	1.000*														
Protein	ns	ns	ns	ns	ns	ns													
Carbo	ns	ns	ns	ns	ns	ns	ns												
Lipid	ns	ns	ns	ns	ns	ns	ns	ns											
CAT	ns	ns	−0.831*	ns	ns	ns	ns	ns	ns										
α-amylase	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns									
Gluc	ns	ns	−0.712*	ns	ns	ns	ns	ns	0.674*	0.751*	ns								
Suc	ns	ns	−0.712*	ns	ns	ns	ns	ns	0.674*	0.751*	ns	1.000*							
Lac	0.81*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns						
Malt	ns	ns	−0.708*	ns	ns	ns	ns	ns	ns	0.750*	ns	0.991*	0.990*	ns					
Amylose	ns	ns	ns	−0.767*	−0.765*	−0.765*	ns	ns	ns	ns	ns	ns	ns	ns	ns				
SOD	ns	ns	ns	ns	ns	ns	0.726*	ns	ns	ns	ns	ns	ns	ns	ns	ns			
LOX	ns	ns	ns	0.855*	0.854*	0.854*	ns	ns	−0.677*	ns	ns	ns	ns	−0.681*	ns	−0.680*	ns		
TFA	ns	ns	ns	ns	ns	ns	ns	−0.863*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
MDA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.785*	ns	ns	ns	ns	ns	ns	ns	ns

Note *indicated significant differences at 5% probability level; ns = non-significant.

the radicle, signifying that germination has been successfully completed. Our observation found that lipid content was significantly decreased for 14 - 16 hours hydro-primed seeds. While protein and carbohydrate content was decreased for 14 hours hydro-primed seeds, even they were increased for 16 hours hydro-primed seeds. This might be the new protein synthesize during the late Phase II, according to **Figure 1**. According to none significantly different of catalase and α-amylase activity, this might be the finished of metabolism process before late of Phase II. Meanwhile, the soluble sugars such as glucose, sucrose, maltose, and amylose were decreased for 14 - 16 hours hydro-primed seeds. These results were supplementary the presumption that food reserve mobilization was occurred at 14 and 16 hours after imbibition of barley seed.

These observations were the evidences that seed priming technique induced advance food reserves hydrolysis process. During germination, the increasing of enzyme activities such as hydrolytic enzymes were essential for the germination process [50]. The water was consumed and induced the starch hydrolysis. The sugars content were also positive correlated with seed germination and vigor [51], which according to **Table 6**. Lee and Kim [52] reported that primed rice seeds were increased in α-amylase activity, fructose content, glucose content, but reduced sucrose content. According to Dhingra [53], glucose was derived from endosperm by amylolytic breakdown process. Then, glucose was mobilized to the scutellum, where the sucrose synthesis occurred. After that, the glucose was transported to the embryonic axis for further seedling development. Hussain *et al.* [48] stated that the better germination and vigorous growth of primed-rice seedlings was associated with 1) higher starch metabolism, 2) enhanced respiration rate, 3) better membrane integrity, 4) higher metabolite synthesis, and 5) increased activities of antioxidants in these seedlings.

Nevertheless, TFA was significantly increased for 14 - 16 hours hydro-primed seeds, while lipid content was significantly decreased. These results were related to Wang *et al.* [54] who reported that degradation of lipids in senescing membranes and the release of FFA initiated oxidative deterioration by providing substrate for LOX in lipid peroxidation process. This mechanism released reactive oxygen species (ROS) from membrane phospholipids in aleurone layer and induced cell senescence [55]. ROS mediated lipid peroxidation, enzyme inactivation, protein degradation, disruption of cellular membranes and damage to genetic integrity as major cause of seed deterioration [56] [57]. Therefore, 14 - 16 hours hydro-primed seeds were induced TFA for lipid peroxidation process. However, the result found that MDA content was significantly decreased. Bailly *et al.* [58] reported that aged seed had accumulated in malondialdehyde, as a result of lipid peroxidation process and led to seed deterioration. However, cellular damage caused by lipid peroxidation process might be prevented by protective mechanism. The protective mechanism or defenses mechanism was activated by antioxidant enzyme activity such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD). These enzymes eradicated hydrogen peroxide (H_2O_2), glutathione, and MDA, which were released from lipid

peroxidation process. Wherewith, our observation indicated that TFA and LOX were significantly increased, while MDA was significantly decreased. Hence, hydro-priming technique strengthened the antioxidant activity. Although, our observation indicated that CAT and SOD were not significantly, even free radicle such as soluble sugars were significantly decreased. Therefore, there might be some enzymes or mechanism to eradicate or inhibit lipid peroxidation process.

4. Conclusion

Hydro-priming technique was one of the effective seed priming techniques to improve germination and vigor, especially speed of germination and germination uniformity. In this study, the best seed vigor occurred at 14 and 16 hours of hydrotime. Primed barley seeds speeded up 25% or 0.5 days to final germination. In addition, primed barley seeds were reduced 50% or 17 hours to reach fifty percentage germination with 36 seedling per day, while 28 seedling per day was found in non-primed seeds, wherewith non-different in final seed germination percentage. Additionally, lipoxygenase activity (LOX) and total free fatty acid (TFA) were increased, but malondialdehyde (MDA) content was decreased for 14 - 16 hours hydro-primed seeds. However, the antioxidant activities such as CAT and SOD were not significantly different from non-primed seeds. Including, free radicles such as soluble sugars were decreased. It might be stated that protective mechanism prevented cellular damage by scavenging free radicle. Then, motivated seed storability of 14 - 16 hours hydro-primed seeds could be stored for 8 - 12 months. Therefore, our experiment could be concluded that hydro-priming technique for 14 and 16 hours could enhance barley seed germination and vigor by reducing germination time, induced defenses mechanism, and could be stored for 8 - 12 months. Nevertheless, hydro-priming technique needed good practice in dry back process and hydro-primed seed needed low storage temperature (5 to −4 degree Celsius).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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