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The Use of Seed Priming Treatments to Improve the Quality of Barley (*Hordeum vulgare* L.) for Malting

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Grain barley (*Hordeum vulgare* L.) is the predominant raw material used to produce malt for industries such as brewing and the food industry. In Thailand, barley has been introduced to Thai farmers to improve farm income and reduce barley imports, but the seed quality of Thai-grown barley is often lower than required for profitable malt production, especially in the aspect of seed germination and vigor, and high pathogen contamination. Thus, this study was conducted to evaluate the effects of different seed priming methods on seed quality of Thai barley for malt production. Three experiments were conducted. The first experiment was hydro-priming: seeds were primed in deionized water for 6, 8, 10, 12, 14, or 16 hours. The second experiment was hydro-priming plus potassium nitrate treatment: seeds were primed in four different concentrations of KNO_3 solution: 2.5, 5, 10, or 20 mg/ml for 6, 8, 10, and 12 hours. The third experiment was osmo-priming by PEG4000: seeds were primed in solutions of three different osmotic potentials: −0.50, −0.75, or −1.50 MPa for 10, 12, 14, or 16 hours. All seed priming methods accelerated barley germination and increased seed vigor. Primed barley seed germinated within 1 day. Barley seeds were primed with hydro-priming plus KNO_3 at 5 mg/ml for 12 hours had significantly high seed germination and speed of germination. Hydro-priming plus KNO_3 and osmo-priming by PEG4000 could speed up seed germination. We conclude that all three of these seed priming techniques could be effectively applied to improve barley seed quality in the Thai malt industry. However, further study is needed to evaluate the effects of seed priming methods on barley seed storability and other attributes of malt quality.

Key words: barley seed, hydro-priming, malt, polyethylene glycol, potassium nitrate

Introduction

Barley (*Hordeum vulgare* L.) is an ancient crop that has played an important role in the development of agriculture and civilization. It is a keenly studied crop in the sciences of agronomy, physiology, genetics, breeding, malting, and brewing. Barley is used commercially for animal feed, malt production, and human consumption. Although, only 13% of the barley produced worldwide is processed into malt, it is well known as the premier malting and brewing grain (Steven, 2011). It is used as the raw material for malt, which is important in many industries such as brewing and manufacturing of non-alcoholic and alcoholic beverages and food. Malted barley products are used commercially for many foods to enhance color, enzyme activity, flavor, sweetness, and nutritional quality (Newman and Newman, 2008).

In Thailand, barley has been introduced to Thai farmers to improve their income and as a substitute for imports (Masjaroon, 1995), yields are relatively low (1.9 t/ha for 'Samerng 2' and 1.7 t/ha for 'Samerng 1') compared to other countries, where 2.4 t/ha is more typical (Prachaya and Attachai, 1998). Currently, Thai maltsters have to import 100% of the barley used for malt production. The quality of the raw seeds is often found to be lower than the standard required for malt production, especially in respect of germination

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percentage and vigor and rate of pathogen contamination (Puangwerakul, 2007; Pedro *et al*., 2012). Barley seeds used in the brewing industry must have high germination capacity, purity of variety, uniformity in grain size, and have low protein content.

Many techniques are used to improve barley seed germination and vigor. Seed priming is a technique that improves germination and crop establishment of several types of crop (Aziza *et al*., 2004). New scientific knowledge of seed physiology and seed biochemistry, combined with new technology, can enhance seed quality. Seed priming is one of the seed enhancement techniques that may allow scientists to develop more innovative efficient procedures for making improvements in crop production. The technique's performance depends on the crop variety, crop imbibition behavior, priming method, and imbibition time.

Seed priming has been widely used to enhance the germination response of agricultural crop species (Bradford 1986) and is the most important physiological seed enhancement method. Seed priming is a hydration treatment that allows controlled imbibition to induce pre-germinative metabolism ("activation"), but radicle emergence is prevented. The hydration treatment is stopped before desiccation tolerance is lost. An important problem is to stop the priming process at the right moment. This time depends on the species and the seed batch. The seeds can be re-dried for storage, distribution, and planting. The speed and synchronicity of germination of primed seeds are enhanced and can be interpreted as though priming increases seed vigor. Priming permits a wider temperature range for germination, releases dormancy and achieves faster emergence of uniform seedlings. A practical drawback of primed seeds is often a decrease in storability and the need for cool storage temperatures.

The rate of water absorption in the initial 24 hours of imbibition may be one of the factors that controls seedling vigor. Seeds that were allowed to imbibe slowly in 25% polyethylene glycol 6000 had increased shoot weight (Ehsanullah and Smith, 2002). Priming is a non-expensive and value added practice that greatly improve yield. This might be due to some biochemical and physiological changes brought about by seed soaking (Khan *et al*., 2002). The improved germination of primed seeds may therefore, be attributed to counteraction of free radicals and re-synthesis of membrane bound enzymes as in unprimed seeds (Srinivasan and Saxena, 2001).

This research addresses the following questions:

1. How does seed priming effect on barley seed quality?
2. For this study, we emphasized on germination percentage within 3 days and evaluated the parameters of slower germination rate, slower rate of growth and development, and decreased uniformity.

Materials and Methods

1. Barley seed sample

The barley seeds used were cv. 'Samerng 2' grown at the Samerng Rice Research Center in Thailand's Chiang Mai province between November 2011 and February 2012. The unprimed seed germination rate was 88% and the seed moisture content was 12%.

2. Priming methods

The experiment was performed in a complete factorial randomized design with four replications (4 priming levels x 6 hydrotime levels x 4 replicates):

Factor A: 4 levels of priming: non-primed barley seed, hydro-priming, osmo-priming by polyethylene glycol (PEG4000, three different osmotic potentials: −0.50, −0.75, or −1.50 MPa for 10, 12, 14, or 16 hours), or hydro-priming plus potassium nitrate (KNO_3, four different concentrations of KNO_3 solution: 2.5, 5, 10, or 20 mg/ml for 6, 8, 10, and 12 hours)

Factor B: 6 levels of hydrotime: 6, 8, 10, 12, 14, or 16 hours.

Priming was performed at room temperature (25℃). Twenty g of seeds was placed in 500 ml of priming solutions. An aeration pump was used during the experiment to provide oxygen from the ambient atmosphere into the chamber. After the end of each hydrotime, the barley seeds were dried in a fan-forced oven at 32℃ for 48−72 hours as required to reach the initial seed moisture content.

3. Seed quality test

Seed moisture content: The moisture content of barley seeds after priming was measured by the hot-air oven-drying method (ISTA 2011). Four replications of 5 g of seed were dried in a hot-air oven at 130℃ for 2 hours. At the end of this period, the samples were transferred to a desiccator for cooling. The samples were then weighed and the percentage moisture content was calculated and expressed on a wet weight

basis (w.b.).

Tetrazolium test: A tetrazolium test was used to estimate vigor as well as viability. A viable seed should show staining in all tissues that require viability for normal seedling development. For the purpose of the test, a viable seed should show, by its biochemical activity, the potential to produce a normal seedling (ISTA, 2011). First, 200 seeds were soaked in distilled water to allow them to hydrate for 8–16 hours at a temperature of 25℃. The hydrated seeds were then sectioned through the embryo and soaked in 0.1% triphenyl tetrazolium chloride for 2–3 hours at 35℃ in the dark. Viable seeds were defined as those that showed red staining of the embryo.

Germination: The percentage germination was determined using the 'between paper' method, according to the standard germination test (ISTA, 2011). Four replicates of 100 seeds of barley were placed between layers of germination paper. The seeds were then incubated in a germination chamber at 20℃ for 3 days. The number of normal seedlings developed from a replicate of 100 seeds represented the germination percentage, and the results were the average of the 4 replicates of 100 seeds.

Germination index (GI): Four replicates of 400 seeds were placed between layers of paper. The number of germinated seeds was counted every day and the cumulative germination index (GI) was calculated from:

$$GI = n_1/1 + n_2/2 + n_3/3 + \cdots + n_x/x$$

where $n_1 \cdots n_x$ is the number of seeds germinated on day 1 to day x and $1 \cdots x$ is the number of days since the start of the test.

A high GI value indicates high seed vigor. Seeds are considered to have germinated when the radicle appears; hence, they should be counted daily and the seeds observed as having germinated should be removed.

Mean emergence time (MET): Four replicates of 400 seeds were placed between layers of paper. The number of germinated seeds was counted every day and the mean emergence time (MET) was calculated using the following formula (Demir *et al*., 2008):

$$MET = \sum nD / \sum n$$

where n is the number of seeds newly germinated on day D from the beginning of the germination test and $\sum n$ = total number of seeds germinated.

4. Statistical analysis

We performed analysis of variance, and when the results were significant, used a least-significant-difference (LSD) test to detect differences between paris of treatments, with significance at $P < 0.05$. Statistical analysis was carried out using version 8 of the SX software.

Results

Barley seed quality

1. Seed moisture content

The initial barley seed moisture content was 10.6%. After the seed priming treatment and re-drying, a significant difference in seed moisture content was detected between the priming techniques and hydrotime, but the differences were very small and overall show that seeds were returned to a moisture content close to that of the non-primed seeds.

2. Seed viability

The initial seed viability was 85%. After seed priming, seed viability significantly increased in all treatments independent of hydrotime (Table 1). The highest seed viability was recorded by osmo-priming with PEG4000 solution at a water potential of −1.50 MPa; however, the result was not significantly different from that of any treatment except hydro-priming.

3. Seed germination

Barley seed germination was not significantly different from that of unprimed seeds for all priming solutions except for PEG4000 solution at water potential of −0.75 MPa (significantly higher) and osmo-priming by PEG4000 at −0.50 MPa (significantly lower). Germination was highest for a hydrotime of 12 hours, which was significantly different to all other times except 8 hours (Table 1).

4. Seed germination index (GI)

Barley's seed germination index (GI) was increased significantly by all priming treatments except hydro-priming. The highest GI values resulted from osmo-priming by PEG4000 at −0.75 MPa and hydro-priming plus KNO_3, with the highest value being at 5 mg/ml KNO_3. Additionally, GI tended to increase as hydrotime increased (Table 1).

5. Mean emergence time (MET)

Untreated barley seeds had a MET of 1.89 days. After seed priming, MET decreased significantly in all priming solutions (Table 1). Hydro-priming plus KNO_3 at 2.5 or 5 mg/ml achieved significantly lower MET than all other solutions. With respect to hydrotime, the

Table 1. Results of four measures of barley seed quality in non-primed seed and after priming as influenced by (A) priming treatment and (B) hydrotime.

Treatment	Seed quality			
	Viability (%)	Germination (%)	GI	MET (day)
Priming methods (A)				
non-prime seed	85 c	88 b	28 e	1.89 a
hydro-priming	93 b	89 b	29 e	1.70 b
hydro-priming plus KNO_3 at 2.5 mg/mL	97 ab	94 a	44 a	1.15 ef
hydro-priming plus KNO_3 at 5 mg/mL	96 ab	95 a	44 a	1.13 f
hydro-priming plus KNO_3 at 10 mg/mL	96 ab	95 a	44 a	1.15 ef
hydro-priming plus KNO_3 at 20 mg/mL	93 b	95 a	41 b	1.28 d
osmo-priming by PEG4000 at −0.50 MPa	94 ab	83 c	33 d	1.42 c
osmo-priming by PEG4000 at −0.75 MPa	95 ab	92 a	41 b	1.22 de
osmo-priming by PEG4000 at −1.50 MPa	99 a	88 b	38 c	1.29 d
$LSD_{0.05}$	*	*	*	*
Hydrotime (hours) (B)				
6	96 a	91 ab	36 b	1.44 a
8	95 a	90 b	35 b	1.45 a
10	95 a	90 b	35 b	1.43 a
12	96 a	93 a	40 a	1.31 b
14	93 ab	90 b	40 a	1.26 b
16	89 b	92 ab	41 a	1.25 b
CV (%)	8.54	4.81	8.77	8.28

† Abbreviations: GI: germination index; MET: mean emergence time
Means followed by the same letter within each column were not significantly different by LSD ($p<0.05$).

lowest METs were achieved for 14 and 16 hours, which were significantly lower than all shorter hydrotimes.

Discussion

Malting is the process of converting cereal seeds, especially barley, into malt. The process starts with imbibition by the seeds in a steeping step. The water enters through the micropyle to activate biochemical mechanisms. The seeds are steeped until the moisture content reaches 42 to 48% (Lewis and Young, 2002). At this point, enzymes are synthesized and food reserves are hydrolyzed (Copeland and McDonald, 1995). Modification (the breaking down of cell walls and converting food reserves to mobile substances) continues until the radical protrudes. The germination process is then stopped before the soluble sugars and soluble proteins are exhausted from the endosperm (Usansa, 2008). Drying and kilning with forced-flow hot air inactivates hydrolase production and all enzyme activities. The kilned malt is stable for storage and has a friable texture suitable for the milling process, which precedes brewing.

Zheng *et al*. (1994) said that seed priming techniques have the potential to improve canola seed germination and increased percentage germination or emergence and/or a reduced time to 50% germination or emergence (T_{50}). Likewise, Sharma *et al*. (2014) reported that hydro-priming for 12 hours and solid matrix (SM) with calcium aluminum and silicate (1: 0.4:1; seed: SM: water) for 24 hours effected on increased germination percentage, seedling vigor, mean emergence time and yield of okra cv. Hisar Unnat. Additionally, Ghazi (1998) reported that reduction in osmotic potential effect on barley seed imbibitions rate by result on germination percentage and decreased

time to 50% germination. Caseiro *et al*. (2004) studied on comparison of three priming techniques (osmo-priming by PEG8000, hydro-priming and drum priming) on percentage and speed of germination, using six lots of onion (*Allium cepa*) seeds. For the osmo-priming method, osmotic potentials of −0.5 MPa and −1.0 MPa and imbibition periods of 24 and 48 h were used. In the hydro-priming method, seeds were moistened between 2, 4 or 6 layers of paper towel, for 48 or 96 h. In the drum priming technique, the optimal amount of water added and treatment period varied among seed lots, which exhibited a wide variation of germination and vigor. All treatments were carried out at 15℃; drum priming was also conducted at 25℃. The response to priming methods varied among seed lots and, in general, less vigor onion seed lots did not respond well to priming treatments. The hydro-priming technique was the most effective method for improving speed of germination for all six lots that were evaluated, especially when 96 h of priming was used. Tarquis and Bradford (1992) studied on the influence of pre-hydration in water or priming in −1.5 MPa polyethylene glycol 8000 solution for various periods, followed by re-drying, on germination rate and longevity of lettuce *(Lactuca sativa* L.) seeds (achenes). Short pre-hydration treatments (up to 1 h) had little effect on either germination rate or longevity, but significantly improved root growth rates. Increasing durations of pre-hydration or priming reduced the mean time to germination by up to 61% relative to untreated seeds, but also reduced mean seed longevity by as much as 84%. Pre-hydration and priming altered the relationships between germination rate and viability and between normal and abnormal seedlings during ageing. Pre-hydration or priming treatments effectively accelerate subsequent germination rates of lettuce seeds, the re-dried seeds are nonetheless highly susceptible to deterioration in storage. Bray *et al.* (1989) studied on osmotic priming treatments reduced both the mean time to germination and the spread of germination for two leek seed-lots of high viability but differing vigor. In addition the differences in germination performance between these two seed-lots were abolished by the priming treatments. In the unprimed seed-lots, differences in germination performance were reflected in differences in rates of protein biosynthesis in leek embryo tissue during germination. Osmo-priming treatments abolished these differences upon subsequent germination of osmotically primed seed and furthermore induced high levels of protein biosynthesis in embryo tissue.

The export standard for malt is a minimum malt extract level of 80%. FAO (2009) specifies that the requirements for good malt quality are a minimum germination percentage of 97% after 3 days of germination; a minimum GI of 6.0; a seed water content of 9 to 11.5%; more than 90% of raw grains ＞2.5 mm; a β-glucan content less than 4%; grain free from pathogens, pesticide residues, ochratoxin, and aflatoxin; a variety purity of at least 99%; and a finished malt with a high malt extract at low modification levels, high diastatic power, pale color, and uniform grain size.

Based on the present results, we conclude that all three seed priming techniques that we examined could be effectively applied as alternative methods to improve barley seed quality in the Thai malting industry.

Conclusions

Our research clearly indicated that;

1. Hydro-priming, hydro-priming plus KNO_3 and osmo-priming by PEG4000 could speed up seed germination.
2. Barley seeds were primed with hydro-priming plus KNO_3 at 5 mg/ml for 12 hours had significantly high seed germination and low MET and T_{50}.
3. Seed priming enhanced barley seed quality in terms of malt quality especially speed of germination.
4. Priming technique can shorten malting process for 64.81%.

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