CHAPTER 3

MATERIALS AND METHODS

Seed sample

Barley seeds (*Hordeum vulgare* L.) of the 'Baudin' variety were obtained from Chiang Mai Beverage Co. Ltd, Chiang Mai in Thailand. The initial seed moisture content was 11.8%. The seeds were then re-wetted to 14% by adding water to a known mass of seeds. The seeds were stored at 4°C for 24 hours in a sealed container, until the moisture content attained equilibrium within and among the seeds. To ensure the moisture content was correct, it was determined again at the end of this period, prior to sampling of the seeds for the experiment.

Radio frequency (RF) treatment

A barley seed sample of 440 g was weighed before each treatment and then transferred into a Teflon container. The seed was treated with an RF heat treatment application unit, developed and built at the Institute of Agricultural Engineering, Georg-August University in Göttingen, Germany. The unit has a variable power supply and an operating frequency of 27.12 MHz. The seed temperatures were measured by a fiber-optic temperature sensor. Experiments were conducted using a Randomized Complete Block Design (RCB), with 4 replications. The factors used in the experiment were temperature of 60, 65, 70 and 75°C, and time periods of 0, 1 and 3 minutes. After each treatment, the samples were examined to evaluate seed-borne fungi infection, seed quality, chemical composition and enzyme activity.

Experiment 1: Identification of seed-borne fungi in barley seed

Before the barley seeds were subjected to RF heat treatment, some 200 seeds were randomly taken from the seed sample, to detect the presence of seed-borne fungi by using the blotter method according to ISTA rules, 2006.

Experiment 2: Effect of radio frequency heat treatment to control seed-borne fungi in barley seed

2.1 Agar method

According to ISTA (2006) for the assessment of seed infection, 200 seeds from each treatment were surface disinfested to eliminate the possible growth of other micro-organisms on the seed surface. The procedure consisted of dipping the seed for 1 minute in 1% sodium hypochlorite solution, rinsing three times in sterile distilled water and then blotting on sterile paper towels. 10 seeds were placed on PDA (Potato Dextrose Agar) in each petri-dish. The plates were incubated at 20-25°C for alternating 12 hour periods of darkness and 12 hour periods near to UV-light (Philips TLD 18W/54), for 7 days. The seeds with colonies of fungi pathogen present were identified according to spore morphology under a stereoscopic microscope**,** and observed colonies on the reverse side of the petri-dishes, as well as the fruiting body, under a compound microscope. The total number of infected seeds with the pathogen were counted and calculated into a percentage of infection.

2.2 Blotter method

For the Blotter method, seed samples were placed in a sterilized petri dish containing 3 layers of blotter paper (Whatman no. 1), which were then soaked well in sterilized water. Barley 10 seeds were placed in each petri dish, then incubated at 20-25°C under a cycle of 12 hours of darkness and 12 hours near UV-light (Philips TLD 18W/54). After 7 days of incubation, the seeds were examined for fungi infection under a stereoscopic microscope. Identification of the fungi was based on the habitual characteristics found on the seeds and by microscopic examination of the conidia, following Mathur and Kongsdal (2003), according to whom, seed bearing a fruiting body is considered as infested by the given fungus. Infestation levels were recorded as a percentage of the infected seeds in each sample.

Experiment 3: Effect of radio frequency heat treatment on seed quality

3.1 Seed moisture content

The moisture content of barley seeds after RF heat treatment was measured by the hot air-oven method (ISTA 2006). Four replications of 5 g of seed were dried by a hot air oven at 130°C 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 of seed moisture content was calculated and expressed based on a wet-weight basis (w.b.).

3.2 Germination test

The percentage of germination was determined by using the 'between paper' method (BP), according to the standard germination test (ISTA, 2006). Barley 100 seeds were placed in between papers for 4 replications. After that, they were incubated in a germination chamber at 20°C for 7 days. The results of the germination test were calculated as the average of 4 replicates of 100 seeds, and expressed as a percentage of the number of normal seedlings.

3.3 Tetrazolium test (TZ test)

A TZ test was used to estimate vigor as well as viability. A viable seed should show staining in all those tissues whose viability is necessary 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 (AOSA, 2002). First, the 200 seeds were soaked in distilled water to let them inflate for 8-16 hours at a temperature of 25°C. After that, the absorbed seeds were sectioned through the embryo and soaked in 0.1% triphenyl tetrazolium chloride for 2-3 hours at 35°C, in the dark. The viable seeds were determined as those seeds which were red at the embryo.

3.4 Accelerated aging test (AA test)

The seeds were put into a screen tray and placed in glass bottle or inner chamber containing 40-50 ml of water. The inner chamber was placed again into an accelerated aging (outer) chamber and the seeds were aged at a specified high temperature (40ºC) for a specified time (72 hours). During the aging process, seeds normally absorb moisture from the humid environment within the chamber and also suffer stress from the high temperatures. After aging the seeds, germination was tested using the standard method (ISTA, 2006).

3.5 Speed of germination test

High speeds of germination are an indication of a vigorous seed lot. Barley 400 seeds for 4 replications were placed between papers. The number of germinated seeds was counted every day and the cumulative index was calculated using the following formula:

Germination index (GI) = n1/1 + n2/2 + n3/3 + … + nx/x

where n1 … nx : were the number of seed germination on day 1 to day x

and 1 … x : were the number of days.

A high value of GI indicates high seed vigor. Seeds are considered to have germinated when the radical appears; hence, they should be counted daily and the seeds observed as having germinated should be removed (AOSA, 2002).

Experiment 4: The effect of radio frequency heat treatment on the chemical composition

4.1 Total protein content

The total nitrogen (N) content was determined using a burning procedure with the Kjeldahl method, as described in AOAC (2000). The nitrogen content in milligrams per gram was calculated using the formula below. The protein content was obtained by multiplying the nitrogen determined by 6.25.

Calculate the results as follows:

$$\% N = \frac{(\text{ml. } H_2SO_4 \text{ for sample} - \text{ml. } H_2SO_4 \text{ for blank}) \times \text{standard } H_2SO_4 \times 0.014 \times 100}{\text{Weight of sample (g)}}$$

4.2 Dehydrogenase activity

The dehydrogenase activity of barley seeds after RF heat treatment was measured according to Haufe (1992) and Achuba and Peretiemo-Clarke (2008).

Reagent:

Tris-buffer 0.1 M, pH 7, 6, 0.2 TTC (Triphenyl tetrazolium chloride) solution.

Procedure:

1. Ground barley seed 1.5 g was weighed into a centrifuge tube
2. Buffer solution 10 ml Tris-buffer pH 7.6, 0.2% TTC solution in MC-II valin- buffers was then added
3. The sample was then mixed by hand with short vibrations, before being incubated at a moderate temperature of 45°C in a water bath
4. The sample was centrifuged at 5000 rounds per minute, for 20 minutes
5. The sample was then filtered using Watman no. 1 filter paper
6. The absorbance level was measured at 480 nm by a spectrophotometer. The analyses activity of the enzyme correlates with the intensity of the extinction.

Statistical analysis

The analysis of variance was performed for data analysis and differentiated with a least significant difference (LSD) comparison at $P<0.05$. Statistical analysis was carried out with SX version 8 (Analytical Software, USA)

Location of the study

- Seed laboratory, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, and
- Post-harvest Technology Institute, Chiang Mai University.