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EFFECTS OF WARM WATER IN BREAKING DORMANCY OF RICE SEED

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ABSTRACT

This small research in order to find out the effect of one of various methods to breakdown seed dormancy, soaking seed in warm water, on two main processes of the seed which are metabolism processes (respiration rate, ethylene production, and α-amylase activity) and germination rate. The result of this experiment showed that soaking seed in ***warm water at 50^{0}C in 15 minutes period not only effectively enhanced germination rate together with enhance respiration rate, ethylene production and α-amylase activity but also limited fungus infection during germination of rice seed****. So far, a further research on methods to breakdown seed dormancy such as scarification and chemical methods is needed.*

INTRODUCTION

Seed dormancy refers to the resting stage of embryo with low germination of viable and freshly harvested grains. It is also defined as inability or failure of perfectly matured seed to germinate even when placed under conditions favorable for germination. This is an important survival mechanism of seeds especially under harsh environmental conditions. Seed dormancy according to Naylor (1983) is a genetically inherited trait whose intensity is modified by the environment during seed development (Copeland and McDonald, 1995). The length of dormancy depends on the type of seed, season and harvest conditions (Johnstone 1989). Wild species are said to be more dormant than the domesticated species. It is a mechanism of adaptation for wild species under abnormal conditions. Seed dormancy may be a problem to seed producers or growers but this is also advantageous to maintain seed quality, but seed dormancy is an important varietals trait in tropical rice where rainfall and high humidity are of frequent occurrence during the maturity and harvest periods. Without dormancy, seed would germinate on the standing crop.

There are many types of dormancy exist in seeds. Dormancy imposed by impermeable seed coats, dormancy induced by environmental light conditions, and dormancy due to immature embryos at dispersal are just a few of the mechanisms which have been described in different seeds. This diversity of mechanisms has contributed to the creation of many classifications of types of dormancy, each based on different criteria. Differences in the time when dormancy is induced led to the distinction between *primary dormancy*, which is present at seed dispersal, and *secondary dormancy,* which is induced by environmental conditions in a seed which was not dormant when shed (Bewley and Black 1983, 1994; Hilhorst 1995, 1998). Another classification system distinguishes *endogenous dormancy* due to immature embryos or "physiological inhibiting mechanism" from *exogenous dormancy,* or dormancy due to seed coats or inhibitors (Nikolaeva 1977). When the seed is dormant, activation of metabolisms, involving synthesis of nucleic acids and proteins, increase in enzyme and respiratory activities, and reserve food breakdown are very low.

Seed dormancy in different species of rice is controlled by a single dominant gene "G", and influenced by duration of maturity and environment (Tipathe et al. 1980). Medium and late maturity of rice tends to show longer dormancy. According to Pili (1969), the number of days or weeks of delay in harvesting correspond to the number of days or weeks that dormancy was shortened. Early maturing varieties have shorter time of dormancy than late-maturing varieties. Chandraratna et al. (1954) estimated that dormancy period is related to photoperiodic reaction. They concluded that relatively insensitive

varieties have short dormancy period. Dormancy also varies among species of rice. Japonica varieties are less dormant than indica varieties (Beachell 1943).

There are various methods in order to breakdown seed dormancy. To overcome exogenous dormancy, the mechanical and chemical removal of the seed coat, a process called scarification. Grinding seeds with abrasives or sand or sacking them (impaction) are often used to scarify seed coats. Other techniques such as heating, chilling, drastic temperature shifts, brief immersion in boiling water, piercing the seed coat with a needle, or exposure to certain radio frequencies alter seed coat integrity, permitting penetration of both water and gases. However, the duration of these treatments may not be sufficient to break dormancy.

Seeds may also be treated with chemical to cause degradation of the seed coat. Sulfuric acid has been used most widely and is effective in industrial and concentrated forms. Other compounds such as sodium hypochlorite and hydrogen peroxide have also been reported to scarify seeds (Hsiao and Quick 1984); nitric acid is also used for this purpose. However, chemical scarification has not been commercially popular because the materials are hazardous to handle, the seed must be thoroughly washed and dried after treatment, and reduction of germination may occur from even slight over scarification. Recent techniques include the use of selective seed coat enzymes such as cellulose and pectinase to degrade seed coats. Seeds that are dormant due to osmotic inhibition (physiological dormancy) can be germinated after removing the seed from the influence of the inhibitor of diluting the inhibitor from around the seed, a process called leaching. Leaching typically requires exposing the seeds to an excess of water that dilutes or removes the inhibitor from the seed.

OBJECTIVES

Observe and determine effects of method to breakdown rice seed dormancy by using **warm water method** on metabolism processes (respiration rate, ethylene production, and α-amylase activity), germination rate.

MATERIALS AND METHODS

Germination test

Hundred newly harvest rice (*Oryza sativa)* seeds, Fujisaka cultivar were placed on two layers of filter paper in Petri dish (9 cm) containing 3 ml distilled water, three replicates. The Petri dishes were incubated at room temperature in 12 hrs fluctuated light (day and night), the plates were wet with 3 ml distilled water after 3 days to maintain moisten condition. Both control and treated seeds were scored as germinated when the radicles were ≥ 1mm.

For the treated seeds, intact grains were soaked in warm water (50^0C) for 15 minutes as dormancy breaking method and transferred to wet filter paper for further germination test.

Determination of respiration rate and ethylene production

Hundred weighted grains were determined the volume by displacement. The seeds were placed in the known volume jars on two layers of wet filter papers. Flushed the jar with a gentle stream of air, (Note 1) and using rubber stoppers fitted with a sampling port seal the jars and allows the jar and its contents to stand undisturbed for 24 hour.

After 24 hrs, five 1-mL gas samples were withdrawn from the jar, and injected into the GC (Note 2). The concentration of ethylene was observed by measured the peak heights 30 seconds after injection by recording equipment connected with GC (Note 3). The concentration of CO_2 was observed after 15 seconds.

Ethylene concentration (nl C_2H_4.g-1.h-1) and respiration rate (mg $CO_2.kg^{-1}.h^{-1}$) were calculated in following formulas:

$$\text{nl } C_2H_4.g^{-1}.h^{-1} = \frac{\dfrac{(\text{peak height x attenuation) Sample}}{(\text{peak height x attenuation) Standard}} \text{ x } [1\text{ ppm}]\text{ standard x } (V_J - V_F)\text{ (ml)}}{\text{WT.Sple. (g) x Time of enclosure (1 hour)}}$$

$$\%CO_2 = \frac{\text{(peak height x attenuation) Sample}}{\text{(peak height x attenuation) Standard}} \times [1\%]\, CO_2 \text{ standard}$$

$$\text{Respiration rate} = \frac{\left[(\%CO_2)_t - (\%CO_2)_0\right]}{100} \times (V_J - V_F) \times \frac{1}{t} \times \frac{1}{w} \times \frac{44 \text{ mg } CO_2}{24 \text{ mL } CO_2}$$

Where: V_J : volume of the jar (ml)
V_F : volume of seed (ml)
t : time interval in hour
w : fresh weight of sample (g)
$(\%CO_2)_t$: percent CO_2 measured after a time interval t (hours)
$(\%CO_2)_0$: percent CO_2 measured at zero time

Determination of α-amylase activity

Preparation of acetone powders. Acetone powders were prepared by homogenizing 2 g of seeds which were taken after observed germination everyday with frozen acetone (1:8 w/w) in a warring blender for 5 minutes. The homogenate was filtered using vacuum and the pellet washed several times with cold acetone. The residue was air dried, ground to a fine powder and stored inside the freezer for further use.

Extraction. α-amylase was extracted from acetone powder (0.2 g), homogenized with 10 ml 0.02 M sodium phosphate buffer, pH 6.9, using a mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 2^0C.

Assay. The activity of α-amylase was determined by the loss in the ability to give a blue color with iodine. The assay mixture (6.5 ml) contained 0.5 ml enzyme extract, 1.0 ml soluble starch 1 mg/ml dissolved in 0.02 M phosphate buffer, pH 6.9, and 5.0 ml 0.005% I_2-0.05% KI solution.

The reaction mixture was incubated for 10 minutes at 20^0C after which the reaction was stopped with the addition of 1.0 ml of 0.1 M HCl. The difference in the absorbance (λ A700 nm) between the control tube (killed enzyme) and the experimental tube gave a measure of the extent of loss of the blue iodine color. One unit of α-amylase was defined as 0.001 values different between absorbance (ΔA) of the control and the experimental sample, followed the formula:

α-amylase activity (units) = ΔA/0.001

All data was analyzed statistically by analysis of variance (ANOVA), and the means were compared by the least significance level of 0.05.

RESULTS AND DISCUSSION

Effect of breaking dormancy (warm water method) on germination

There was no germination occurred after 1 day incubation in both control and treated seeds (Table 1 and Fig. 1). This is in agreement with the basic principle of seeds germination, during the imbibition phase (24 hrs) there is no germination, it just a first step of seed germination involving rapid absorption of water by the seed. Once

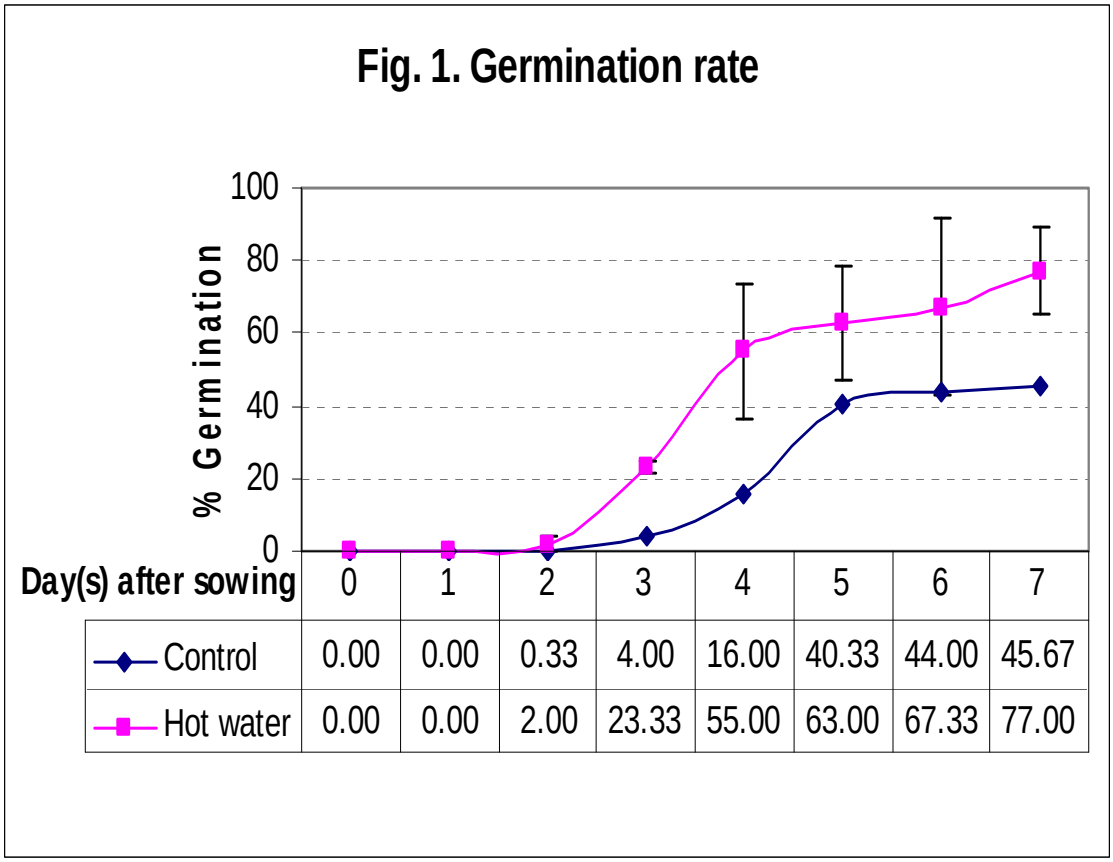
germination begins the rate of the cell respiration increases greatly because the need for energy has suddenly become tremendous. The ATPs yielded by the cellular respiration is needed to support the rapid growth of the germinating seed.

The percentage of germination was observed at the 2nd day in both treatments, and the germination percentage of seeds treated with warm water (2%) was higher compared to the control (0.33%). The germination rate in warm water treatment increased rapidly and remarkable higher than that in the control which also increased after 2 days to 7 days after sowing. This result implied that warm water method effectively enhanced germination rate of newly harvested rice seeds.

Table 1. Germination rate (%)

DAS*	0	1	2	3	4	5	6	7
Control	0.00	0.00	0.33	4.00	16.00	40.33	44.00	45.67
Warm water	0.00	0.00	2.00	23.33	55.00	63.00	67.33	77.00
LSD5%			1.8	1.8	18.3	15.5	24.6	11.9
CV%			70	6	19.4	10.7	28.8	8.7

* day after sowing



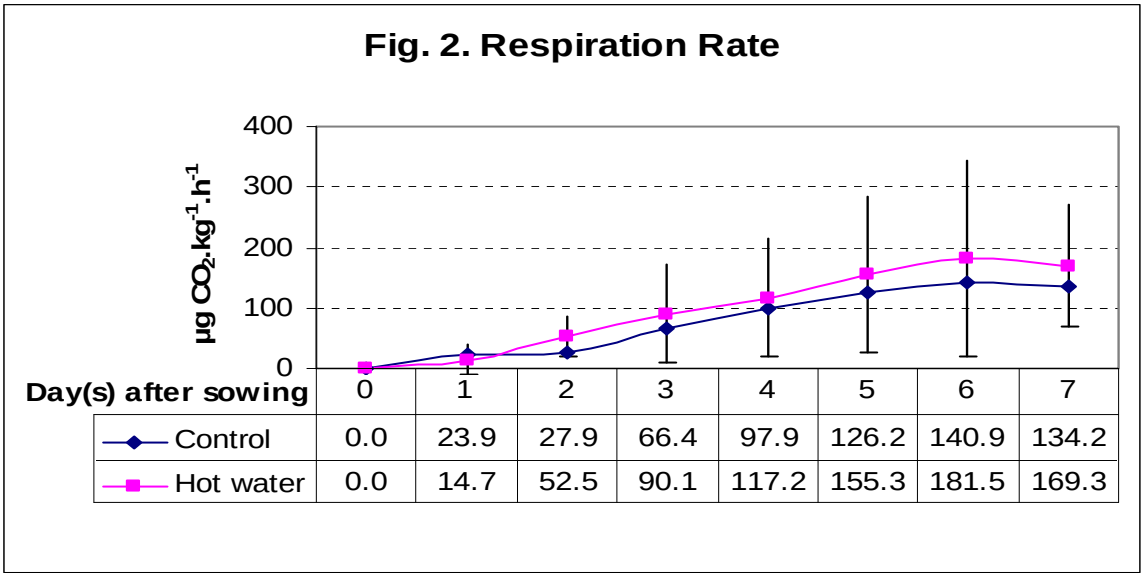
Effect of breaking dormancy on respiration rate and ethylene production

The respiration rate of both treatments were increased and correlated with germination rate when the germination increased but slightly slow down after sowing 6 days at both treatments (Table 2 and Fig. 2). This result implied that the respiration rates decreased due to the concentration of supplying oxygen in the enclosed jars during incubation period (24 hrs) was reduced. It consisted with theory: "Once germination begins, the rate of cell respiration increases greatly during germination and more oxygen is required". If the oxygen supply during germination is limited or reduced, germination can be severely retarded or inhibited. The respiration of untreated seed is lower when compared to which in breaking dormancy treatment after 3 days further to 7 days after sowing, but not significant different.

Table 2. Respiration rate (µg CO_2.kg^{-1}.h^{-1})

DAS*	0	1	2	3	4	5	6	7
Control	0.0	23.9	27.9	66.4	97.9	126.2	140.9	134.2
Warm water	0.0	14.7	52.5	90.1	117.2	155.3	181.5	169.3
LSD5%		***23.8***	***33.4***	***80.9***	***98.7***	***128.6***	***161.2***	***100.2***
CV%		***54.6***	***36.7***	***45.7***	***40.6***	***40.4***	***44.2***	***29.2***

* days after sowing

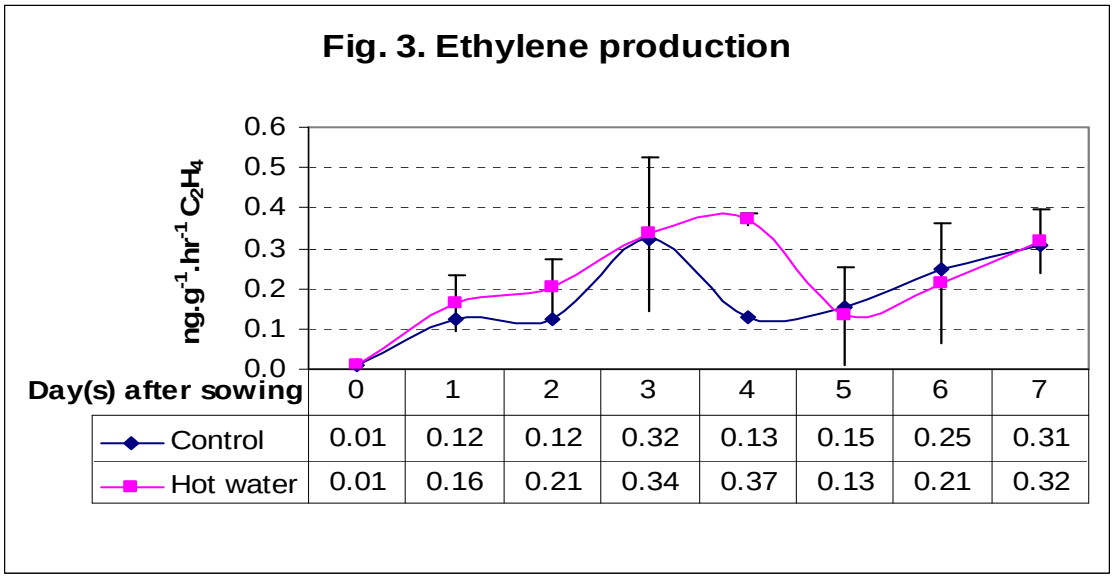


The ethylene evolution was increased and highest at 4 days after sowing in warm water treatment during germination (Table 3 and Fig. 3). The ethylene production decreased after it reached a peak at 4 days after sowing then increased again from 5 to 7 days after sowing. In the control, the same pattern of ethylene production was obtained, but the maximum rate of ethylene production occurred one day earlier and at a lower rate of ethylene production. Although this result was different from previous studies - germination of rice seeds were always accompanied by an increase in ethylene synthesis - it still indicated breaking dormancy methods also enhanced ethylene production.

Table 3. Ethylene production ($ng.g^{-1}.h^{-1}$ C_2H_4)

DAS*	0	1	2	3	4	5	6	7
Control	0.01	0.12	0.12	0.32	0.13	0.15	0.25	0.31
Warm water	0.01	0.16	0.21	0.34	0.37	0.13	0.21	0.32
LSD5%		0.07	0.065	0.19	0.014	0.12	0.15	0.08
CV%		21.6	17.5	26	25.4	37.4	28.9	11.9

*day after sowing



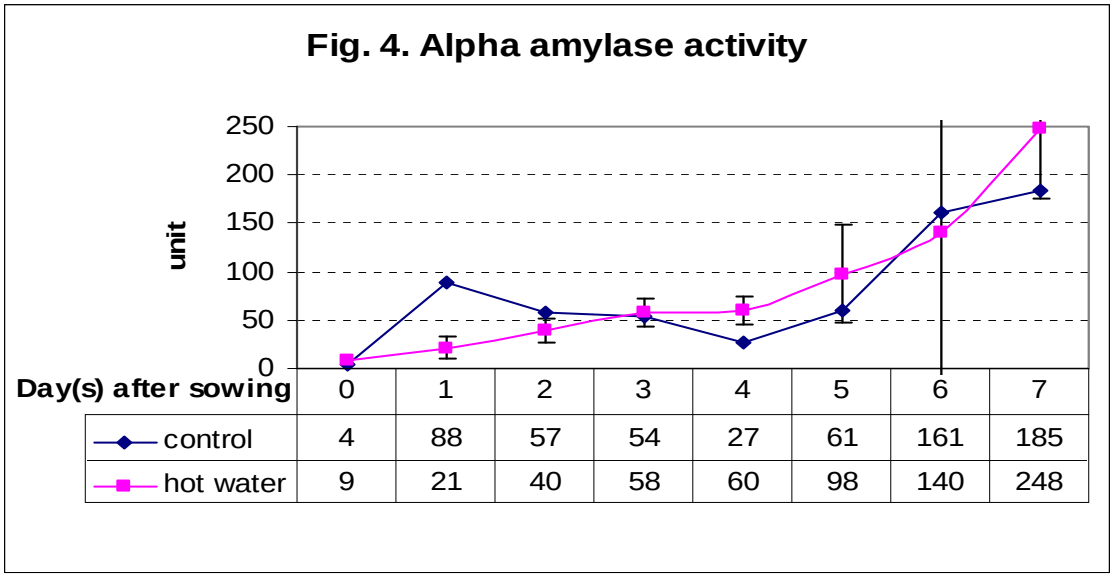
Effect of breaking dormancy on α-amylase activity

α-amylase activity increased rapidly in water treatment during germination of rice seed, whereas the increasing of the untreated seeds were slow and irregular (table 4 and Fig. 4). It can be explained that might be due to the activity of fungus, which occurred and attached the untreated seeds after 24h of incubation, caused disordered α-amylase activity in untreated seed while almost none of fungus was found on seeds were treated with warm water. In general, the α-amylase activity of the untreated seed was lower than that in seeds were treated with warm water during germination.

Table 4. Alpha amylase activity

DAS*	0	1	2	3	4	5	6	7
control	4	88	57	54	27	61	161	185
warm water	9	21	40	58	60	98	140	248
LSD 5%		11.9	12.2	14.7	13.7	50.5	159.4	72.1
CV%		9.6	11.1	11.7	13.9	28.1	46.7	14.7

*day after sowing



Day(s) after sowing	0	1	2	3	4	5	6	7
control	4	88	57	54	27	61	161	185
hot water	9	21	40	58	60	98	140	248

CONCLUSION

The method of breaking rice seed dormancy in this experiment not only effectively enhanced germination rate together with enhance respiration rate, ethylene production and α-amylase activity but also limited fungus infection during germination of rice seed.

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Phá miên trạng hạt lúa bằng nước ấm

Thí nghiệm này được thực hiện nhằm mục đích tìm hiểu tác động của phương pháp phá vở miên trạng của hạt lúa, bằng cách ngâm chúng trong nước ấm, đến quá trình trao đổi chất (hô hấp, giải phóng ethylene và hoạt động của enzyme α-amylase) và tốc độ nảy mầm như thế nào. Kết quả của thí nghiệm cho thấy rằng việc ngâm hạt lúa trong nước ấm 50^0C trong vòng 15 phút không những đẩy nhanh tốc độ nảy mầm của hạt lúa rỏ rệt cùng với thúc đẩy quá trình hô hấp, giải phóng ethylene và hoạt động của enzyme α-amylase mà còn hạn chế được sự tấn công của nấm bệnh trong suốt quá trình nảy mầm của hạt lúa. Cho đến nay, một nghiên cứu sâu hơn đối với những phương pháp phá vở miên trạng hạt như vật lý, hóa học hay sử dụng hormone thực vật cần phải thực hiện.