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Research Article
Effect of Seed Dormancy Breaking Treatment and Storability on Seed Quality of Indigo (*Indigofera tinctoria* L.)

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Abstract

Background and Objective: Indigo seeds are small with hard and thick seed coat, affecting seed germination. The objectives were to study seed dormancy breaking and storage period that affect the quality of indigo seeds. **Materials and Methods:** The experiment was a completely randomized design with 4 replications in 15 treatments, control, seeds soaking in water (80°C) for 15, 30, 45 and 60 sec, seeds soaking in water at room temperature for 24, 48 and 72 hrs, seeds incubation in hot air oven (50°C) for 24, 48, 72, 96, 120 and 144 hrs and smashing of seeds using mortar. The next experiment was to study the storage period that affects the quality of indigo seeds by storing indigo seeds at room temperature and refrigerator for 1, 2, 3, 4 and 5 months. **Results:** The result showed that the treatments of soaking the seed at room temperature for 72 hrs can break the dormancy of the seeds the most. This resulted in the highest germination (71.75%) and control had the lowest germination, 15.25% ($p<0.01$). For the storage period: It was found that the percentage of germination and the highest germination index were 38.50 and 10.40% respectively when indigo seeds were stored at room temperature for 1 month. **Conclusion:** Indigo seeds dormancy could be broken by soaking for 72 hrs and seeds could be stored at room temperature. This knowledge was increasing the germination percentage and preserving of quality and yield of indigo seeds.

Key words: Indigo, seed, dormancy, germination, storage, soaking

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indigo (*Indigofera tinctoria* L.) is a leguminous plant shrub (Family Fabaceae) that was found to have 750 species worldwide[1]. In Thailand, 30 species of indigo were found[2]. Two of these species are used for dyeing: *Indigofera tinctoria* and *Indigofera arrecta* due to the special properties of indigo, which are durable and have a distinctive colour. In Thailand, indigo is exported and planted a lot in the northeast region, especially in Sakon Nakhon province. People have been planting and indigo dyeing for a long time, increased production requires more indigo planting and requires a lot of seeds to get enough indigo to dye fabric.

Indigo seeds are small with hard and thick seed coat affecting seed germination. Dormancy and integument thickness have significant effects on the emergence and establishment of seeding from the soil. Hydro-priming, mechanical or chemical scarification can break seed coat where metabolic inhibitor of seed is located[3]. Therefore, seeds dormancy breaking was studied to increase germination: soaking indigo seeds in water resulted in a higher percentage of vigour and germination[4]. Also, soaking coriander seeds[5] and report of Singh *et al.*[6], confirmed that hydro primed Cowpea seeds had higher total germination (66.00%) than unprimed seeds (53.00%) and soaking seeds in water have accelerated Faba bean germination and seedling emergence[7] as well as soaking rice in water[8]. Moreover, it was found that soaking seeds in hot water (80°C) for 60 sec increased the germination of *Iliamna remota*[9] similar to Mavengahama and Lewu[10] study who reported that the *Corchorus olitorius* L. was soaked in hot water (80°C) for 10 min. There was a report from Maesaroh and Demirbağ[3] which mentioned that scarification by sandpaper could be an alternative to break the hard seed coat of *Indigofera zollingeriana*. Besides, to break the dormancy, doing scarification with the blade to cut the indigo seed coat are applied[11]. Seed dormancy breaking is not only able to increase higher seed germination but also causes plants to have good growth and be more productive. It can increase the number of branches and pods and the height of plants[12] as well as increase grain yields[13]. Forever, dormancy breaking method on germination rate depends on the plant species[14]. Therefore, this study aimed to find out the methods to break the dormancy of indigo seeds to increase seed germination and study the storage period affecting indigo seed quality to get high-quality seeds for further planting.

MATERIALS AND METHODS

Study area: All the experiments were performed from October, 2015 to September, 2016 in the Plant Science Laboratory, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Thailand.

Seed sample: Indigo seeds were harvested from the field of Plant Science, Sakon Nakhon Rajabhat University, Thailand. There were 2 experiments, experiment1: studying the methods of breaking dormancy of indigo seeds by completely randomized design with 15 treatments, 4 replications. Treatment 1: control, treatment 2, 3, 4 and 5: Soaking of seeds in hot water (80°C) for 15, 30, 45, 60 sec, treatment 6, 7 and 8: Soaking of seeds in water at room temperature for 24, 48 and 72 hrs, treatment 9, 10, 11, 12, 13 and 14: Incubation of seeds in a hot air oven (50°C) for 24, 48, 72, 96, 120 and 144 hrs and treatment 15: Smashing of seeds by using a mortar for 2 min, then testing of the germination percentage to find normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. Experiment 2: studied the storage period of indigo seeds by storing them in a zipped plastic bag and placed in a plastic box, then put in two different conditions, refrigerator (10°C) and room temperature (25°C) for 0, 1, 2, 3, 4 and 5 months. After that, they were tested for moisture, germination and seed germination index.

Seeds moisture: Five grams of indigo seeds per replication (2 replications) were weighed before drying in a hot air oven, then incubated with the temperature of 105°C for 48 hrs. After drying, the seeds were weighed and found out the moisture content from the formula of Siddique and Wright[15]:

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}}$$

Seed germination: Germinated indigo seeds by using between paper method (BP), 100 seeds per replication and 4 replications. Placed them at room temperature, germination was counted after 4 and 7 days of seedling after germinating. The number of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds was counted and then the total germination percentage was calculated from the formula of Elouaer and Hannachi[16]:

$$\text{Germination (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total seed}} \times 100$$

Germination index: Indigo seeds were germinated by using between paper method (BP), 100 seeds per replication and 4 replications afterwards placed them at room temperature, counted the number of seedlings every day for 7 days. Then GI was calculated from the formula of Akkajit and Nuamkongman[17]:

$$\text{Germination index (GI)} = \sum \frac{\text{Number of seeds that emerged on the day}}{\text{Days after planting}}$$

Data analysis: The data were statistically analyzed using the ANOVA (Analysis of Variance) technique. Mean were separated using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Indigo harvesting: Harvested 170 days of indigo in Fig. 1a by cutting the whole trees. Then removed the pods from the trees as shown in Fig. 1b, cracked the seeds as in Fig. 1c and prepared for the next step test.

Seed dormancy breaking: There were 14 treatments in breaking the dormancy of indigo seeds which could increase germination of indigo seeds in Table 1. Besides, it was found that breaking of dormancy of indigo seeds affected seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds were statistically different in Fig. 2 as follows.

Seed germination (%): soaking the seeds in water at room temperature for 72 hrs gave the highest germination (71.75%). Followed by 24 and 48 hrs were 52.25 and 52.00%, respectively. For the control seeds, it was found that the indigo seed germination had the lowest germination (15.25%). When analyzing the variance, it was found that there were significant differences (Table 1). For the seeds soaked in hot water (80°C), the germination was 26.00-49.75%. Then in smashing the seeds with a mortar, it was found that seed germination was 34.25%. Incubation of seeds in a hot air oven (50°C), the seeds had the lowest germination (Table 1). This was consistent with the research of Singh *et al.*[6], which confirmed that hydro primed Cowpea seeds for 10 hrs had higher total germination (66.00%) than unprimed seeds (53.00%) and seeds hydro-priming have accelerated Faba bean germination[7]. Moreover, from the research of Sy *et al.*[4] indigo seeds soaking in water could cause the highest germination. Because soaking seeds in water could soften the seed coat and allow the water to penetrate inside the seeds faster. Using hot water (80°C) for 30 sec could increase germination (49.75%) because hot water could crack the seed coat, help dissolve the waxy cuticle and soften the seed coat. Water could penetrate the seeds easily. However, hot water could cause the most abnormal seedlings. It also affected seedling weight[9]. On the other hand, smashing seeds could increase indigo seed germination. This was consistent with the study of Maesaroh and Demirbağ[3], who found that using sandpaper could break seed dormancy. Whilst using a hot air oven (50°C) was not suitable to break indigo seeds dormancy because the germination of the seeds was low compared with other treatments (18.75-26.50%).

Abnormal seedlings, hard seeds, fresh seeds and dead seeds: The highest percentage of abnormal seedlings (8.00%) was found after soaking the seeds in hot water (80°C) for

Table 1: Germination percentage of indigo seeds using different seed breaking dormancy treatments

Treatments	Germination (%)
Control	15.25^{g}
Soaking the seed in hot water at 80°C (15 sec)	26.00^{ef}
Soaking the seed in hot water at 80°C (30 sec)	49.75b^{c}
Soaking the seed in hot water at 80°C (45 sec)	40.25^{d}
Soaking the seed in hot water at 80°C (60 sec)	42.75^{cd}
Soaking the seed in water at room temperature (24 hrs)	52.25^{b}
Soaking the seed in water at room temperature (48 hrs)	52.00^{bc}
Soaking the seed in water at room temperature (72 hrs)	71.75^{a}
Seed incubation at 50°C (24 hrs)	18.75^{fg}
Seed incubation at 50°C (48 hrs)	20.50^{fg}
Seed incubation at 50°C (72 hrs)	26.50^{ef}
Seed incubation at 50°C (96 hrs)	20.50^{g}
Seed incubation at 50°C (120 hrs)	22.75^{fg}
Seed incubation at 50°C (144 hrs)	22.50^{ef}
Smashing the seed	34.25^{de}
F-test	**
CV (%)	16.75

Means within a column followed by the same letter are not significantly different by DMRT, **Significant at p<0.01. hrs: hours, sec: seconds

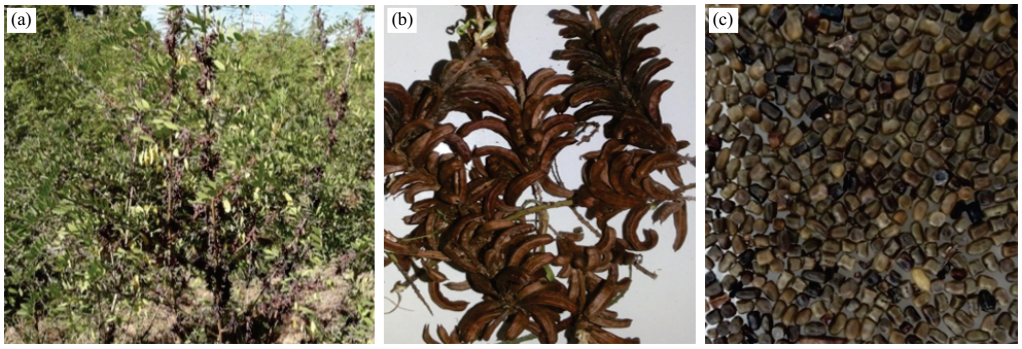

Fig. 1(a-c): Indigo plants on the field at
(a)170 days , (b) pod and (c) seeds

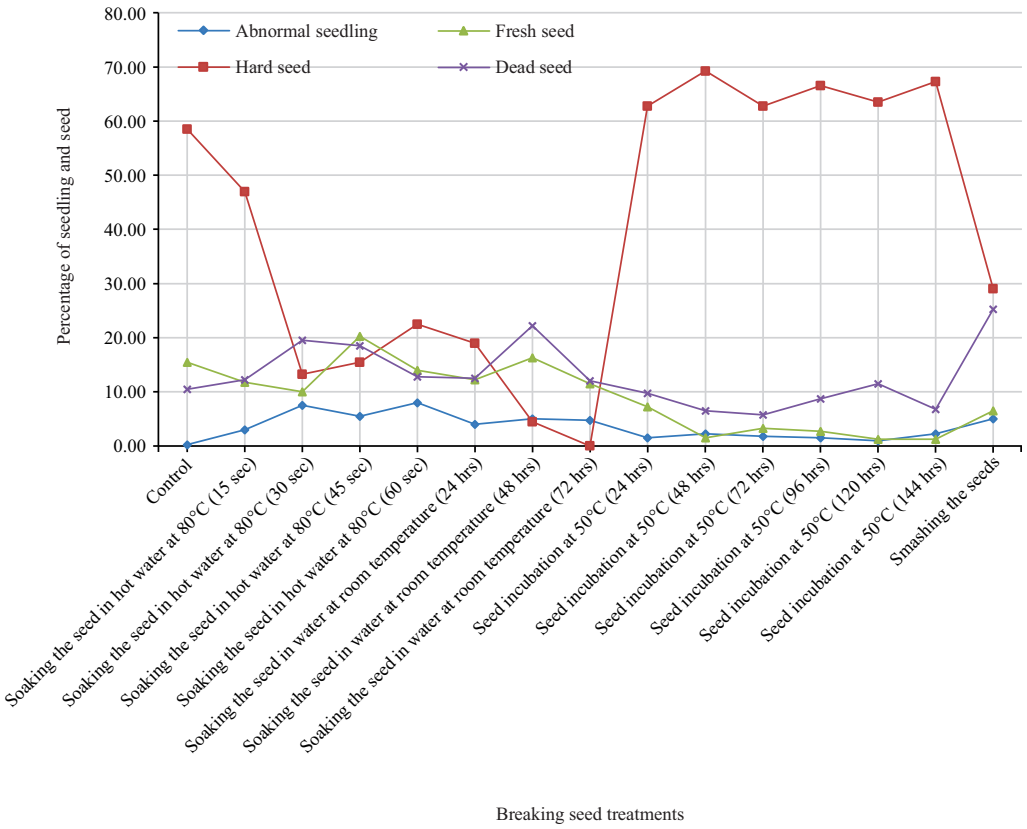

Fig. 2: Result of breaking dormancy of indigo seed using different treatments

60 sec while the lowest percentage of abnormal seedlings (0.25%) was found in the control treatment (which is untreated). These data are shown in Fig. 2. This suggests that hot water caused abnormal seedling germination and damaged the seed structure. Hard seeds were also noted in this study. The highest percentage of hard seeds was from incubating the seeds in a hot air oven (50°C) while no hard seeds were recorded from soaking the seeds in water at room temperature. The highest percentage of fresh seeds (20.25%) was gained from soaking in hot water (80°C) for 45 sec while the lowest (1.25%) was gained from incubating in a hot air oven (50°C). Moreover, dead seeds were also noted. The highest percentage of dead seeds (25.25%) were caused by smashing the seeds using a mortar while incubating seeds in a hot air oven (50°C) yielded the lowest percentage of dead seeds which was 5.75% (Fig. 2). Furthermore, the scarification treatment can cause excess water absorption, which can lead to degeneration and death of seeds.

Table 2: Seed moisture, germination and germination index of indigo seeds stored in the refrigerator for 0, 1, 2, 3, 4 and 5 months

Storing period (month)	Seed moisture (%)	Germination (%)	Germination index (GI)
0	9.85	15.25	5.50
1	11.10	16.00	3.91
2	10.80	17.50	4.07
3	11.4	17.00	3.40
4	10.90	18.75	3.95
5	10.70	19.75	3.81
F-test	ns	ns	ns
CV (%)	6.62	25.28	22.50

Means within a column followed by the same letter are not significantly different by DMRT, ns: Non-significant at p>0

Table 3: Seed moisture, germination and germination index of indigo seeds stored at room temperature for 0, 1, 2, 3, 4 and 5 months

Storing period (month)	Seed moisture (%)	Germination (%)	Germination index (GI)
0	9.85^{b}	15.25^{b}	5.50^{c}
1	11.40^{a}	38.50^{a}	10.40^{a}
2	11.70^{a}	21.75^{b}	5.11^{c}
3	11.10^{a}	18.25^{b}	4.17^{c}
4	10.90^{a}	35.75^{a}	7.52^{b}
5	10.80^{a}	20.50^{b}	4.49^{c}
F-test	*	**	**
CV (%)	3.44	17.76	16.89

Means within a column followed by the same letter are not significantly different by DMRT, * Significant at p<0.05, **Significant at p<0.01

Storage duration on seed quality

Seed moisture: Before storing, seeds moisture was 9.85%. The seeds were stored in the refrigerator, the average moisture was 10.98% over the 5 months storage period with no statistical difference in Table 2. At room temperature, it was found that the seeds had average moisture throughout the storage period of 11.18% with statistically significant differences in Table 3. To store the indigo seeds, the moisture of the seeds changed slightly when stored in the refrigerator. The moisture before storing was 9.85%. After 1 month of storage, the moisture of the seeds changed to 11.10%. After that, the moisture was lessened because the moisture of seeds could be controlled. This also occurred with seeds stored at room temperature.

Seed germination: before storing, seed germination was 15.25%. Then after having stored in the refrigerator for 1, 2, 3, 4 and 5 months, the indigo seed germination percentage increased slightly without statistical differences (Table 2). For the indigo seeds stored at room temperature, they had higher germination with statistically significant differences (Table 3).

Germination index: Before storing, the germination index of the seeds was 5.5. Then after storing, the seed germination index showed no statistical differences (Table 2). At room temperature, the germination index was significantly different in statistics (Table 3).

In terms of germination and germination index of indigo seeds that were stored in both conditions, the results showed that after the first month of room temperature storing, the seed germination increased from 15.25-38.50%, then decreased in the third month of storage. This was consistent with Saltana *et al.*[18], germination percentage obtained after 2, 4 and 6 months of storage were 77.87, 29.21 and 8.90%, respectively to its initial value before storage. It was decreased with an increase in storage duration.

From the study of treatment in breaking dormancy of indigo seeds to increase seed germination, it was found that all 14 treatments could break indigo seed dormancy. They were divided into 4 groups as follows: group 1: Seeds soaked in water at room temperature could break the seed dormancy, group 2: Seeds soaked in hot water (80°C), which could moderately break the seeds' dormancy, group 3: Seeds smashed could break the dormancy of seeds, group 4: Seed incubated in hot air oven (50°C), which could break the seed dormancy the lowest.

Indigo seed dormancy breaking in water at room temperature for 72 hrs could give the highest germination (71.75%). Also, this was similar to Chickpea seeds which were soaked in water for 12 hrs helps increase seedling emergence and seedling vigour index as compared to seed not soaked[19], also corn[8] and coriander[5], including, tomatoes, soybean, peppers, wheat, salads and cabbage included[20]. However, the duration of soaking seeds in water to increase the germination

was dependent on the species, size, structure and composition of the seeds. If the seeds have a thick seed coat, they may take a longer time to soak.

CONCLUSION

The most effective method in breaking the indigo seed dormancy was soaking them at room temperature for 72 hrs. The highest germination of the seeds was 71.75%, followed by soaking the seeds in water at room temperature for 24 and 48 hrs with germination of 52.25 and 52.00%, respectively. The indigo seeds could be stored for up to 5 months, if stored in a zipped plastic bag placed in a plastic box at room temperature condition. The percentage of seed germination and the highest germination index were 38.50 and 10.40%, respectively after one month of storage.

SIGNIFICANCE STATEMENT

This study discovers the dormancy breaking of indigo seeds by soaking them in the water at room temperature for 72 hrs and it can be stored at room temperature that can be beneficial for academicians, farmers and the general public. This study will help the researcher to uncover the critical areas of the Sakon Nakhon province that many researchers were not able to explore. Thus, a new application using for increasing the germination percentage and preserving of quality and yield of indigo seeds may be arrived at.

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