Effects of Seed PEG Immersion And Hydration-Dehydration on Rice Physiological Quality

N Rozen¹, M Kasim², A Anwar¹

¹ Department of Agrotechnology, Andalas University, Padang city, Indonesia

Abstract. Storage of seeds really determines the storability and quality of seeds, because Long-term storage, the seeds will deteriorate. Deterioration of seed quality (deterioration) is inevitable but can be slowed by good and proper storage. To maintain seed quality, proper storage methods need to be carried out. The deterioration of the seeds can be improved by seed PEG immersion and hydration-dehydration. The objective of this research is to obtain an appropriate invigoration method to improve physiological seed quality. Method: In this research, we used factorial two factors, we examine the effects of PEG immersion and hydration-dehydration treatment on three rice varieties; Batang Piaman, PB42, and IPB3S. Results: The results showed that PEG immersion and hydration-dehydration of three cultivars interacted with the index value. Conclusion: Our findings indicate that the invigoration method significantly improves the viability and vigor of Batang Piaman from 50% to 70% and 46.67% to 70% (PEG immersion), respectively. Keywords: Deterioration; Storage Method; Batang Piaman; PB42; IPB3S

1. Introduction
Seed quality is determined by four quality categories namely genetic quality, physiological quality, physical quality and pathological quality. Quality seeds will provide high yields with good maintenance of plants. Quality seeds are characterized by upright sprouts that grow fast and simultaneously, sturdy sprouts and grow normally. In maintaining the quality of seeds, it is important to consider how to process seeds properly and correctly because if it is wrong in handling seeds, the quality will decrease.

Deterioration of seed quality could not be stopped but can be slowed down with proper handling. According [1] seed setbacks can be identified biochemically and physiologically. Biochemical indications of seed decline are marked by decreased enzyme activity, decreased food reserves, decreased respiration rate and increased conductivity values. The indicative physiology of seed setback is decreased vigor and viability. Physiological symptoms are also influenced by decreased enzyme activity (dehydrogenase, glutamate decarboxylase, catalase, peroxidase, phenolase, amylase, cytochrome oxidase) and decreased respiration (low O2 consumption, low CO2 production, low ATP production). While the main conditions for increasing crop production are using quality seeds.

Efforts that can be made to overcome the problem of seed setback are invigoration. Seed invigoration is the treatment given to seeds before planting with the aim of improving germination and germination growth [2]. Invigoration methods that have been carried out on sorghum plants include: osmoconditioning, bio-matriconditioning, and hydration-dehydration [3].
One method of invigoration is hydration-dehydration. According to [3] hydration-dehydration is the treatment of moisturizing or soaking the seeds within a certain time followed by drying the seeds until returning to their original weight. Seeds treated with hydration-dehydration turned out to have a percentage of germination, maximum growth potential, first count germination, and soil emergent tests, and a higher index value than seeds without hydration-dehydration. This shows that invigoration by hydration-dehydration can actually optimize the viability of seeds that have been stored for 8 months [4].

Seed deterioration will occur during storage and can be slowed down by proper storage. Seed deterioration is more common at retailers and farmers than at distributor level so that seeds planted in the crop are less likely to grow properly. Seed quality is generally indicated by seed vigor and viability. [5] high-quality seeds have a viability of at least 80%. A decrease in the value of seed viability is usually caused by deterioration factor. Many studies have been conducted invigorating the seeds to improve seed viability after long term storage. Seed Viability improved from 76% to 83% and plant growth from 72% to 78% in hydration-dehydration treatment. Seed germination of pepper increased from 76% to 88% in the presence of KNO3 (1%).

A decline in physiological function is characterized by a decrease in germination, an increase in the appearance of abnormal sprouts, a decrease in the appearance of normal sprouts, slow growth and development of plants, and increased sensitivity to the environment. According to [6] seed deterioration is indicated on the decline in seed quality and the ability of seeds to germinate. Several factors that affect the rate of seed decline are seed type, seed mass, seed damage, ambient temperature and humidity, harvest handling, and seed storage. Seed storage is affected by several factors: genetic variation, pre-harvest conditions, seed and its chemical structure and composition, seed maturity, seed size, seed moisture content, mechanical damage and seed vigor. Seed invigoration is an alternative to improve the quality of seed in long-term storage by activating seed germination. Seed invigoration can improve seed ability to grow, reduce the rate of seed deterioration, and increase in growth rate. According to [7] PEG immersion techniques on seed invigoration proved to be effective for improving viability, Seedling emergence, and absorption of nutrients in wheat germ provided with hormone and vitamin. The aim of this study was to investigate the effects of PEG immersion/hydration-dehydration treatment on three rice varieties; Batang Piaman, PB42, and IPB3S and improve seed quality.

2. Materials And Methods

2.1. Location and Time of Experiment
The research was conducted at the Seed Science and Technology Laboratory, Agrotechnology Department, Faculty of Agriculture, Universitas Andalas and at the LLDIKTI region X Laboratory in Padang, West Sumatera, Indonesia from September to November 2019.

2.2. Research Design
This research was based on the completely randomized design (CRD) with two factorial arrangement in 4 replicates. The first factor is consisting of 3 levels, i.e. PB42, Batang Piaman, and IPB3S. The second factor is consisting of 3 levels, i.e. without invigoration, hydration-dehydration and PEG immersion. The observational data were quantitatively analyzed by the 5% significance level, any significant difference will be continued by the 5% Tukey's honestly significant difference (HSD).

2.3. Materials and tools
The materials used are the seed rice that has been stored for 1 year i.e. PB - 42, Batang Piaman, and IPB3S, stencil paper, distilled water, detergent, sodium hypochlorite 1%, tissue, 100% alcohol, 96% alcohol, 80% alcohol, 70% alcohol, sand, 1% safranin, FAA solution, hard paraffin, formalin 4%, acetic acid, xylol, HCl solution, KOH solution, methyl orange indicator, phenolphthalein indicator, label paper selenium mix, concentrated sulfuric acid, benzene, fast green solution, Whatman paper, 1% starch substrate, DNS, and cotton, while the tools used are handsprayer, analytical balance, germinator, seedbed, cup glass, cup set, oven, desiccator, camera, aspirator, vial bottle, measuring cup,
dropper, burette, jar, erlenmeyer, aluminum cloud, porcelain cup, electric furnace, blanket, kjedahl flask, refrigeration, object glass, hot plate, mortal, spectrophotometer, stationery, plastic box, and microscope.

2.4. Seed treatments
The 2000 rice grains were stored for 1 year in plastic at ± 20-25°C. Seeds used in this study had a decline in percent germination of 40-50%. Rice seeds were surface sterilized in aquadest for 2 minutes, then soaked in 1% sodium hypochlorite for 2 minutes, and soaked again in aquadest for 2 minutes. The seeds were then air-dried for 5 minutes at room temperature.

Treatment without invigoration is done without PEG immersion and without hydration-dehydration. The 600 rice seeds were used for hydration-dehydration treatment. The seeds were weighed and hydrated in aquadest for 1 hour and the seeds were then air-dried for 5 minutes. Then, the seeds were dehydrated in an oven for 6 hours at 40°C. Finally, The seeds were removed from the oven and weighed. The seeds were treated in PEG 6000 and the first count test, normal/abnormal sprouts, dead sprouts, maximum growth potential, and index values were measured.

2.5. Sterilization Equipment
The tools used such as aluminum plates, goblets, tweezers, hand sprayers, and germinators, washed thoroughly using detergent, then sprayed with 1% sodium hypochlorite, then washed with aquadest, sprayed with 70% alcohol and dried with tissue paper.

3. Results and Discussion
3.1. Invigoration Effects on the First Count
The results (Table 1) showed there was a significant effect of all three varieties with invigoration for the first count test. Each variety had a different response to the invigoration and Batang Piaman was more responsive than PB42 and IPB3S. The PEG immersion technique showed a higher first count value than the hydration-dehydration technique. The Batang Piaman showed a higher first count value than PB42 and IPB3S. The softened Batang Piaman seed after soaking may affect seed imbibition to be much faster. It is likely that the soaking softened the seed, therefore water and oxygen easily entered the seeds. Batang Piaman has thinner seed compared to PB42 and IPB3S, thus accelerate imbibition rate and seed germination as stated by [8] that priming has proven useful in improving viability in agricultural crops.

| Cultivars     | Invigoration                   | Mean   |
|---------------|--------------------------------|--------|
|               | Without Invigoration | PEG Immersion | Hydration-Dehydration |
| Batang Piaman | 46,67 | 70,00 | 42,00 | 52,89 a |
| PB42          | 14,67 | 40,00 | 14,00 | 22,89 b |
| IPB3S         | 2,00  | 24,67 | 4,67  | 10,44 c |
| Mean          | 21,11 B | 44,89 A | 20,22 B |

C.V = 10,79 %

Different uppercase letters in same row and lowercase letters in same column indicate statistically no significant difference (5%) according to Tukey HSD. C.V= Coefficient of variation

Low quality seeds will be difficult to germinate normally because during storage there is already enzyme activity that secretes electrolytes so that food reserves have been reduced due to respiration.
Batang Piaman varieties where viability is initially higher than PB42 and IPB3S varieties can increase the first count test from 46.67% without invigoration to 70% with PEG treatment. PEG treatment is better than dehydration-dehydration on rice seeds that have been stored this year in unfavorable conditions. Although peg granting can be profitable but only at a certain level. [9] stated that too high PEG levels would cause seed viability to decrease even if it did not result in death. [4] further stated that too high PEG levels will cause osmotic pressure in cells to become negative so that water is difficult to absorb by the seed because the water absorbed by the seed in small amounts.

3.2. Invigoration Effects on Abnormal Sprouts
Abnormal sprouts value with invigoration on the three cultivars showed no real interaction, but the varieties had a significant effect. Each variety had a different response to the invigoration and the abnormal sprouts will also be significantly different. Batang Piaman and PB42 were relatively similar and significantly different from IPB3S. It is very apparent that the response of each variety is different from the given invigoration method. The invigoration method can improve seed viability so that for seeds that have been stored for a long time and have fallen in quality, they can be treated with invigoration so that seed quality can be improved. Seeds that have suffered setbacks will form a lot of abnormal sprouts. This is because the seeds have experienced respiration during storage. Thus the food reserves have been overhauled as a result of germination of seeds that will become abnormal sprouts, even the seeds become dead. During storage when respiration occurs, the enzyme will actively overhaul food reserves so that electrolytes will come out of the seed.

| Cultivars    | Invigoration                          | Mean   | C.V = 10.25 % |
|--------------|---------------------------------------|--------|---------------|
|              | Without Invigoration                  |        |               |
| Batang Piaman| 6,67                                  | 6,00   | 6,00          | 6,22 a         |
| PB42         | 8,67                                  | 6,67   | 6,67          | 7,33 a         |
| IPB3S        | 3,33                                  | 6,00   | 3,00          | 4,11 b         |
| Mean         | 6,22                                  | 6,22   | 5,22          |

Different uppercase letters in same row indicate statistically no significant difference (5%) according to Tukey HSD. C.V= Coefficient of variation.

3.3. Invigoration Effects on Dead Sprouts
The dead sprouts showed no real interaction compared to the single factor (significant effect). Table 3 showed that the dead sprouts were higher in IPB3S compared to PB42 and Batang Piaman. It is because of IPB3S incapable for long term storage where the dead sprout already had high value with/without invigoration. Each variety had a different response to the invigoration and the dead sprouts will also be significantly different. Seed PEG immersion or hydration-dehydration significantly reduces dead sprouts. Seed invigoration in pre-sowing can greatly improve seed quality. Abnormal and dead sprouts are indicators of low viability and physiological quality because metabolic activities have taken place in the cell during storage and the germination will produce abnormal or dead sprouts. Low-quality seeds will not provide growth or good results.
Table 3. Effects of Invigoration on Dead Sprouts in Batang Piaman, PB42, and IPB3S

| Cultivars    | Without Invigoration | PEG Immersion | Hidration-Dehydration | Mean     |
|--------------|----------------------|---------------|-----------------------|----------|
| Batang Piaman| 43,33                | 24,00         | 24,00                 | 30,44 a  |
| PB42         | 69,33                | 55,33         | 55,33                 | 60,00 b  |
| IPB3S        | 76,67                | 65,33         | 68,33                 | 70,11 c  |

Mean: 63,11 A, 48,22 B, 49,22 B

C.V = 9,36%

Different uppercase letters in same row and lowercase letters in same column indicate statistically no significant difference (5%) according to Tukey HSD. C.V= Coefficient of variation.

3.4. Invigoration Effects on Maximum Potential Growth

The maximum growth potential of several rice varieties with invigoration shows that there is no real interaction, but a single factor has a significant effect. The maximum growing potential reflects the viability of the seed, where the higher the value, the higher the viability of the seed. From the above data it is seen that Batang Piaman varieties have the highest maximum growth potential value and differ manifestly from PB42 and IPB3S varieties. This is because genetically the Batang Piaman variety is more of a response to the given method of invigoration. This indicates that batang piaman varieties are indeed higher viability initially so that by providing invogiation to the seeds then the Batang Piaman variety will increase its viability more quickly.

Table 4. Effects of Invigoration on Maximum Potential Growth in Batang Piaman, PB42, and IPB3S

| Cultivars    | Without Invigoration | PEG Immersion | Hidration-Dehydration | Mean     |
|--------------|----------------------|---------------|-----------------------|----------|
| Batang Piaman| 56,67                | 76,00         | 76,00                 | 69,56 a  |
| PB 42        | 30,67                | 44,67         | 44,67                 | 40,00 b  |
| IPB 3S       | 23,33                | 34,67         | 31,67                 | 29,89 c  |

Mean: 36,89 B, 51,78 A, 50,78 A

C.V = 10,05%

Different uppercase letters in same row and lowercase letters in same column indicate statistically no significant difference (5%) according to Tukey HSD. C.V= Coefficient of variation.

3.5. Invigoration Effects on Index Value

The index value of Batang Piaman was higher than PB42 and IPB3S with PEG immersion and hydration-dehydration. Each variety had a different response to the invigoration. Seed PEG immersion and hydration-dehydration treatment positively increase the index value. The higher index value indicated high vigor seeds. The result showed that invigoration on three rice varieties can improve the viability of low-quality seeds. it is because PEG immersion and hydration-dehydration are able to recover damaged seeds.
### Table 5. Effects of Invigoration on Index Value in Batang Piaman, PB42, and IPB3S

| Cultivars   | Without Invigoration (%) | PEG Immersion (%) | Hidration-Dehydration (%) |
|-------------|--------------------------|-------------------|----------------------------|
| Batang Piaman | 7.24 b                   | 8.48 b            | 14.14 a                    |
| PB 42        | 2.78 b                   | 3.20 b            | 5.20 a                     |
| IPB 3S       | 1.29 b                   | 1.60 b            | 3.25 a                     |

C.V = 4.32%

Different uppercase letters in same row and lowercase letters in same column indicate statistically no significant difference (5%) according to Tukey HSD. C.V = Coefficient of variation.

Many plants accumulate or produce proline as a protective or stress avoiding osmolyte. Priming enhances proline content in seeds. Leachate was increased with the treatment of hydration dehydration treatment than with hydro primed seeds [10]. Hydration dehydration induced cessation of leaching of essential components and increased the seed quality indicate the potential quality enhancement property of this unique cost-effective treatment which consequently hardened the seed coat or seed membrane resulted in enhanced seed germinability, plant growth, and metabolism [11].

### 3.6. Protein Content

The following are the protein content in seeds in several rice varieties with invigoration (Figure 1). In the figure, it can be seen that protein content increases with invigoration treatment compared to control in all three varieties. However, the hydration-dehydration treatment is higher than that of PEG. The IPB3S variety has higher protein content than PB42 and Batang Piaman varieties.

![Figure 1. Protein content in seeds of several rice varieties with invigoration](image)

Membrane proteins alongside phospholipids function to perform membrane functions. Decreased membrane phospholipid levels will affect the decrease in membrane function. Decreased levels of phospholipids and membrane proteins reflect the decline of seeds [12]. In addition, there has also been an increase in some metabolite results such as free fatty acids and sugar reduction resulting in damage...
to the protein [13], [14] mention that proteins in seeds are synthesized as sources of carbon, nitrogen and sulfur for the next generation of plants.

3.7. Carbohydrate content

Carbohydrate content in seeds in several rice varieties with invigoration treatment showed that the control was higher in carbohydrate content compared to invigoration treatment. This is caused by invigoration, imbibition will occur so that water enters the seed and will activate enzymes so that the enzyme will destroy carbohydrates into glucose. While in the control where the seeds are of low quality so the slow imbibition process occurs, as a result the enzyme activity is reduced, especially as many electrolytes have come out of the seed on the low quality seeds, so that an overhaul of food reserves has occurred while the seeds are stored. Carbohydrate levels are presented in Figure 2 below.

![Carbohydrate content in seeds of several rice varieties with invigoration treatment.](image)

The decrease in carbohydrates indicates a decrease in seed defense, namely a decrease in seed viability characterized by a decrease in seed germination power during storage [15]. [14] mentioned that the decrease in carbohydrate, protein and fat content can be caused by an overhaul of food reserves due to the increased respiration process due to high levels of seed water.

4. Conclusion

It was concluded that the seed PEG immersion and hydration-dehydration greatly improve the seed quality that experienced physiological degradation during long term storage. It is recommended to do invigoration in pre-sowing to enter the germination phase. In order for the seed to still give maximum results must be kept in correct and good condition.

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