Biochemical Test of Rice Seeds (*Oryza sativa* L.) that have been Invigored

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Abstract. Storage of seeds will affect the age of the seeds, so the storage of seeds must be done properly and correctly. During storage, the seeds will experience deterioration. Deterioration cannot be avoided but can be slowed as long as the condition of storing the seeds is well maintained. Deterioration the quality of rice seed greatly affect the growth and yield of rice in the field, to the need to find a solution with the method invigorasi such as the treatment of priming and hydration-dehydration. The purpose from Research of this is to know the biochemistry of seeds already in invigoration. This research has been carried out at LLDIKTI Laboratory Region X West Sumatra, Riau, Jambi and Riau Islands from September to October 2019. Experiment is this use Design Randomized Complete (RAL) with a two-factor factorial pattern. The first factor are varieties of rice (Batang Piaman, PB42, and IPB3S) and the second factor are methods invigorasi (priming and hydration-dehydration). Variables measured either be a seed moisture content, seed germination, soil emergent test, the content of ash, fat content, and respiration rate. The observational data were analyzed with F test of 5% significance level. If it is significantly different, it is continued with further testing of BNJ at the 5% level. The results showed that invigoration could be increase seed moisture content, seed germination, and soil emergent test. Treatment of seeds with invigoration can activate the biochemical processes in the seed, there are by increasing fat content, and respiration rate but decreasing ash content.

Keywords: Rice Seeds, Deterioration, Quality of Seed, Hydration-dehydration, Priming

1. Introduction

One of the problems in increasing rice production is the use of superior seeds that have experienced a decline in seed quality. Deterioration the quality of rice seed could not be avoided but can be slowed in a manner which is correct and proper storage. Storage at the retailer and farmer level is not as feasible as at the distributor level, so that deterioration in seed quality can occur. Superior high-quality seed, also will decline because of improper storage, so that after the seed sown in the field the seed viability is already low. Physiological quality is reflected by seed viability and vigor. [1] said that the seed is of good quality if the viability of the seed is at least 80%.

Seed quality deterioration is a gradual process of diminishing quality and cannot be reversed due to physiological changes caused by factors in the seed. The decline of a seed can be explained as a decrease in the quality / viability of the seed resulting in low vigor and low crop production [2]. A seed decline is a decrease in seed viability either by natural factors (deterioration) or by factors that are intentionally made (devigorated) [3].
The aging process or physiological retreat of vigor is characterized by a decrease in germination capacity, an increase in the number of abnormal sprouts, a decrease in the appearance of sprouts in the field, stunted plant growth and development, increased sensitivity to the environment [2]. The decline of a seed can be seen from the decline in seed quality and also the ability of seeds to germinate [4]. The seeds reach the highest vigor and viability during physiological maturity, after which the seeds begin to experience a decrease in vigour and viability, and eventually the seeds will die.

The factors that influence the length of a seed's life include the internal factors of the seed and the external factors of the seed [2]. Seed internal factors include genetic traits, physical condition and physiology of seeds. Seed that is damaged, cracked or broken will experience a decline more quickly than seeds that did not experience damage. Genetic traits that affect seed decline can be seen from the chemical composition of the seeds, such as seeds with high fat content will experience a faster decline when compared with seeds that contain high carbohydrates. External factors that affect the length of a seed's life period in storage include temperature, humidity and air pressure.

Some of the factors that influence the rate of deterioration of seeds in such as are: seeds, weight and part of the seed injured, humidity and temperature of the environment in the field, handling the harvest and seed storage conditions [5]. Low temperatures and humidity can increase the life period of seeds (for orthodox seed types) [6]. The shelf life of seeds is influenced by several factors, including genetic influences, pre-harvest conditions, chemical structure and composition, seed maturity, seed size, seed moisture content, mechanical damage and seed vigor.

Metabolic symptoms can be shown from biochemical analysis, while growth symptoms are known through physiological indications that include maximum growth potential, normal dry weight of germination, and germination. Power germinate seen from comparison of the number of seeds that germinate normal in certain conditions and germination period. Seeds with high viability will produce strong seedlings with rapid root development, resulting in healthy and steady planting.

Based on research that state the germination of seeds at 0 weeks of storage by 95% was not significantly different from the value of germination at 3 weeks, 6 weeks and 9 weeks after saving, with successive germination values of 94%; 93.3%; and 90.66%. After 12 weeks of storage, the germination value has decreased significantly compared to the beginning of the storage period. The value of germination in 12 weeks of storage and 15 weeks of storage were 89.66% and 84.66%, respectively [7].

Physiological treatment of seeds to improve seed germination through water imbibition has become the basis for seed invigoration. Invigoration treatment is currently one alternative that can be used to overcome the low quality of seeds, namely by treating seeds before planting to activate seed metabolic activities so that the seeds are ready to enter the germination phase. Some seed invigoration treatments are also used to uniform the growth of the sprouts and increase the rate of sprout growth [8]. The invigoration treatment on seeds that have been stored for a long time, is expected to improve the ability to grow and prevent the rate of decline of the seeds. Invigoration generally aims to prevent and reduce the rate of seed decline [9].

2. Methods

2.1 Place and time
This experiment was already implemented at the Laboratory of Science and Technology Faculty of Agriculture Seed Andalas University and at the Laboratory LLDIKTI Region X of West Sumatra, Riau, Jambi and the Riau Islands. The implementation time starts from September to October 2019.

2.2 Materials and tools
The materials used are the seed rice that has been stored for 1 year (variety description PB - 42, Batang Piaman, and IPB-3S, distilled water, stencil paper, sand, detergent, sodium hypochlorite 1%, tissue, 100% alcohol, 96% alcohol, 80% alcohol, 70% alcohol, 1% safrnin, FAA solution, hard paraffin, formalin 4%, acetic acid, xylol, KOH solution, HCl solution, phenolphthalein indicator, methyl orange indicator,
2.3 Experimental design
The experiment used a completely randomized completely factorial design with two factors with 3 replications. The first factor is the variety consisting of 3 levels (PB-42, Batang Piaman, and IPB-3S). The second factor is that invigoration consists of 3 levels (hydration-dehydration and priming). The observational data were quantitatively analyzed using the F level of 5% significance level, if there were significant differences, it was carried out by further testing using the 5% BNJ test, while the qualitative data were analyzed descriptively.

2.4 Research Implementation

2.4.1 Seed Preparation
The rice seeds used as experimental material were Batang Piaman, PB-42 and IPB-3S varieties which had been stored for 1 year, with 2000 seeds. The rice seeds were obtained from the Andalas University Seed Technology Laboratory and stored in plastic at room temperature (± 20 °C-25 °C). Seed criteria used in this experiment are seeds that have undergone a decline with a germination of 40% -50% (Mutia, 2018).

The seeds are then surface sterilized by soaking the seeds in aquadest for 2 minutes, then the seeds are soaked in a container containing 1% sodium hypochlorite for 2 minutes, and soaked again in a container containing aquadest for 2 minutes. The seeds are then air-dried for 5 minutes.

2.4.2 Sterilizer
The tools used, such as aluminum plates, goblets, tweezers, handsprayers, and germinators, are washed using detergent, sprayed with 1% sodium hypochlorite, then washed with distilled water until clean and dried. After that spray with 70% alcohol and dried with tissue.

2.4.3 Seed Treatment
For treatment without invigoration, the seeds are not treated with hydration-dehydration or priming (PEG). For hydration-dehydration treatment, 600 seeds of rice are taken, the weight of the seed before hydration-dehydration is weighed, then put in a container containing aquadest for 1 hour for dehydration. After that the seeds are removed from the container containing aquadest and dried for 5 minutes, followed by dehydration of the seeds for 6 hours in an oven at 40 °C. The seeds are removed from the oven and weighed. For priming treatment, the seeds are immersed in PEG 6000. Observations made in the form of seed water content, seed germination capacity, and soil emergence test.

3. Results and Discussion
The results of the experiment found that by invigorating treatment both priming and hydration-dehydration of seeds that have suffered setbacks can increase seed viability. Invigoration treatment on several varieties provides a real interaction with the seed water temperature. Data on seed moisture content is presented in Table 1.

Table 1. Seed water contents of several rice varieties with invigoration

| Varieties       | No Invigoration | Priming (%) | Invigoration (%) |
|-----------------|-----------------|-------------|------------------|
| Batang Piaman   | 4.83 c          | 14,30 b     | 27,80 a          |
| PB 42           | B               | B           | B                |
| PB 42           | 4.90 c          | 13,37 b     | 29,60 a          |
Invigoration treatment on several rice varieties gave interaction to seed water content. This is because each variety will give a different response to the invigoration given. It can be seen in Table 1 above that the Batang Piaman variety shows water content without invigoration (4.83%), but with invigoration treatment the water content increases on priming (14.30%) and hydration-dehydration (27.80%). PB42 variety has a water content without invigoration (4.90%) increased to 13.37% (priming) and 29.60% (hydration-dehydration). In the IPB3S variety the water content of 5.17% (without invigoration) increased to 16.83% (priming) and 22.00% (hydration dehydration). All three varieties show that invigoration with hydration-dehydration is better than priming. This is due to the hydration-dehydration treatment given to dry seeds that have deterioration in seed quality can increase water content, due to immersion of seeds in water (hydration), resulting in imbibition of the seeds and then dried again (dehydration).

The germination of some rice varieties with invigoration showed no real interaction, but the treatment of varieties gave a real difference, as did the invigoration treatment. Seed germination data is presented in Table 2.

Table 2. Germination of several rice varieties with invigoration

| Varieties   | Invigoration | Average |
|-------------|--------------|---------|
|             | No Invigoration | Priming | Hydration-Dehydration |     |
| Batang Piaman | 50.00        | 70.00   | 70.00                    | 63.33 a |
| PB 42       | 22.00        | 38.00   | 38.00                    | 32.67 b |
| IPB 3S      | 20.00        | 28.67   | 28.67                    | 25.78 c |
| Average     | 30.67 b      | 45.56 a | 45.56 a                  |       |

Note: The numbers followed by the same lowercase letters in the same row and the same uppercase letters in the same column are not significantly different according to BNJ level of 5%

Some rice varieties show a real effect on seed germination, as well as invigoration. Batang Piaman variety shows higher germination and significantly different from PB-42 and IPB-3S varieties. This is because the Batang Piaman variety is more responsive to the invigoration given.

Invigoration treatment given both of priming and hydration-dehydration is significantly different from without invigoration, this is because giving invigoration can increase seed germination. From the data presented above, it turns out that invigoration treatment on several rice varieties can improve seed viability that has decreased. This is caused by invigoration both of priming and hydration-dehydration can restore seeds that have begun to be damaged. The physiological treatment of seeds to improve seed germination through water imbibition has become the basis for seed invigoration [10]. Currently, invigoration treatment is an alternative that can be used to overcome the low quality of seeds, namely by treating seeds before planting to activate the activities of seed metabolism so that the seeds are ready to enter the germination phase. Some seed invigoration treatments are also used to uniformly sprout growth and increase the rate of sprout growth [8]. The priming is a slow and controlled water uptake process that results in the strengthening of the plasma membrane, minimizing electrolyte loss and increasing seed viability and vigor [11].

The soil emergent test is presented in Table 3, where the data after analysis of variance shows a real interaction between invigoration treatments given to three rice varieties.

Table 3. The Soil emergent test of several rice varieties with invigoration
Invigoration treatment can increase soil emergent test in Batang Piaman and PB42 varieties, but in IPB3S variety it actually decreases from 40% (without invigoration) to 16.00% (priming) and 26.00% (hydration-dehydration). This is because priming results in imbibition due to osmotic pressure applied to the seed, so that metabolic activity in the seed will take place. Then assimilation occurs and cells extend on the embryonic axis so that the radicles will penetrate the seed coat. Likewise, with hydration treatment where after the seeds are soaked the seeds contribute to the seed water content then increases, and the seeds will swell. After that the seeds are dried so that metabolic activity occurs in the seeds. This treatment makes the seeds germinate so that there is an increase in seed vigor. An invigoration treatment on seeds that have been stored for a long time, is expected to increase the ability to grow and prevent the rate of decline of the seeds. Invigoration generally aims to prevent and reduce the rate of seed decline [9].

Ash content after analysis in the laboratory has decreased. The ash content in the varieties of Batang Piaman control of 19.91 into 15.12 (hydration-dehydration) and 17.76 (priming). PB42 control varieties from 14.05 to 12.63 (hydration-dehydration) and 12.96 (priming), but in the IPB3S control varieties from 17.78 rose to 18.41 (hydration-dehydration) but dropped to 15.8 (priming).

![Figure 1. Ash content of several rice varieties with invigoration](image-url)

Ash content will decrease due to seeds undergoing metabolic processes during germination. The ash content in the seeds is overhauled so that the levels will be reduced. Fat content of some rice varieties after analysis in the laboratory increases with invigoration treatment. The increase is presented in Figure 2.
Fat content of several seed varieties with invigoration

Fat content of some rice varieties with invigoration treatment increases due to enzyme activity that occurs in the seeds during the germination process. Food reserves in the form of fat will be overhauled by lipase enzymes into fatty acids so that fat levels will rise. The priming treatment is better than hydration-dehydration, because osmotic pressure changes so that water will enter the seeds and metabolism activities occur in the seed.

Seed respiration rates in several rice varieties after analysis in the laboratory increased with invigoration. Figure 3 displays the rate of seed respiration.

Respiration rates of the three varieties with invigoration increased compared to controls. This is because priming given can increase the rate of respiration in the seed because the seeds soaked in PEG will change the osmotic pressure and water will enter the seed. [11] that when osmotic pressure increases, water availability decreases but certain ions especially sodium and magnesium affect
germination more than water availability. Water functions to activate enzymes so that metabolic activity takes place, as well as hydration-dehydration treatment. Seeds soaked in water will contribute to water so that the water content increases. Water serves to activate enzymes to remodel food reserves. Increasing the rate of respiration will produce energy for cell division and cell extension in the radicles, so the seeds will germinate. The seeds that are germinating do respiration quickly. Aerobic respiration consumes O₂ and releases CO₂ [11].

4. Conclusion
Based on the results obtained in this study, it can be concluded that the invigoration given to the three varieties provides interaction with the seed water content and soil emergent test. Priming and hydration-dehydration can improve seed quality which has decreased physiologically. Invigoration increases seed moisture content, seed germination, and soil emergence test. Given invigoration can activate the biochemical processes in the seed so that it increases fat content, and respiration rate, but decreases ash content.

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