



Research Article

Effect of Seed Hydration-Dehydration Integrated with Rhizobacteria on Viability and Vigor of Deteriorated Soybean Seed

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Abstract

Background and Objective: Soybeans undergo rapid deterioration due to its chemical composition and unfavorable storage conditions. The objective of the research was to determine the duration of seed hydration and dehydration integrated with rhizobacteria affecting the viability and vigor of deteriorated soybean seeds. **Materials and Methods:** A complete randomized design with four replicates were used in this experiment. Seeds were treated with various combinations of hydration duration in a rhizobacteria suspension and followed by dehydration time. Data were analyzed using the F-test and significant differences were further tested using Least Significant Difference with a $p < 0.05$. **Results:** Results showed that the seed hydration and dehydration period in a rhizobacteria suspension could improve the seed viability and vigor about 12.28 % resulting in 59.5 % initial germination percentage. Additionally, this seed pre-treatments exhibited index value was 0.56 with 25.4 % growth uniformity and T_{50} value about 1.4 days compared to no invigoration. The 60 minutes hydration followed by 60 minutes dehydration displayed the best results on seed viability and vigor.

Key words: Invigoration, physiological quality, rhizobacteria suspension, seed quality, soybean.

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Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the strategic commodities that frequently undergo the availability problem. Indonesia had great demand on soybean, thus the importation reached more than 70% of the total demand, while the production had decreased by 8.4% (Kompasiana, 2015). The high importation of soybean is due to the lack of soybean supply that can be met by soybean farmers and the lack of high quality seeds that can be planted to meet the needs of soybean producers.

Soybean contains about 37% protein and 16% fat (Mustika *et al.*, 2014). The high content of protein and fat in the seeds causes rapid physiological deterioration of soybeans, especially when the seeds are stored in unfavorable storage condition. Farmers often faced with low quality seeds problem (Braccini *et al.*, 2000). Seed deterioration is indicated with the decline of seedling performance followed by retardation of growth and development of seeds in the field (Nurmauli and Nurmiaty, 2010).

One of the effort that can be done to overcome seed deterioration problems is seed invigoration treatment, which is seed priming or seed hydration and dehydration. Basically, seed priming activate internal and external factors of seeds to maximize the plant growth and yield improvement (Ilyas, 2012). Seed priming will provide physiological improvements, such as higher, faster, and simultaneity seed germination in addition to more tolerant to unfavorable environmental condition because the seeds have a higher germination rate.

Application of seed priming and its benefit has been widely reported by several researchers. Hydration-dehydration of seeds increased the viability and vigor of deteriorated soybeans (Dewi-Hayati, 1995) and improved germination characteristics (Nurmauli and Nurmiaty, 2010). Seed hydration and dehydration has also reported to improve the viability of deteriorated resin and mahogany seeds (Zanzibar and Makodompit, 2007).

Rhizobacteria or popularly known as Plant Growth Promoting Rhizobacteria (PGPR) is a group of beneficial bacteria located in the area of plant rhizosphere that provides benefits for plant growth. Rhizobacteria could facilitate the absorption of various nutrients in the soil, synthesize and modify the concentrations of various growth-promoting phytohormones as well as to act as a biocontrol agent against diseases (Glick, 1995; Sutariati *et al.*, 2006). The application of rhizobacteria to seeds could also improve plant viability and vigor (Gholami *et al.*, 2009), as well as its growth and yield (Sutariati and Safuan, 2012). The objective of this research was to determine the effect of seed hydration and dehydration duration integrated with rhizobacteria on the viability and vigor of deteriorated soybean seeds.

Materials and Methods

The experiment was conducted using complete randomized design applied for two factors (duration of rhizobacteria hydration and dehydration) with four replicates. The seed hydration in rhizobacteria suspension was performed for 30 and 60 minutes. Both durations were then subjected into dehydration for 60 and 180 minutes. Longer dehydration period (300 minutes) was also tested after 60 minutes rhizobacteria hydration. As the negative control, no hydration and dehydration treatment applied to the seeds.

The rhizobacteria used in this experiment was *Bacillus sp.* suspension. Before being subjected into hydration treatment, seeds were dried on stencil paper for 5 minutes. The

seeds were then hydrated in rhizobacteria suspension followed by the dehydration treatment in a pre-heated oven at 40°C according to its corresponding durations. After removing from the oven, the seeds were cooled in a desiccator for 15 minutes.

Several seed quality parameters, including moisture content, germination percentage, the number of dead seeds, first count test, T₅₀ value (day of 50% seed germinate) and index value were evaluated. Seed viability and vigor assessments were conducted according to ISTA (2006) by soaking the seeds on a moistened rolled stencil paper (30 x 42 cm) then incubated in a germinator. Fifty seeds were used for each replicate, thus 200 seeds were required for four replicates in each treatment. The percentage of germination was observed on the 5th and 8th day of germination. First count test was conducted on the 5th day, while index value test were conducted from the 1st until the 8th day of germination. Data were analyzed using the F-test and significant differences were further tested using Least Significant Difference at the 5% level.

Results

Effect of rhizobacteria hydration followed by dehydration treatments revealed insignificantly different moisture content between the treated and non-treated seeds. The resulted seeds moisture contents varied between 15.87 – 16.46 % (Table 1). The similar moisture content between hydrated-dehydrated seeds and non hydrated-dehydrated seeds (15.25 %) suggesting that the durations of hydration and dehydration applied to the seed was appropriate as its initial weight was well maintained and align with its initial moisture content.

Table 1. Percentage of moisture content, germination, and dead seeds at various durations of hydration-dehydration in a rhizobacteria suspension.

Hydration in a rhizobacteria suspension	Moisture Content (%)	Germination (%)	Dead Seeds (%)
Hydration 30 mins, dehydration 60 mins	16.28±0.9 a	73.0±7.3 b	26±6.7 b
Hydration 30 mins, dehydration 180 mins	16.05±0.8 a	63.5±5.7 a	36.5±5.7 a
Hydration 60 mins, dehydration 60 mins	16.46±1.7 a	81.5±4.1 b	18.5±4.1 b
Hydration 60 mins, dehydration 180 mins	15.91±1.2 a	76±1.9 b	24±1.6 b
Hydration 60 mins, dehydration 300 mins	15.87±0.4 a	67.5±15 a	32.5±15 a
No hydration-dehydration	15.25±2.1 a	59.5±3.0 a	38.5±2.5 a
CV (%)	10.0	9.9	25.4

Similar notation within column with no hydration-dehydration treatment was significant based on LSD at α 0.05. \pm SD.

The combination of rhizobacteria hydration and dehydration treatment could improved the germination rate by 12.8 %, ranging from 4 to 22 % (Table 1). The seeds treated with 60 minutes hydration followed by 60 minutes dehydration exhibited the highest increase in the germination rate about 81.5 % (Table 1). This improvement was in line with the decline of abnormal and dead seeds quantities. Number of dead seeds recorded in this study ranging from 2 – 20 % (Table 1). The lowest dead seeds were found in seeds treated with 60 minutes hydration and followed by dehydration for 60 minutes (18.5 %) (Table 1). The number of abnormal seedlings also reduced about 1 % after being hydrated for 30 minutes and then dehydrated for 60 minutes (Table 1).

Prolonged duration of hydration and dehydration treatments showed no abnormal seedlings (data not shown).

The application of hydration-dehydration treatment with rhizobacteria suspension could shorten the period required to achieve a total of 50 % germination (T_{50}) by 1.4 days, ranging from 0.5 to 2 days (Table 2). Seeds treated with 60 minutes hydration and both durations of dehydration resulted in T_{50} value at 3 days, while 30 minutes hydration and 180 minutes dehydration achieved its T_{50} value at 3.5 days (Table 2). This results suggested that the utilization of rhizobacteria suspension contributed to better germination rate compared to the non-treated seeds.

Table 2. Time required for 50 % germination (T_{50}), index value, and first count test at various durations of hydration-dehydration in a rhizobacteria suspension.

Hydration in a rhizobacteria suspension	T_{50} (days)	Index Value	First Count Test (%)
Hydration 30 mins, dehydration 60 mins	3.5±0.6 b	13.46±1.9 b	69.50±9.6
Hydration 30 mins, dehydration 180 mins	4.25±1.0 a	10.55±2.7 a	69±15.46
Hydration 60 mins, dehydration 60 mins	3.00±0 b	14.08±1.8 b	71.5±11
Hydration 60 mins, dehydration 180 mins	3.00±0 b	13.88±1.3 b	70±8.2
Hydration 60 mins, dehydration 300 mins	4.5±1.2 a	11.5±2.8 b	67.0±15.8
No hydration-dehydration	5.00±0.81a	8.14±1.7 a	44±9.9
CV (%)	19.9	17.8	18.5

Similar notation within column with no hydration-dehydration treatment is significant based on LSD at α 0.05. \pm SD.

Similar to T_{50} value, the hydration-dehydration in a rhizobacteria suspension also elevated the index value of the seed about 4.6, ranging from 2.4 to 5.9 (Table 2). Combination of 30 minutes hydration and 60 minutes dehydration as well as 60 minutes hydration followed by dehydration in all durations exhibited higher seed's index values compared to non hydrated-dehydrated seeds (Table 2). All treatments of hydration-dehydration combined with rhizobacteria suspension showed significant effect on the increasing of growth simultaneity by 25.4 % or ranging from 23 – 25.5 %. This result was in line with the results of vigor and viability assay obtained from the combination of 60 minutes hydration and 60 minutes dehydration.

Discussions

Hydration-dehydration was a treatment of soaking seeds for a period of time followed by drying until it returned to its initial weight (Nurmauli and Nurmiaty, 2010). The high moisture content of non hydrated-dehydrated seeds showed that the initial moisture content was high as the seeds were not stored in an ideal condition that could maintain its physiological qualities. Environmental factors, such as temperature and humidity, post-harvest storage conditions greatly affected the rate of seed deterioration (Shelar, 2008; Imad *et al.*, 2017). Initial moisture content could also decline the germination rate where the magnitude was in line with the initial moisture content of the seed (Ndimande *et al.*, 1981). Even at the seed initial moisture content of 9 %, high germination percentage could only be maintained for two months and slowly decreased

during the longer period of storage. Within 5 months, soybean lost its germination rate by 54.6 % (Manggung *et al.*, 2014).

The low germination percentage by 59.5 % showed that the seed has been deteriorated physiologically (Odaba *et al.*, 2016). The increasing of germination rate due to the application of rhizobacteria hydration and dehydration was consistent with the increasing of seed viability and vigor reported in soybeans (Miladinov *et al.*, 2014), maize and wheat (Arief and Koes, 2010). Additionally, Sutariati *et al.* (2014) also reported that combination of rhizobacteria hydration and dehydration successfully increased the viability and vigor of upland rice about 88 and 47 %, respectively.

The period of seed soaking in a rhizobacteria suspension for 30 – 60 minutes was sufficient to allow the water imbibition into the seed tissues without inhibiting of seed respiration. The imbibition process would activate the digestive enzymes within the tissue. The result of these enzymatic activities would result in various amino acids, fatty acids and glucoses. These molecules would be transported into embryonic axis to be re-synthesized as the materials to build new cells supported by the energy resulting from the previous processes (Kamil, 1979). The process of dehydration or removal of water out of the tissue would stop the seed physiological activities. But as soon as the seed re-imbibide, the physiological process would resume the cell division and extension processes. Growth could be seen morphologically by the emergence of plumule and radicle.

Dehydration period for 60 – 180 minutes at 40 °C was an ideal duration to stop the physiological activity in an embryonic axis marked by high percentage of seed germination, index value, and germination rate. Eventhough the dehydration period was extended up to 300 minutes, the moisture content remained the same as the initial moisture content. This condition was assumed as the causal factor of the decreasing of *Bacillus sp.* activity due to growth reduction after unfavorable temperature exposure. Rhizobacteria is known for its ability as the pytohormones producers and biocontrol agents against various diseases (Astuti, 2008).

The deteriorated seeds are vulnerable for the environment as it is potentially to be pathogenic. The dead seed is marked by its watery texture and often covered by fungal hyphae. The decreasing number of dead seeds due to the application of rhizobacteria hydration and dehydration was in line with the increasing of germination percentage. This increasing percentage indicated the indirect implication of rhizobacteria activity as biocontrol or antifungal agents (Astuti, 2008). Seeds treated with this methods combination also exhibited the increasing on germination rates, simultaneity and seedling vigor.

Conclusions

The combination of rhizobacteria hydration and dehydration has been proven able to increase germination rate and percentage, index value and growth simultaneity of physiologically deteriorated soybean seeds. The best invigoration treatment was hydration in a rhizobacteria suspension for 60 minutes followed by 60 minutes dehydration.

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