Effect of soaking duration in hydropriming on seed vigor of sorghum (*Sorghum bicolor* L. moench)

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Abstract. Priming or invigoration is treatments on seeds to enhance and improve the appearance of seeds that germinate. Research on priming was conducted in Indonesian Cereal Research Institute (ICERI) seed laboratory from May 2017 until October 2018 to evaluate the effect of soaking duration in hydropriming on sorghum seed vigor. Seed quality parameters includes germination percentages, germination rate, shoot and primary root length, shoot and root dry weight, electric conductivity of seed leakage. Sorghum seed variety of Numbu and Super1 were used as varieties tested. Results showed that priming treatments significantly effect on increasing germination percentage, germination rate, shoot and root length, shoot and root dry weight. The priming treatment with soaking duration 2 hours has positive significant effect on seed vigor, in terms of germination rate, shoot and root length and shoot and root dry weight, and electrolyte leakage.

1. Introduction

Seed quality is a major factor in crop establishment and subsequent productivity. Losses in seed quality occur during field weathering, harvesting and storage. Seeds get damaged if they are exposed to high temperature and high humidity. Planting sorghum seeds on dry land depends on water availability. Appearance of delayed germination can occur due to land preparation and growing conditions environment. Sub optimal growing condition could causes an increase abnormal seedling growth and seedling vigor is weak. The emergence of seedling can be slow or very diverse, depending on the vigor of seeds that are sowed, and environmental conditions to grow. Seed vigor reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Seeds may be classified as viable in a germination test which provides optimum temperature, moisture and light conditions to the growing seedlings; however, they may not be capable of continuing growth and completing their life cycle under a wide range of field conditions.

Generally, seeds start to lose vigor before they lose their ability to germinate; therefore vigor testing is an important practice in seed production programs. Vigor testing becomes more important for carryover seeds, especially if seeds were stored under unknown conditions or under unfavorable storage conditions. Seed vigor testing is also used as indicator of the storage potential of a seed lot and in ranking various seed lots with different qualities. One way to enhance and improve the appearance of seeds that germinate is to provide treatment of priming or invigoration [9]. Priming is a process that controls the process of hydration of seeds for the ongoing metabolic processes before germination. The general purpose of seed priming/conditioning is to hydrate partially the seed to a point where germination processes are initiated but not completed. Priming treatments involve imbibing seed with
restricted amounts of water to allow sufficient hydration and advance of metabolic processes but preventing the protrusion of the radicle. Some research results showed that priming causes growth rate of seedling faster and uniform [5]. In addition, priming treatments could increase plant vigor, seed yield and harvest index [7]. Furthermore [3] also states that priming treatment with CaCl₂ increase wheat grain yield compare to control treatment, but did not affect plant height, spikelet number, seed number and 1000 grain weight. Priming treatment on the seed is one alternative to increase seedling resistance against environmental conditions that are less optimum growth [2]. Primed/conditioned seeds usually would exhibit rapid germination when absorb water under field conditions [2].

According to priming method, [9], divided priming into: hydropriming, osmopriming, halopriming, biopriming, and solid matrix priming/matriconditioning. Hydropriming is a conditioning seed using water, osmoconditioning is a seed conditioning using osmotic solution, while matriconditioning uses a damped solid material. Osmopriming technique refers to soaking of seeds for a certain period in solution of sugar, PEG followed by air drying before sowing. Hydropriming refers to alternate wetting and drying of seeds under laboratory conditions to withstand and continue productive plant growth even under adverse field condition of drought, cold and salt. Pre-sowing hydropriming brings about physiological reorganizations, metabolic, anatomical and morphological changes. Physiological reorganization that takes place are increase in hydrophilic property of the protoplasmic colloids namely the viscosity and elasticity, increase in osmotic pressure, changes in the quality of proteins and overall increase in the water holding capacity of seed. Metabolic changes that result due to hydropriming were respiration, synthetic reactions, phosphorylation activity of mitochondria, and rate of photosynthesis were increased even during drought, more starch in leaves of hardened plants.

Hydroprimed seeds produce plants with xeromorphic morphology more extensive and dense network of veins and ribs, epidermic and stomatal cells are smaller, number of stomata per unit, leaf area is greater, foliage area is increased, faster recovery from atmospheric drought, greater total and absorbing surface into root system, as well as more number of primary roots and leaves of these plants will have more starch [13]. Seed soaking in water could increased water absorption, size of cell walls and better root development, which could enhance germination, seedling length, and vigour index. Seed priming has been a common seed treatment to reduce the time between seed sowing and seedling emergence and the synchronization of emergence [12]. Aim of the research was to evaluate the effect of soaking duration in hydropriming on sorghum seed viability and vigor.

2. Materials and Methods
The experiment was conducted from May 2017 until October 2018, in the laboratory and greenhouse of Indonesian Cereal Research Institute. Seeds multiplication was held in Bontobili Experimental Farm, Gowa district of South Sulawesi. Sorghum of Numbu and Super 1 varieties were prepared from 16 months storage seed lots and fresh harvest seed lots. Priming treatments were conducted by soaking 250 g seeds (for three replications) for each treatment in distilled water according to the treatments, after which the dried-air for 2 hour. The treatments arranged in a completely randomized design with 2 factors, repeated 3 times, and statistical analysis was according to [8]. Priming treatments was performed by soaking sorghum seeds in distilled water of appropriate treatment is given, as follows:

Priming duration :
1. Check (without priming)
2. Priming for 2 hours
3. Priming for 4 hours
4. Priming for 6 hours
5. Priming for 8 hours
6. Priming for 16 hours

Variety/Seed lot:
1. Fresh harvested of Numbu
2. 6 month storage of Numbu
3. Fresh harvested of Super 1
4. 16 months storage of Kawali

Before priming treatments determination of seed moisture content were carried out. After all priming treatments had completely finished, seed quality determination were carried out in ICERI seed laboratory. All determinations for seed quality analysis were carried out according to International Rules for Seed Testing [10]. Moisture content: 100 seed samples were ground and oven-dried at 130 °C for two hours. Calculations were based on the wet basis. Germination: 100 seeds from each treatment and each replications were germinated in rolled germination towels moistened with water in an incubator at 25°C. The first count was made 5 days later. Normal seedlings were evaluated after 7 days. Germination percentage was expressed by the percentage of seed germinating normally after 7 days [10]. Germination rate: Data obtained from substrate of seed germination test. Every observation time, total percentage of normal shoot is divided by etmal (24 hours). Cumulative etmal value is obtained when seeds are planted until the time of observation. The formula used as follows:

\[
KT = \frac{(X_i - X_{i-1})}{T_i}
\]

where

- \(KT\) = germination rate (%/etmal)
- \(X_i\) = the percentage of normal seed etmal i
- \(T_i\) = time of observation (etmal)

Shoot length: About 10 normal seedlings were taken at random from each replicate at the end of standard germination test to evaluate shoot length (cm). Root length: The same 10 normal seedlings of shoot length evaluation test used to evaluate root length (cm). Shoot dry weight: 10 normal seedlings were taken and dried in a forced air oven at 110°C for 17 h [1] to obtain shoot dry weight and expressed as grams. Root dry weight: 10 normal seedlings were taken and dried in a forced air oven at 110°C for 17 h [1] to obtain root dry weight and expressed as grams. Electrical conductivity: Three replications of 50 seeds of each treatment were weighted and moisture content recorded. The seeds of each replication were placed in 200ml beaker and 50ml of deionized water was added. Seeds were stirred gently to ensure that all seeds were completely immersed and evenly distributed. The beakers were placed at temperature of 20°C for 24 hours. The electrical conductivity of the leachates of each replication was measured by using a conductivity meter (sension5) and conductivity per gram of seed weight was calculated [10]:

\[
\text{Conductivity (μScm}^{-1}\text{g}^{-1}) = \frac{\text{conductivity reading - blank reading}}{\text{weight (g) of replicate}}
\]

3. Results and Discussion
The response of seedling growth for different seed treatments were interpreted in terms of germination percentage, germination rate, shoot length, root length, shoot length, shoot dry weight, root dry weight. Data in table exhibited a significant variation in germination of sorghum. It is evident from the table 1, that significantly maximum increase in total germination and germination rate occurs by soaking duration 2 hours (95.08% germination, 30.623%/etmal germination rate), while lowest germination and germination rate occurs by soaking duration 16 hours (Table 1). The interaction effect of variety/seed lots and treatments results were found significant.
Table 1. Germination percentage and germination rate after soaking treatments*)

| Varieties              | Soaking duration (hours) | Control | 2     | 4     | 6     | 8     | 16    |
|------------------------|--------------------------|---------|-------|-------|-------|-------|-------|
|                        | Germination Percentage   |         |       |       |       |       |       |
| Super 1 (fresh harvest)| 95.66c                   | 98.33a  | 94.33c| 93.66cd| 93.66cd| 87.66g|
| Super 1 (16 months storage)| 93.66cd                | 97.00ab | 83.66h| 81.33i| 81.66i| 74.33jk|
| Numbu (fresh harvest)  | 89.66f                   | 98.00a  | 94.33c| 92.00de| 90.33ef| 90.00ef|
| Numbu (16 months storage)| 72.66k                  | 87.00g  | 75.66j| 69.66i| 66.00m| 64.33m|
| Average germination (%)| 87.91                    | 95.08   | 86.99 | 84.16 | 82.91 | 79.08 |
| Germination rate (%)   | 30.91b                   | 31.83a  | 30.64bc| 29.93c| 28.96d| 26.81ef|
| Super 1 (16 months storage)| 26.61efg               | 30.28bc | 26.78ef| 25.88ghi| 25.23i| 23.57j|
| Numbu (fresh harvest)  | 30.45cb                  | 32.03a  | 27.04e | 25.96ghi| 26.09efg| 25.39hi|
| Numbu (16 months storage)| 23.95j                  | 28.35d  | 22.74k| 20.610| 18.38m| 17.29n|
| Average germination (%)| 27.98                    | 30.62   | 26.80 | 25.59 | 24.67 | 23.27 |

*) Means followed by the same letters are not significantly difference by DMRT .01

Evaluation of seedlings showed that shoot length, root length, shoot dry weight, root dry weight had significant difference on soaking duration treatment for both sorghum varieties (Numbu and Super 1). The results showed shoot length after treated with 2 hours soaking durations, gave the highest shoot and root length and significantly different with others treatments on varieties treated. (table 2). The same results found in shoot and root dry weight which showed that 2 hour soaking duration were give the highest shoot and root dry weight and they were significantly difference with others soaking treatments on varieties tested Table 3). Metabolic changes that result due to hydropriming were respiration, synthetic reactions, phosphorilation activity of mitochondria, and rate of photosynthesis were increased even during drought, more starch in leaves of hardened plants. Hydroprimed seeds produce plants with xeromorphic morphology more extensive and dense network of veins and ribs, epidermic and stomatal cells are smaller, number of stomata per unit, leaf area is greater, foliation area is increased, faster recovery from atmospheric drought, greater total and absorbing surface into root system, as well as more number of primary roots and leaves of these plants will have more starch [13]. Seed soaking in water could increased water absorption, size of cell walls and better root development, which could enhance germination, seedling length, and vigour index. Seed priming has been a common seed treatment to reduce the time between seed sowing and seedling emergence and the synchronization of emergence [12]. In this research, enlargement of cell could cause root length and shoot length increased soon after imbibiton processed.

Table 2. Shoot length and root length after soaking treatments*)

| Varieties              | Soaking duration (hours) | Control | 2   | 4   | 6   | 8   | 16  |
|------------------------|--------------------------|---------|-----|-----|-----|-----|-----|
|                        | Shoot length (cm)        |         |     |     |     |     |     |
| Super 1 (fresh harvest)| 11.70b                   | 13.32a  | 12.11b| 11.71b| 11.03c| 11.05c|
| Super 1 (16 months storage)| 9.87de                  | 11.12c  | 9.63def| 9.49fg| 9.79de| 9.10ghi|
| Numbu (fresh harvest)  | 9.81de                   | 11.86b  | 9.72de| 9.26ghi| 8.98hi| 8.94hi|
| Numbu (16 months storage)| 9.13ghi                 | 9.97d   | 9.13ghi| 8.78i| 8.73i| 7.43j|
|                        | Root length (cm)         |         |     |     |     |     |     |
| Super 1 (fresh harvest)| 11.15j                   | 11.11j  | 12.34g| 11.84h| 13.89c| 13.84c|
| Super 1 (16 months storage)| 9.10n                   | 9.05n   | 8.87o| 9.40l| 11.89i| 11.11j|
| Numbu (fresh harvest)  | 8.78p                    | 9.31m   | 8.70q| 9.27m| 12.89f| 10.55k|
| Numbu (16 months storage)| 8.77p                   | 13.13e  | 13.58d| 14.15b| 14.80a| 9.46l|

*) Means followed by the same letters are not significantly difference by DMRT .01
Table 3. Shoot dry weight and root dry weight after soaking treatments

| Varieties                     | Soaking duration (hours) | Control     | 2           | 4           | 6           | 8           | 16          |
|-------------------------------|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                               |                          | Shoot dry weight (g) |             |             |             |             |             |
| Super 1 (fresh harvest)       | 0.0058bcd               | 0.0078a      | 0.0058bcd   | 0.0056cde   | 0.0052def   | 0.0031jk    |
| Super 1 (16 months storage)   | 0.0044gh                | 0.0073a      | 0.0043h     | 0.0050fg    | 0.0051ef    | 0.0032ij    |
| Numbu (fresh harvest)         | 0.0059bc                | 0.0064b      | 0.0054gh    | 0.0044gh    | 0.0033i     | 0.0026jk    |
| Numbu (16 months storage)     | 0.0044gh                | 0.0059bc     | 0.0036i     | 0.0031ij    | 0.0026jk    | 0.0025k     |
| Super 1 (fresh harvest)       | 0.0040cd                | 0.0047a      | 0.0043b     | 0.0040cd    | 0.0038de    | 0.0027g     |
| Super 1 (16 months storage)   | 0.0037ef                | 0.0042bc     | 0.0036efg   | 0.0035fg    | 0.0034gh    | 0.0025j     |
| Numbu (fresh harvest)         | 0.0038de                | 0.0043b      | 0.0040cd    | 0.0038de    | 0.0032hi    | 0.0020k     |
| Numbu (16 months storage)     | 0.0031i                 | 0.0036efg    | 0.0031i     | 0.0031i     | 0.0031i     | 0.0015l     |

*) Means followed by the same letters are not significantly different by DMRT .01

Table 4. Electric conductivity of seed leakage after soaking treatments*)

| Varieties                     | Soaking duration (hours) | Control | 2 | 4 | 6 | 8 | 16 |
|-------------------------------|--------------------------|---------|---|---|---|---|----|
|                               |                          | 9.44t   | 9.74t | 35.45m | 39.42j | 42.04i | 46.03g |
| Super 1 (16 months storage)   | 21.65o                   | 14.40q | 41.85i | 47.47t | 50.79e | 55.02d |
| Numbu (fresh harvest)         | 10.50s                   | 11.60r | 37.54l | 38.24k | 61.81c | 68.81b |
| Numbu (16 months storage)     | 24.03n                   | 20.02p | 42.31i | 43.49h | 43.46h | 78.42a |

*) Means followed by the same letters are not significantly different by DMRT .01

Electric conductivity of seed leakage measures the integrity of cell membranes, which is correlated with seed vigor. It is well established that this test is useful for maize, garden beans, and pea. It has been also reported that the conductivity test results are significantly correlated with field emergence for corn, and soybean [11]. As seeds lose vigor, nutrients exude from their membranes, and so low quality seeds leak electrolytes such as amino acids, organic acids while high quality seeds contains their nutrients within well structured membranes. Therefore, seeds with higher conductivity measurement are indication of low quality seeds as vice versa [6].

In this research, electric conductivity of seed leakage showed significant difference on soaking treatments and varieties/seed lots. The lowest value of electrolyte leakage were found on fresh harvest of Super 1, and the highest value of electrolyte leakage on Numbu (16 months storage). Its indicated that there were deterioration of stored seeds along storage. [4] proposed that electrolyte or ion quantifications in seed immersed water were counted by electric conductivity. At imbibition process, soaked maize seeds released some ions, amino acids and others electrolytes. In deteriorated seeds, there were many free radicals, enzymatic dehydrogenase, aldehyde oxidation from proteins, and Maillard reaction were found [3]. Free radical will fractured cell membran and cause decreased its viscosities and permeabilities [14]. Increasing solution in seed immersed water were indicated that seed membrane cell had already fractured.

4. Conclusion

From the all parameters observations, it showed that 2 hours soaking treatment on both variety tested gave significantly highest germination percentage, germination rate, highest shoot and root length, shoot and root dry weight, and the lowest electrolyte leakage in immersed seed water. Interaction between soaking duration and varieties/seed lots was significant on electrolyte leakage of seed immersed water, germination rate, root and shoot length, and root and shoot dry weight.
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