Influence of seed priming on germination characteristics of sorghum (*Sorghum bicolor* L. Moench)

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Abstract. Seed priming or invigoration is presowing treatments of improving germination aimed to reduce the time from sowing to emergence and improving emergence uniformity. Research on seed priming was conducted Indonesian Cereal Research Institute (ICERI) seed laboratory from January until March 2021 to evaluate the effect of seed priming on sorghum seed vigor. Seed quality parameters includes germination percentages, germination rate, shoot and primary root length, seedling dryweight, Sorghum seed variety of Suri 4 were used as seed material. Results showed that among the priming treatments that give positive effect on germination percentage, germination rate, seedling dry weight were priming treatment with KNO₃ 1.5%.

1. Introduction

Sorghum in Indonesia was used as food, feed, and seed. Sorghum was planted in some areas of Indonesia: East Nusa Tenggara, South East Sulawesi, some areas of Sumatera and Java Province. Indonesian Cereal Research Institute has delivered sorghum breeder seed to these areas to be multiplied as foundation seed in order to distribute and use as planting material around the neighbor areas. Although sorghum was still not well developed as large industrial scale, but in some areas of South East Sulawesi (District of East Kolaka), small scale industry of sorghum for food has already developed since 2019. Successfull sorghum crop productivity was supported by qualified planting material i.e.high vigor seed. The use of poor seed quality was one of factors that responsible for low level of yielding. Related with the seed, there are many factors resposible for the low level of yielding: some of these are low quality seed, poor seed germination and poor seedling vigour depending on the agro-ecological zones [1]. Some farmers in remote areas often find difficulties to obtain qualified seeds in the planting period. Thus, farmers usually stored the existing available seeds for the next planting seasons [2].

Generally seeds are very susceptible and sensitive to the adverse environmental conditions, thus the storage environment where the seeds are stored, greatly influences the time period of the survival of the seeds. Unfavorable storage enviromental might deteriorate the seeds resulting the decrease of seed vigor. The deterioration of seeds might be due to interactions of several factors, like initial moisture content, oxygen, temperature and relative humidity (RH) of storage room, pest and disease attacks and others [3]; [4]; [5].

Based on the reports of [6], [7], the seed deterioration due to high oxidation reaction might result in the decrease of vigor and viability of seeds, especially when stored in longer period. Efforts have been
made to prevent and minimize the seed deterioration during storage and one promising method is called invigoration. Invigoration was pre-planting treatment for seed in order to increase the rate of germination, vigor and viability [8]; [9]. Invigoration can also be referred as conditioning or priming. Depending on the seed types, the conditioning methods can be applied through hydro priming or hydration-dehydration, osmoconditioning or osmopriming that using osmotic solution, and matricconditioning which is also called a solid matrix priming that using a damped solid material [10]; [11]. The method that can be done in navigation is priming [12] can also be called seed conditioning. [13] divides seed conditioning into two types, namely osmoconditioning and matricconditioning. Osmoconditioning is the addition of controlled water by adding a salt solution that has a low osmotic potential and a negligible matrix potential. Whereas matricconditioning is the addition of controlled water during inhibition of germination on solid media which has a low matrix potential and negligible osmotic potential.

   The method of hydropri ming can be done by softening the seeds in various ways, such as soaking the seeds, dipping the seeds, spraying the seeds and throwing the wind in the air saturated with moisture. While the process of returning water can be done by drying the seeds with direct sunlight, with an oven at 30 ° C or by aerating the seeds to a heavy start, also known as hydration-dehydration method [14].

   Osmoconditioning begins when the seed is accumulated in the dissolution process with low air potential and air content that can produce after balance [14]. Osmoconditioning success is determined by the amount of water that enters the seed, the osmotic potential and the type used. The usual solutions are PEG, KNO3, K3PO4, MgSO4, NaCl, glycerol and mannitol [13]. Many reports has revealed the succesfull the application of conditiniong on the increase of gemination percentage, uniformity of germination and vigor improvement of seedlings and yield on several crops, like maize [15], rice [16], soybean [17]; [18], wheat [19]; [20], black gram [21], [22]. [23] reported that hydropriming significantly improved germination rate and root weight of lentil (Lens culinaris Medik). compared to other seed treatments. Seed priming has been a common seed treatment to reduce the time betweenseed sowing and seedling emergence and the synchronization of emergence [24]. Sorghum seeds soaking in distilled water or salt solution reduced inhibitory activities of trypsin and chymotrypsin although the effect of the latter treatment was greater [25]. Similar results were obtained when seed of redgram (Cajanus cajan) were pre-soaked in distilled water or salt solution [26]. The research aimed to evaluate influence of the use of KNO3 solutions as priming material to some germination characteristics of sorghum.

2. Methodology
   The research was conducted in the Seed Laboratory of Indonesian Cereal Research Institute (ICERI), Maros, South Sulawesi, Indonesia. Sorghum obtained from 3 seed lots of Suri 4 variety which was harvested in 2019, 2020 and 2021. The teatments were arranged using randomized completely design, thrice, with two factors:
   (1) Seed storage period :
      a. freshly harvested seeds
      b. 12 months storage
      c. 24 months storage
   (2) priming treatment :
      a. control (unprimed)
      b. priming with aquadest
      c. priming with KNO3 0.5%
      d. priming with KNO3 1%
      e. priming with KNO3 1.5%
      f. priming with KNO3 2%.
2.1. Preparation of seeds and application of priming treatments

The seeds were obtained from the cool storage room (temperature 22-24°C; RH 45-50%) with 3 storage periods (12 months, 24 months, fresh harvested seeds). Each sample from seed lots then treated with priming solution (base on the above treatments) for 30 minutes and then aerated until seeds were dried.

Sand as planting media was put into porous plastic box (50 x 16 x 13 cm) at the depth of 1.5 cm. Three replications of 50 seeds of each treatment combination was planted in planting media. Each box were then gently poured with 500 ml water to facilitate humidity. Normal seedlings were evaluated after 7 days.

The observed parameters included percentage of germination, germination rate, shoot length, root length, seedling dry weight and electrical conductivity. Percentage of germination was calculated by the comparison of number of germinated seeds and number of planted seeds after 7 days. Germinated rate was expressed by the margin germinated seed in every observation time. In every observation, total percentage of normal seedling is divided by etmal (24 hours). The germinated rate was calculated using the following formula [27]:

\[ KT = \frac{\text{Xi} \times \text{Ti}}{24} \]

Where,
- \( \text{KT} \) = germination rate (%/etmal)
- \( \text{Xi} \) = the percentage of normal seed etmal i
- \( \text{Ti} \) = time of observation (etmal)

Shoot and root lengths were observed from selected 10 normal seedlings from each treatment combination after 7 days germination periods. Seedling dry weight was measured from the weight of 10 normal seedlings after being forced dried using oven at 1100°C for 17 h. Electrical conductivity was measured weighing 50 seeds of each replication. The seeds of each replication were placed in 200 ml beaker and 50 ml 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 using the following formula [28].

3. Results and Discussion

Analysis of variance revealed that the single effects of priming and seed storage periods treatments were observed significant in all parameters observed. Similar results were also observed in the interaction effects of the treatment being applied.

3.1. Germination percentage

Before the seeds were stored, the seeds underwent certain treatments to lower their moisture contents. These low seed moisture contents was dedicated to control respiration rates and prevent the physiological integrity of seeds during storages [29]. These indicated that under prolonged storages, the moisture content of seeds were decreased until certain periods, the seed moisture content was unable to facilitate and maintain the supply of energy, resulting the death of the seed embryos [30]. The process of water imbibition is the initial process on seed germination and the process was dedicated to stimulate other biochemical process regarding seed germination [31]. Percentage of sorghum, seed germination from different storage periods under various concentration of KNO3 priming treatments was presented in Table 2. In all level of KNO3 concentration treatments, percentage of germination was higher in freshly harvested seed, followed by 12 and 24 months. On the seed lots from freshly harvested, the percentage of germinated seed were not significantly affected by all level of KNO3 priming treatments. On the seeds from 12 and 24 months storages, however, only priming with KNO3 1.5% treatment significantly induced higher percentage of seed germination.
Table 1. Effect of priming treatments on percentage of seed germination (%) of sorghum seed that previously stored in different periods storage period (months)

| Priming Treatment | Seed Storage Period (month) | Fresh harvested (0) | 12    | 24    |
|-------------------|----------------------------|---------------------|-------|-------|
| Control (unprimed)|                            | 98.66 a             | 82.00 d| 68.66 f|
| Aquadest          |                            | 98.66 a             | 84.00 d| 76.00 e|
| KNO₃ 0.1%         |                            | 98.66 a             | 87.33 c| 81.33 d|
| KNO₃ 0.5%         |                            | 98.66 a             | 87.33 c| 83.33 cd |
| KNO₃ 1%           |                            | 98.66 a             | 90.00 b| 87.33 b|
| KNO₃ 1.5%         |                            | 99.33 a             | 97.33 a| 93.33 a|
| KNO₃ 2%           |                            | 98.66 a             | 87.33 c| 84.67 c|

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT (α ≤ 5%).

**) Values in the same column followed by different undercase letters differ significantly by DMRT (α ≤ 5%).

Primming treatments involves the soaking of seeds in osmotica of low water potential to regulate the amount of water supplied to the seed. Priming ensures that some of the metabolic processes required for the germination to occur without the actual germination taking place. Primed seeds, usually pass the imbibition stages and lag stage of germination and are ready for germination [10]. A few processes take place at the time of priming at cellular level, such as the activation of cell cycle [31] and utilization of storage proteins [32]. Priming of aged-seeds gradually restores the primary germination ability and it also reduces the lipid peroxidation levels [3]. Priming usually includes the soaking of seeds in osmotica of low water latency such as salicylic acid. Priming usually improves crop yields substantially [33], impedes ethylene bio-synthesis and it increases the chlorophyll content [34]. Polyamines (PAs), plant phenolic substances of pervasive nature, play different roles in plant metabolism that include cell division, differentiation and proliferation, DNA, cell death, protein synthesis and gene expression [4, 35]. Xu et al. The results in this sorghum priming research was in line with [36], who reported that tobacco (Nicotiana tabacum L.) seed priming with putrescine improved the germination percentage, germination index, seedling length and dry weight of the seed.

3.2. Germination rate

Germination rate of sorghum seeds from various storage period after priming treatments was presented in Table 2. In all priming treatments, the germination rates of sorghum seeds decreased along with the lengthened storage periods, tough in certain priming. Germination rate of sorghum from different storage periods under various concentration of KNO₃ priming treatments was presented in Table 2. In all level of KNO₃ concentration treatments, germination rate was higher in freshly harvested seed, followed by 12 and 24 months. On the seed lots from freshly harvested, germination rate were not significantly affected by all level of KNO₃ priming treatments. On the seeds from 12 and 24 months storages, however, only priming with KNO₃ 1.5% treatment significantly induced higher germination rate. Higher percentage of seed germination and germination rates of sorghum seeds taken from longer storage period when treated with KNO₃ 1.5% indicated that KNO₃ solutions as priming treatment more suitable to induce embryo response and promote seed viability. These conditions ensure the water imbibition process would be slower and take place longer in aerobic conditions than direct water soaking or submersion [37]. Slower and longer imbibition process prevented the disruption of cell membran, thus the membrane stability is maintained to facilitate further biochemical and physiological processes for seed germination [34].
Table 2. Effect of priming treatments on germination rate (%/etmal) of sorghum seed that previously stored in different storage period (months)

| Priming Treatment | Seed Storage Period (month) |
|-------------------|-----------------------------|
|                   | Fresh harvested (0) | 12  | 24  |
| Control (unprimed)| 32.46 b               | 27.14 e | 25.38 e |
| Aquadest          | 32.94 a               | 28.79 d | 26.85 d |
| KNO$_3$ 0.1%      | 33.26 a               | 29.86 c | 26.53 d |
| KNO$_3$ 0.5%      | 32.66 ab              | 30.54 b | 27.53 c |
| KNO$_3$ 1%        | 32.93 ab              | 31.47 a | 28.53 b |
| KNO$_3$ 1.5%      | 32.93 ab              | 32.04 a | 29.93 a |
| KNO$_3$ 2%        | 32.79 ab              | 31.56 a | 28.80 b |

Remarks:*) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$).

**) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

3.3. Seedling dry weight

In line with percentage of germination, germination rate, the dry weight were higher at the seedlings derived from the fresh harvested seeds, followed by 12 and 24 months storages (Table 3). These phenomena were detected in all treatments. Among the treatments, priming with KNO$_3$ 1.5% was apparently still to be the most suitable substances to improve seedling growth quality derived from the stored seed, especially dry weight. Higher seedlings dry weight indicated that tissues and organs formations were optimally promoted through active respiration and biosynthesis from the carbohydrate mobilization [38] and perhaps a part from photosynthesis from the newly developed leaves. Priming repairs damage of aged seeds [39] or seeds exposed to abiotic stresses such as salinity [40], improving germination performance. Priming treatment consists of soaking seeds in an osmotica of low water potential to control the amount of water supply to the seed. At the cellular level, few processes have been described to act during priming some of these being: activation of cell cycle (De Castro et al., 2000 [41]) and mobilization of storage proteins (Gallardo et al., 2001[42]). The priming-induced increase in the rate of seed germination has been associated with the initiation of germination-related processes [43], repair processes [44] and increase in various free radical scavenging enzymes, such as superoxide dismutase, catalase and peroxidase have also been demonstrated [42]. This priming with KNO$_3$ solutions was in line with [45], proposed that halopriming treatment can lead to better germination and establishment in many crops such as maize, wheat, rice and canola.

Many recent researchers suggested that seed priming of crop seeds might be a useful way for better germination, seedling growth, establishment and yield [46]. [47] indicated that during seed hardening process, several physiological and biochemical changes occur; changes in the properties of colloids and the hydrophobic colloids change to hydrophilic, the viscosity of protoplasm and plasticity of plasma lemma increases, increase in osmotic potential which will helps in drought withstanding capacity, activation of enzymes and rate of respiration and synthetic activity increases, increased levels of nucleo-proteins and organic phosphorus, increased in the activity of RNA and rate of protein synthesis, smaller epidermal and stomatal cells and increased total absorbing surface of the root system. Amylases are key enzymes that play a vital role in hydrolyzing the seeds starch reserve, thereby supplying sugars to the developing embryo. Effects of hydropriming on water potential, the driving force for water up-take during imbibition and the activity of $\alpha$-amylase were examined in wheat and rice kernels [48]. In this research, hydopriming with aquadest indicate an increase of germination rate and seedling dry weight (Table 2, 3).
Table 3. Effect of priming treatments on seedling dry weight of sorghum seed that previously stored in different storage period (months)

| Priming Treatment | Seed Storage Period (month) | Fresh harvested (0) | 12 | 24 |
|-------------------|-----------------------------|---------------------|----|----|
| Kontrol (unprimed)| 2.47 f                      | 1.71 e              | 1.22 f |
| Aquadest          | 2.63 e                      | 1.91 d              | 1.43 e |
| KNO₃ 0.1%         | 2.72 cd                     | 1.97 d              | 1.68 d |
| KNO₃ 0.5%         | 2.78 bc                     | 2.10 c              | 1.86 e |
| KNO₃ 1%           | 2.84 b                      | 2.59 b              | 2.16 b |
| KNO₃ 1.5%         | 2.96 a                      | 2.79 a              | 2.53 a |
| KNO₃ 2%           | 2.68 de                     | 2.59 b              | 2.11 b |

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT (α ≤ 5%).
**) Values in the same column followed by different undercase letters differ significantly by DMRT (α ≤ 5%).

4. Conclusions
Priming treatments improved the seed and seedling qualities of sorghum seeds derived from different storage periods. Shorter seed storage period (freshly harvested seed; 0 months) produced better seed and seedling qualities for average higher of seed germination, 12.4-20.3%, higher germination rate, 8.8-19.2% higher seedling dry weight, 21.9-46.8%. Priming treatments improved seedlings qualities in any seed storage period. Priming with KNO₃ 1.5% induced higher seed germination 5.1-16.3%, higher germination rates, 1.9 - 12.5%, higher dry weight and 12 - 55.6%.

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