



# Effect of Different Priming Methods on Seed Viability and Seedling Growth of Upland Rice under Drought Stress

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors MK and MRY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BD and SM managed the analyses of the study. Author Syamsia managed the literature searches and revised the manuscript. All authors read and approved the final manuscript.

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## ABSTRACT

This study aims to determine the effect of different priming methods on the viability and early growth of upland rice under drought stress. Priming is an important approach to improve the resistance of upland rice plants to drought stress from the germination phase to growth, especially on sub-optimal land. There are several efficient priming methods as seed pretreatment to increase germination and tolerance to drought stress. An effective priming method is needed to increase the germination and growth of upland rice seedlings for application in dryland agriculture. Therefore, an

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experiment was conducted at the Seed laboratory of Pangkep State Polytechnic of Agriculture, Indonesia, from September to October of 2022 to investigate the effect of several priming methods on the germination and growth of upland rice seedlings under drought stress. The experiment consisted of 2 stages, germination testing and seedling growth phase testing with the addition of water stress treatment. Experiment 1 was conducted in a completely randomized design (CRD) with four replications, including Control (no priming), Osmopriming with 15% and 20% PEG solution, Redox Priming with 3% and 6% H<sub>2</sub>O<sub>2</sub> solution, and Organic Priming using 50% and 75% Moringa leaf extract. Experiment 2 was conducted using a two-factor of factorial in Randomized Block design (RBD) with three replications. The first factor is the Seedling results of stage 1 experiments (selected the best of each priming method) includes 4 treatments each Control treatment, Osmopriming with 15% PEG solution, Redox Priming with 3% H<sub>2</sub>O<sub>2</sub> solution, and Organic Priming with 50% Moringa Leaf Extract. The second factor is the level of Drought Stress conducted by 100%, 60%, and 30% of Field Capacity. The results showed that the priming method with Osmopriming 15% PEG solution gave the best results on Seed germination percentage (87.5%) followed by Organic priming with 50% Moringa Leaf Extract (SGP 85%). Under drought stress conditions with 30% field capacity, the highest increase in proline levels was observed with H<sub>2</sub>O<sub>2</sub> redox priming 3% (10.3  $\mu\text{-mol. g}^{-1}$ ), while the average root growth of all primed seedlings showed better root growth than seeds without priming treatment. Seed priming gives better results on the growth and physiological activities of upland rice at several levels of drought stress, in the early growth phase of seedlings

**Keywords:** Priming; osmopriming; germination; drought; upland rice.

## 1. INTRODUCTION

Rice is the staple food of most Indonesians, so the economic aspect of rice is a strategic commodity and a supporter of Indonesia's rapid economic growth. In addition to the issue of seeds and the development of crop cultivation technology, rice production is highly dependent on the availability of land area and how the threat of natural disturbances affects it [1]. The data from [2] shows that the harvest area of paddy rice in 2021 is 10,411,801.22 hectares with Milled Dry Grain (MDG) production reaching 54,415,294.22 tons or equivalent to approximately 31.36 million tons of rice. If the average Indonesian needs approximately 130 kg of rice per year, then the consumption of 273,879,750 Indonesians could reach 35,604,367.5 tons [2]. Rice production is still vulnerable to production shortages, especially in conditions where there are climate disruptions and environmental disasters that can cause crop failure and thwart national rice production targets such as the threat of flooding during the La Nina disaster, the threat of drought during the *el-nino* period, pest attacks and various other biotic and abiotic disorders cannot be avoided. Global warming conditions (*climate change*) also pose a threat to national rice production [1].

Upland rice is a type of rice that has the potential to be developed to support Indonesia's rice production, especially in drylands. Indonesia has 53,963,705 Ha of drylands, or 28.67% of the total

Indonesian land area, and is a huge asset in realizing rice self-sufficiency [2,3] suggested that drylands can be utilized for rice extensification by developing more intensive upland rice cultivation.

Potential drylands for upland rice are areas with ecosystems that tend to be more critical than the ecosystems of paddy fields and tidal lands that have been used for upland rice production. Therefore, the development of upland rice has many problems that must be faced, in addition to low productivity, erosion, and degradation of soil fertility. Drought is one of the most disastrous stressors for rice cultivation in the world. It causes serious yield losses in annual rice production [4]. The threat of drought stress to rice plants has become a major concern, and various experiments have been conducted to evaluate the resistance of rice plants to drought stress and conduct selection to obtain drought resistance indices of rice [4] and [5]. Physiological and biochemical changes in rice seeds after priming treatment are still not widely reported or conducted. Sun et al. [6] conducted priming experiments to improve drought resistance in rice, and found the dynamic changes of some physiological characteristics in the rice seeds during germination after hydro priming and Osmopriming using Polyethilen Glycol (PEG).

Priming is the treatment of seeds with natural or synthetic compounds before germination to induce a certain physiological state in the plant

that makes the plant competent enough to undergo cellular defense responses to all types of stresses. It has proven to be an effective method in imparting stress tolerance. Different seed priming methods have been found to show a significant impact on germination, seedling growth, and crop yield under normal as well as stress influenced by factors such as aeration, temperature, light, duration of treatment, and seed quality [7]. The benefits offered by seed priming include faster germination rates, seedling vigor, and plant establishment under adverse conditions. It also reduces soil-borne diseases and increases the production of enzymes that protect plants against oxidative damage caused by free radicals thereby reducing stress-induced adverse effects on plants [8]. The defense mechanisms developed as a result of seed priming also form a 'primary memory' that helps achieve greater tolerance to abiotic stress upon subsequent exposure [9]. Priming treatment on seeds as a treatment before seeds are planted aims to increase endogenous plant activity so that it is expected that the performance of physiological and biochemical plant activities will change to become more survivable [10]. The use of various methods is more widespread trying to see how effective it is in triggering plant tolerance to stress. To induce stress in drought experiments can be done by manipulating water application (irrigation intervals, field capacity), or in some studies, drought stress is obtained from differences in osmosis pressure levels obtained by applying PEG as conducted in [11].

Priming is becoming an important approach to enhance plant defense against biotic and abiotic stresses [12]. It is defined as the pre-exposure of seeds or young seedlings to chemical agents or abiotic stresses (salinity, drought, cold, etc.) making the plant more resistant to stresses in the next phase and more able to detect a second signal quickly [13]. Many efficient priming compounds and priming methods are used. One study reported that seed pre-treatment with Gibberellic Acid (hormonal priming) increased germination and improved tolerance in *Trifolium* plants grown in heavy metal-contaminated soil [14]. Ouhibi et al. [15] explained that exposure to the UV radiation of lettuce seeds strengthens the resistance of this species to salt stress (salinity). Osmopriming treatment (Using Sorbitol, mannitol, or PEG) is a beneficial pretreatment for cottonseed and provides this species with subsequent oxidative stress tolerance [16]. Besides Osmopriming, there are various priming methods to increase plant tolerance to drought or

other abiotic stresses reported, including Halopriming (using NaCl media/agents, NaCl, KNO<sub>3</sub>, CaSO<sub>4</sub>), Hydropriming (using H<sub>2</sub>O media/agents), Chemical Priming (Ethanol, Choline, ZnSO<sub>4</sub>, BABA, etc.), Redox Priming (H<sub>2</sub>O<sub>2</sub>, Glutathione, Cysteine), Biopriming (using biological agents *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp) and Priming using plant extracts (Essential oils) [17].

The effectiveness of various priming methods is related to the materials used so that the results obtained to increase the tolerance of rice plants to drought stress will potentially be different, where one method may be better to increase biochemical and physiological activities towards better growth in upland rice plants, therefore this study aims to see the effectiveness of several priming methods to increase germination and tolerance of upland rice plants to drought stress in the early vegetative growth phase of upland rice seedlings.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

This experiment was conducted at The Seed and Plant Laboratory Department of Agricultural Production Technology, Pangkep State Polytechnic of Agriculture, Mandalle Pangkep Regency from August 2022 to October 2022. It is located on Latitude 4°33'59"S and Longitude 119°35'50"E, 57 m above sea level.

### 2.2 Planting Material

The Planting material used in this experiment was one Upland rice Variety namely "Situbagendit", an upland rice variety produced by the Indonesian Rice Research Center in Sukamandi, Subang West Java. This Upland rice variety was chosen because it is a superior upland rice variety with productive tillers of 12 - 13 stems/shrub, and it is resistant to blast disease, moderately resistant to leaf blight, and resistant to Rice *Tungro Bacilliform Virus* (RTBV) diseases. Other materials used were Polyethylene Glycol (PEG) 6000, Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>), Aquadest, Ethanol 95%, Moringa Leaf Extract, Compost + Topsoil, Whatman filter paper No.1, petri-dish pot, and plastic Cups

### 2.3 Experimental Design and Layout

The experiment was carried out in 2 stages, i.e. the germination stage and the phase of seedling growth with water stress treatment. The First stage of the experiment was laid out in a

complete randomized design (CRD), with four replications, in the seed laboratory, that is Control (no priming), Osmoprimer with 15 % PEG Solution [18], and 20 % PEG Solution [19], Redox Priming with 3% H<sub>2</sub>O<sub>2</sub> Solution [20] and 6% H<sub>2</sub>O<sub>2</sub> Solution, and then Organic Priming with 50% and 75 % Moringa Leaf Extract. A total of 56 petri-dish were used in the experiment, with 20 seeds being sown and placed per petri-dish on Whatman filter paper softened inside each petri-dish with a diameter of 90 mm. The Second stage of this experiment was laid out in a Factorial 2 factor of Randomized Block Design (RBD) with three replication. The first factor was the seedlings from the first stage of the experiment (selected the best from each priming method) so there were 4 treatments namely Control treatment (no priming), Osmoprimer with 15% PEG solution, Redox Priming with 3% H<sub>2</sub>O<sub>2</sub> solution, and Organic Priming with 50% Moringa leaf extract. The second factor was Drought Stress consisting of 100%, 60%, and 30% of the Field Capacity of planting media. there are a total of 12 treatment combinations. The germination phase observation and calculation were daily performed for seven days after sowing the seeds [21].

## 2.4 Measurement and Data Collection

Data were collected on the seed germination phase in the laboratory and the seedling growth phase in the screen house. In the Seed Germination stage, measured Data were collected on the following;

**The germination percentage (%)**: The germination percentage was calculated based on the total number of germinated seeds divided by the total number of seeds used.

**Mean germination time (MGT) or Germination Rate (Day)**: Expressed in days was calculated based on the number of additional normal germinated each day. The germinated rate was calculated daily for 7 days on normally growing seeds, Following equation (1) [21] (Where N is the number of seeds germinated on the day, and T is the number of days counted from the beginning of the germination test.)

$$MGT = \frac{N_1T_1 + N_2T_2 + \dots + N_xT_x}{N_1 + N_2 + N_3} \times 100\% \quad (1)$$

**Seedling Vigor Index (%)**: Vigour Index was performed on the number of normal germinated seeds on the first count, on day 5 [21] following equation (2):

$$SVI = \frac{\text{Number of Normal First Count Germinated}}{\text{Total number of Seeds Used}} \times 100\% \quad (2)$$

**Germination Simultaneity (%)** was measured based on the number of strong normals on day 6 after the seeds were germinated. Following equation (3)

$$GS = \frac{\text{Number of Normal Germinated day6}}{\text{Total number of Seeds Used}} \times 100\% \quad (3)$$

Seedling growth stage measured Data were collected on the following:

**Seedling Root length (cm)**: The average length of roots for seedling were measured using the centimeter rule and the values were recorded in centimeter (cm).

**Proline Content (μ-mol. g<sup>-1</sup>)**: measured using the troll and Lindley method [11]. A total of 0.5 g of leaf sample was crushed in 10 ml of ninhydrin acid, 2 ml of glacial acetic acid, heated at 100°C for 1 hour, added 4 ml of toluene, measured by spectrophotometry /Spectronic 21-D (UV spectrophotometer) at a wavelength of 520 nm. Proline concentration was compared with the proline standard.

## 2.5 Statistical Analysis

Data collected were subjected to statistical analysis (ANOVA), using STAR (Statistical Tools for Agricultural Research by IRR) software (ver.2.0.1). The data analysis on parameters that have a significance is continued with a posthoc test of means separated using Duncan multiple range test (DMRT) and Least Significance Different (LSD)

## 3. RESULTS

### 3.1 Effect of Priming Methods on the Viability of Upland Rice Seeds

#### 3.1.1 Seed Germination percentage (SGP) and Days Germination Time (DGT)

The analysis showed a significant (P<0.05) influence of different priming methods on the seed germination percentage and Days Germination Time of Upland Rice Seed. The results regarding the seed germination percentage and Days Germination Time of Upland rice seed as influenced by different

priming methods have been demonstrated in Table 1.

The results of posthoc test (5% level of DMRT ) on Seed germination percentage (Table 1) showed that the Osmopriming treatment with 15% PEG solution gave the highest percentage of germination with an average of 87.5%, significantly different from the control. Another treatment that also shows the percentage of germination significantly different from the control is the organic priming treatment of moringa leaf extract at 50% with an average of 85.0%. The data also showed that the 3% H<sub>2</sub>O<sub>2</sub> redox priming treatment was better than the same priming at the 6% level. Osmopriming treatment with 15% PEG solution had the fastest Days Germination Time (an average value of 2.4 days), also with Days Germination Time Other priming treatments showed better Days Germination Time and were significantly different from the control (no priming).

### 3.1.2 Seedling Vigor Index (SVI)

The analysis showed a non-significant (P>0.05) influence of different priming methods on the

Seedling Vigor Index of Upland Rice Seed. The results regarding the Seedling Vigor Index (%) as influenced by different priming methods have been shown in Fig. 1.

An interesting observation on the Seedling Vigor Index (Fig.1) is that the Organic Priming of *Moringa Leaf Extract* 50% (SVI=0.89) is higher than the same priming material with 75% content (SVI= 0.78), as well as for Osmopriming with PEG 15% (SVI=0.89) is higher than Osmopriming with PEG 20% (SVI=0.78).

### 3.1.3 Germination Simultaneity (GS)

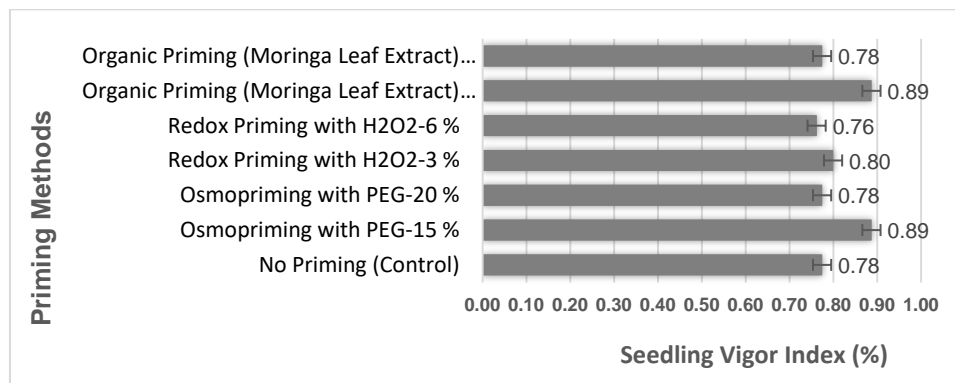
The analysis showed a non-significant (P>0.05) influence of different priming methods on the Germination Simultaneity of Upland Rice Seed. The results regarding the Germination Simultaneity as influenced by different priming methods have been shown in Fig. 2.

The diagram in Fig. 2 shows that Organic Priming with 50% *Moringa Leaf Extract* has the highest average value of Germination Simultaneity with an average value of GS=90%, also Osmopriming with PEG 15 % (GS=0.89).

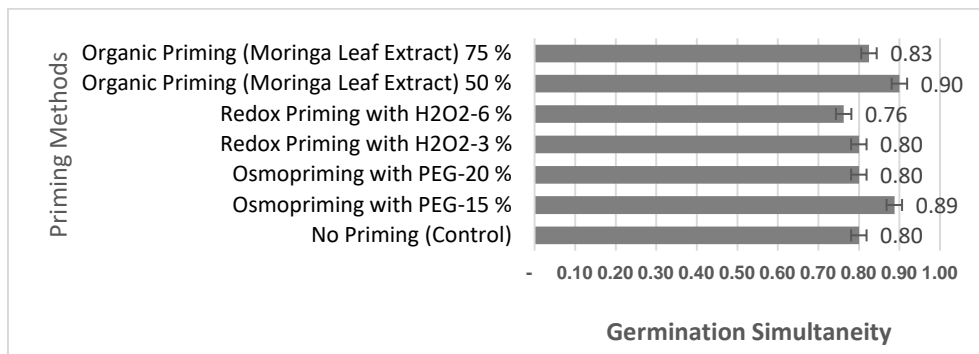
**Table 1. Effect of different priming methods on seed germination percentage and days germination time of upland rice seed**

Priming method	Seed Germination Percentage (%)	Days Germination Time (Day)
No Priming (Control)	75.00	3.04
Osmopriming with PEG-15 %	<b>87.50</b>	<b>2.40</b>
Osmopriming with PEG-20 %	80.00	<b>2.37</b>
Redox Priming with H <sub>2</sub> O <sub>2</sub> -3 %	<b>81.25</b>	2.65
Redox Priming with H <sub>2</sub> O <sub>2</sub> -6 %	78.50	2.93
Organic Priming (Moringa Leaf Extract) 50 %	<b>85.00</b>	<b>2.37</b>
Organic Priming (Moringa Leaf Extract) 75 %	82.50	<b>2.41</b>

*This means having a similar letter(s) is statistically non-significant and those having a different letter(s) are significantly at a 5% level of DMRT Test*



**Fig. 1. Effect of different priming methods to upland rice seedling Vigor Index (SVI)**



**Fig. 2. Effect of different priming methods to upland rice seed's germination simultaneity (GS)**

### 3.2 Effect of Priming Method and Drought Stress on Upland Rice Seedling

H<sub>2</sub>O<sub>2</sub> Redox Priming treatment (average Proline Content = 10.3  $\mu$ -mol. g<sup>-1</sup>).

#### 3.2.1 Seedling root length

The analysis showed a significant effect ( $P < 0.05$ ) of different priming methods on the root length of upland rice seedlings under drought stress conditions induced by the level of Field Capacity Percentage (%) applied to the planting medium.

Table 2 shows that at 60% Field Capacity (Drought Stress Condition), the longest roots were shown in seedlings with 3% H<sub>2</sub>O<sub>2</sub> Redox Priming treatment (average Root Length = 16.4 cm), and at 30% Field Capacity (More Drought stress Condition), all priming method treatments gave longer root lengths than seedlings without priming (Control). The longest roots in 30 % Field Capacity were shown by the treatment of Osmopriming with PEG 15% (average Root Length 15.87 cm), significantly different from seedlings without priming treatment (Control) (14.1 cm).

#### 3.2.2 Proline content

The analysis showed a significant effect ( $P < 0.05$ ) of different priming methods on the Proline Content in the Leaf of upland rice seedlings under drought stress conditions induced by the level of Field Capacity percentage (%) applied to the planting medium.

Table 3 shows that at 60% Field Capacity (Drought Stress Condition), the highest proline content was shown in seedlings with 15% PEG Osmopriming treatment (average Proline Content = 9.33  $\mu$ -mol. g<sup>-1</sup>), and at 30% Field Capacity (More Drought stress Condition), the highest proline content was shown in seedlings with 3 %

## 4. DISCUSSION

Germination of rice seeds as the beginning of growth requires optimal environmental conditions, this is because germination is a very decisive process for further growth. Actually, under normal conditions including good physiological conditions of seeds (known as the quality and superior seeds), this will not be a significant problem. Under certain conditions such as land conditions and micro-climatic conditions that are less than optimal and when physiological deterioration of seeds occurs, some aspects of biotechnology need to be done to spur the physiological activity of seeds to be better in the face of stress, especially drought stress. This often occurs in the development of food crops on marginal lands such as upland rice, where aspects of seed viability regarding germination, vigor, and uniformity need to be stimulated so that there is plant resistance in its growth in the face of environmental conditions. Several seed treatments can be carried out before sowing. In this case, one important treatment that can be done as an initial treatment is priming. There is a strong basis and evidence on how seed polishing is a very useful treatment and effective method that leads to improved plant performance to grow and develop under stressful conditions [12].

Seed priming is a common pre-sowing step in some vegetable and flower crops in some countries. The mechanism of seed priming is to initiate a membrane repair system and metabolic preparation for germination by controlling the rate of water absorption in seeds. So that the results of this study are in line with the study results of [22] who stated that the ability of germination and seed resistance to unfavorable seed conditions

**Table 2. Root Length (cm) of upland rice seedlings at various priming methods and level of field capacity**

Priming Methods	Field Capacity (%)					
	30		60		100	
No Priming (Control)	14.10	c	15.27	b	15.12	c
Osmopriming with PEG-15 %	<b>15.87</b>	a	15.12	b	16.00	b
Redox Priming With H <sub>2</sub> O <sub>2</sub> 3 %	15.12	b	<b>16.40</b>	a	15.20	c
Organic Priming with Moringa Leaf Extract 50 %	<b>15.73</b>	ab	15.57	b	16.97	a

*This means having a similar letter(s) is statistically non-significant and those having a different letter(s) are significantly at a 5% level of LSD Test*

**Table 3. Proline content in leaf ( $\mu\text{-mol. g}^{-1}$ ) of upland rice seedlings at various priming methods and levels of field capacity**

Priming Methods	Field Capacity (%)					
	30		60		100	
No Priming (Control)	9.40	b	8.71	b	8.40	a
Osmopriming with PEG-15 %	9.45	b	<b>9.33</b>	a	8.38	a
Redox Priming With H <sub>2</sub> O <sub>2</sub> 3 %	<b>10.30</b>	a	8.68	b	8.47	a
Organic Priming with Moringa Leaf Extract 50 %	9.16	b	8.85	ab	8.44	a

*This means having a similar letter(s) is statistically non-significant and those having a different letter(s) are significantly at a 5% level of LSD Test*

can be significantly improved by priming treatment. It is further explained that hydro priming and PEG priming have an effect in stimulating rice seed metabolism, accelerating germination, and increasing seedling tolerance to drought. Rice seeds after hydro or PEG priming showed higher germination and better seedling quality than without priming treatment when exposed to drought-stress conditions. However, according to [23], the regulatory effect of priming is at a certain level, and there are differences between genotypes or the reaction of each genotype to seed priming treatment will vary from one another.

Priming technology enhances antioxidant activity, respiration, gene expression, and DNA repair through pretreatment while simultaneously altering biochemical and physiological activities after seed germination. As a result, oxidative stress associated with most abiotic stresses is reduced. In addition, the synthesis of genes and proteins involved in water transport and translocation, changes in the cell wall structure, cytoskeleton, and cell division are accelerated [24]. In this regard, some studies have shown that priming may involve heritable epigenetic changes [25] This plant's innate defense system is induced by several inorganic or organic molecules and various biological stimulants to protect plants from the resulting stress. Over the past few years, seed priming has emerged as a cost-effective and amenable approach to biotic

and abiotic stress management without resorting to genetic/transgenic modification. It increases germination percentage, enhances uniform seedling emergence, and also increases crop production [22]. This study also showed the effect on germination uniformity and simultaneity.

The important aspect of priming treatment is also highly expected in ecologically unfavorable field conditions that result in low seed germination rates and uneven seedling growth in dryland rice cropping systems (upland rice) or direct seed transplanting systems (*Tabela*). Generally, farmers will tend to increase the volume of seeds to be planted or sown to ensure the number and density of seedlings that grow in the field is sufficient to be planted on a certain land area, this will certainly increase the need for seeds per area which has an impact on higher costs. An effective seed priming treatment can overcome this problem. A proper priming treatment can stimulate seed germination, improve seedling quality, and increase seedling tolerance to drought. These advantages directly result in increased germination rate, seedling rate, and vigorous seedling rate, which can be a solution to reduce seed quantity. The optimal concentration of priming inducers is related to rice cultivars. Thus, it should be determined before practical application. According to [22], medium-concentration PEG priming resulted in higher tolerance to drought stress than water priming.

Priming treatment of rice seeds is generally aimed at accelerating the time required to start the germination process, increasing the rate of germination and synchronization, increasing the length of shoots and roots, and thus increasing the fresh and dry weight of seedlings [26]. Pretreatment of seeds with H<sub>2</sub>O<sub>2</sub> priming is expected to improve plant growth starting with good germination and of course when plants are exposed to stress. Priming using H<sub>2</sub>O<sub>2</sub> plays an important role in the regulation of seed germination [27]. It has also been previously found that the pretreatment of rice seedlings with H<sub>2</sub>O<sub>2</sub> priming is more efficient than without priming because priming properties can improve tolerance to subsequent stresses [28]. The effectiveness of priming strategies depends largely on the nature of the priming agent, plant species, and seed physiology. Priming with an H<sub>2</sub>O<sub>2</sub> solution stimulates beneficial growth and antioxidant defense. The effect of priming with H<sub>2</sub>O<sub>2</sub> suggests that during early stages, seeds acquire the ability to store information that is recalled after the imposition of stress and prepare themselves to respond aggressively to severe osmotic stress, thus better performance was recorded in seedlings raised from seeds when compared to those from seeds primed with mannitol solution [29].

The accumulation of proline in plant tissues that experience drought stress is an indicator or marker of the level of reaction or response of plants to the level of tolerance to drought stress [30]. In the process of cellular homeostasis, redox reactions, and cellular energy status proline also has an important role as stated by the results of research from [31], which also suggests that proline content increases under water stress conditions so that it can be interpreted that priming treatment increases stress tolerance by increasing the proline content of seed priming combinations effectively reduce drought stress for plants. Plant growth, development, and yield are significantly affected when the germination stage is under adverse environmental conditions. Drought stress markedly inhibits seed germination by creating a low osmotic potential that prevents water uptake [32].

## 5. CONCLUSION

The different priming methods given to the seeds gave different results on upland rice seed viability and seedling growth under drought conditions. The priming method with osmopriming 15% PEG

solution gave the best results on seed germination percentage (87.5%) followed by organic priming with 50% moringa leaf extract (85%). Under drought stress conditions with just 30% field capacity, all priming methods gave longer root lengths than seedlings without priming. The longest roots were shown by osmopriming with PEG 15% (average root length 15.87 cm). The highest increase in proline levels was observed with H<sub>2</sub>O<sub>2</sub> redox priming at 3% (10.3 μ-mol. g<sup>-1</sup>). Seed priming gives better results on the growth and physiological activities of upland rice at several levels of drought stress, in the early growth phase of seedlings.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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