

## Effect of seed priming on the germination of upland rice (*Oryza sativa* L.) sown in acidic soil



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**Abstract.** Upland rice is a type of rice that can be grown in Ultisol. The obstacles to cultivating plants in Ultisol are low pH and high aluminum content which hinders seed germination. Seed priming is a technology that can overcome problems with Ultisol. The aim of this research was to determine the effect of priming on the germination of upland rice seeds in acid soil conditions. The experiment conducted on non-factorial Completely Randomized Design (CRD) consisting of 8 treatments with 3 replications. The data were analyzed for variance and followed by Honest Significant Differences (HSD) at  $\alpha$  5% using the Statistic R Program. Seed priming treatments tested were (1) Untreatment; (2) Hydropriming; (3) priming GA<sub>3</sub> 25 ppm; (4) priming GA<sub>3</sub> 50 ppm; (5) priming PEG 6000 10%; (6) priming PEG 6000 20%; (7) priming KNO<sub>3</sub> 0.5%, and (8) priming KNO<sub>3</sub> 1%. Rice seed are soaked in priming for 24 hours. The upland rice seeds of the Inpago 13 Fortiz variety were planted in Ultisol soil media with a pH of 4.45, Al content of 0.44%, and Fe 1.37%. The results showed that the priming treatment increased the germination and the most effective treatment was priming GA<sub>3</sub> 50 ppm, each value of showed germination (92.38%), germination speed (19.71% day<sup>-1</sup>), vigor index (83.81%), and time of appearance of plumule (2.96 day).

**Keywords:** Enhancement, invigoration, paddy, seed, ultisol

### INTRODUCTION

Upland rice is a rice variety well-suited to be cultivated in dryland areas. In Indonesia, the productivity of upland rice is lower, ranging from 2.32 to 4.5 tons ha<sup>-1</sup>, compared to rice that has reached 5.12 tons ha<sup>-1</sup> [1]. The upland rice variety Inpago 13 Fortiz has an anti-stunting nutritional Zinc (Zn) content of  $\pm$  34 ppm, protein content of 9.83%, and high yield (8.11 tons ha<sup>-1</sup>). This variety is also resistant to blast disease and rice brown planthopper [2]. Climate change is expected to reduce rice productivity up to 20 % ha<sup>-1</sup>. The upland rice is an alternative for increasing rice production and food security [3] [4].

Plants on dryland is faced with challenges due to the attributes of the land, such as low pH and high aluminum (Al) and iron (Fe) content [5]. This condition causes plants' low productivity, including the germination process delay at sowing time. Low germination of upland rice on acidic soils results in more seeds than the quantity employed in lowland cultivation. Planting upland rice requires 5-7 seeds hole<sup>-1</sup> [6]. Consequently, the need for upland rice seeds reaches 30 kg of seeds ha<sup>-1</sup> compared

to lowland rice, which is only approximately  $\pm$  20 kg ha<sup>-1</sup>.

Seed priming improves germination and abiotic stress tolerance. Priming seeds allows seed metabolism to start, but the seed germination process does not occur. The seeds can germinate perfectly when sown in less favorable land because the metabolic process begins before planting. Seed priming can be used to quickly improve quality and uniform seed emergence [7][8].

Priming increases the metabolism of seed germination so that the radicle emerges more quickly. In immature seeds, they were priming repairs the membrane [9][10][11]. Seed priming can regulate physiological activities such as cell division, elongation, and activation of stress response proteins [12][13]. Seed priming techniques could improve seed germination under less normal and adverse environmental conditions such as salinity, alkaline, and drought stress [14][15][16]. Some priming techniques have been reported to be successful in various crops including maize [17][18][19], tomato [20], sorghum [21], cucumber [22], and rice [23][24]. Various priming techniques include hydropriming, halopriming, osmopriming, and hormonal priming. The



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hydropriming type involves soaking seeds in water, while the halo entails immersing seeds in solutions of inorganic salts such as NaCl, KNO<sub>3</sub>, CaCl<sub>2</sub>, and CaSO<sub>4</sub>. Osmopriming is a treatment where seeds are soaked in solutions containing sugar and polyethylene glycol (PEG). Moreover, priming can also be achieved using gibberellic acid (GA) [25].

Gibberellin (GA<sub>3</sub>) is involved in plant growth and acquiring mineral nutrients [26]. GA<sub>3</sub> plays a role in plant growth and development under different environmental conditions. Seed germination, root growth, stem elongation, and water uptake can be improved by gibberellin treatment. Seed invigoration with gibberellins is adequate for plant seedling growth and nutrient absorption [22][27][28]. Priming with GA<sub>3</sub> improves seed germination by increasing the activity of carbohydrate metabolism enzymes and chlorophyll biosynthesis, increasing embryo development and plant seedling growth [29][30][31].

Nitrogen is an essential nutrient for plant growth. The availability of essential nutrients such as nitrogen and its uptake are essential and influence growth. Plants have a comprehensive transport system involved in the uptake and transport of nitrate ions from the soil through the roots to the shoots [32]. Nitrogen, whose source is KNO<sub>3</sub>, functions in wall structure, cell elongation, and division of plants [33]. On the other hand, potassium which contained in KNO<sub>3</sub> functions in balancing membrane potential, which activates enzymes and regulates osmotic pressure in cells [34][35].

In plants, nitrogen functions in nutrient absorption across cell membranes and increases water absorption [36]. Priming rice seeds with KNO<sub>3</sub> increases protein and soluble sugars during germination under drought stress conditions. In addition, the activity of catalase enzyme, ascorbate peroxidase, and superoxide dismutase of rice under stress was increased by KNO<sub>3</sub> priming treatment [15]. Nitrogen is the main limiting factor in plant production. Nitrogen stress reduces grain yield by inhibiting plant growth and development [37][38].

Polyethylene Glycol (PEG) increases the vigor of seeds germinated under stress conditions. The water potential around the seed becomes lower so that PEG can regulate the rate of water uptake during imbibition [39]. PEG is a compound that can reduce the osmotic potential of a solution and bind water molecules with hydrogen bonds [12]. The PEG germinated in aluminum stress responds well because PEG can secure aluminum, so water remains available. This aim of this research was to determine the effect of priming on the germination of upland rice seed sown in Ultisol media.

## METHODOLOGY

### Place, Time, and Research Design

This research was conducted at the Seed Technology Laboratory and Greenhouse, Faculty of Agriculture, University of Lampung, from January to May 2022. Seed priming was conducted in laboratory and seed planting carried out in the greenhouse. This experiment was a non-factorial design arranged in a Completely Randomized Design (CRD), consisting of 8 treatments

and 3 replications. The treatments tested were (1) without priming, (2) hydropriming, (3) GA<sub>3</sub> priming at 25 ppm, (4) GA<sub>3</sub> priming at 50 ppm, (5) PEG 6000 priming at 10%, (6) PEG 6000 priming at 20%, (7) KNO<sub>3</sub> priming at 0.5%, and (8) KNO<sub>3</sub> priming at 1%.

### Priming and Germination of Seeds

The priming application was carried out by immersing fifty (50) the upland rice seeds of the Inpago 13 Fortiz variety into priming solutions. Rice seed are obtained from Indonesia Center for Rice Research. Hydropriming involves soaking the seeds in water for 24 hours [40]. GA<sub>3</sub> priming was carried out at 25 and 50 ppm concentrations, involving soaking for 24 hour [23]. PEG priming was administered at 6000 10% and PEG 6000 20% for 24 hours [41], and KNO<sub>3</sub> priming at 0.5% and 1% for 24 hours [42]. After seed priming, fifty (50) the upland rice seeds were dried and sown in trays (25 cm x 45 cm) containing acidic soil at a planting density of 1 seed per planting hole. The planting medium used was Ultisol soil with a pH of 4.45, Al<sup>3+</sup> content of 0.44%, and Fe<sup>2+</sup> content of 1.37% kg<sup>-1</sup> of soil.

### Observational Variables

Variables of viability and vigor of rice seeds observed were:

#### Germination (%)

Germination was obtained by calculating the number of seeds germinating normally (NG) in the first count, 7 days after planting (DAP), and the second count, at 14 DAP (International Rules for Seed Testing, 2022 and Handbook on Seedling Evaluation, 2009). Seed germination was calculated using the formula expressed below:

$$\text{Germination (\%)} = \frac{\sum \text{NG first count} + \sum \text{NG second count}}{\sum \text{planted seeds}} \times 100\%$$

Information:

NG = Seed germinating normally

#### Speed of Germination (%NG day<sup>-1</sup>)

Speed of germination was calculated by the percentage of sprouts that grew normally (NG) every day for 14 days, the formula:

$$\text{Speed of Germination (\%/day)} = (\% \frac{\text{NG}}{\text{day}}) = \sum_0^{\text{tn}} \frac{\text{N}}{\text{t}}$$

#### Vigor Index (%)

The vigor index was observed on the number of normal sprouts on the first count, at 5 DAP. The vigor index was calculated using the formula expressed below:

$$\text{Vigor index (\%)} = \frac{\sum \text{normal sprouts on the first count}}{\sum \text{planted seeds}} \times 100\%$$

#### Plumule Appearance Time (days)

The time of plumule appearance was observed on the day of the first appearance on the soil surface for each treatment. The time the plumule appeared was calculated using the formula:

$$\text{PAT (days)} = \frac{\text{N1T1} + \text{N2T2} + \text{N3T3} + \dots + \text{N14T14}}{\text{The total number of seeds that germinated}}$$

Information:

N = Total plumule that appears in a certain time unit

T = Total time between the start of the test to the end of a certain interval of an observation

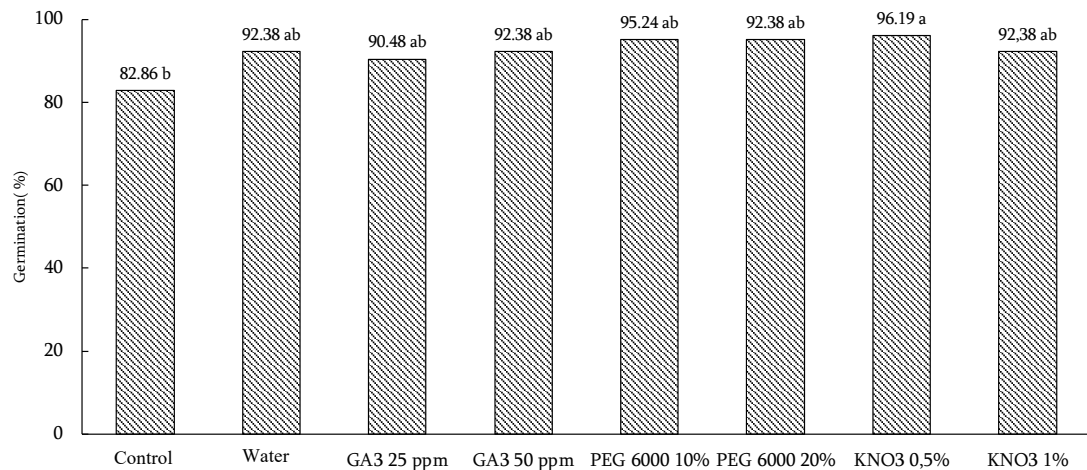


**Figure 1.** Effect of priming treatment of upland rice seeds on germination sown in acid soil 14 days after sowing.

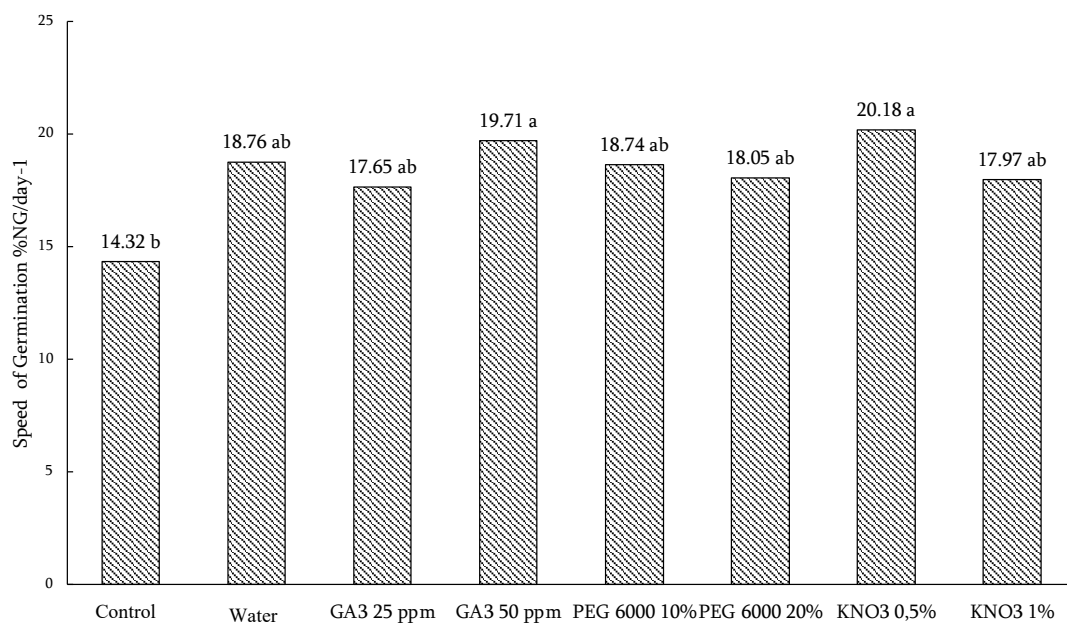


**Figure 2.** Effect of priming treatment of upland rice seeds on growth speed sown in acid soil 14 days after sowing.

#### *Sprout Length (cm)*

Sprout length was measured from the base of the coleoptile to the tip of the longest leaf, carried out on the last day of observation, the 14th day. Measurements were made on normal sprouts for each repetition.

#### *Root Length (cm)*

Root length was measured on day 14, utilizing the tip to the base of the root on all normal sprouts from each replication.

#### *Normal Sprout Wet Weight (g)*

Normal wet weight of sprouts was carried out on the 14th day. The wet weight of normal sprouts was obtained by weighing normal sprouts 14 DAP using a digital scale

#### *Normal Sprout Dry Weight (g)*

The dry weight of normal sprouts was obtained by weighing normal sprouts at 14 DAP, which had been dried in an oven at 80°C for 3 x 24 hours and weighed using a digital balance.

#### **Data Analysis**

Based on 8 treatments which were repeated 3 times, there were 24 experimental units. Fifty seedlings from 24 experimental units were taken as samples for all observation variables. The data were analyzed for variance and subjected to an HSD test at a 5% using *RStudio* statistical program.

## **RESULTS AND DISCUSSION**

#### **Germination (%)**

Figure 1 showed that priming treatment enhanced the germination of upland rice seeds sown in acidic soil. The 0.05% KNO<sub>3</sub> priming treatment yielded the highest germination percentage at 96.19%. Although this percentage was not significantly different from other treatments, it was higher than the non-primed seeds, which only achieved 82.86% germination. Previous research by Olaynika et al [43] on maize seeds, wheat seeds, [44], and tomato seeds [20], showed that KNO<sub>3</sub> priming enhanced seed germination under salinity stress conditions. Conversely, [15][45] reported that KNO<sub>3</sub>

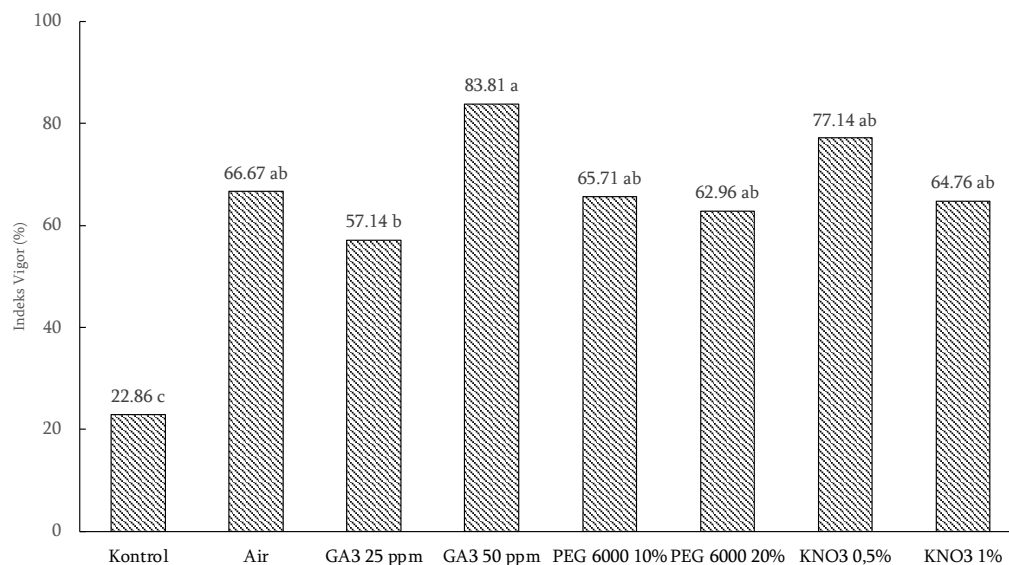


**Figure 3.** Effect of priming treatment of upland rice seeds on the vigor index sown in acid soil 14 days after sowing.

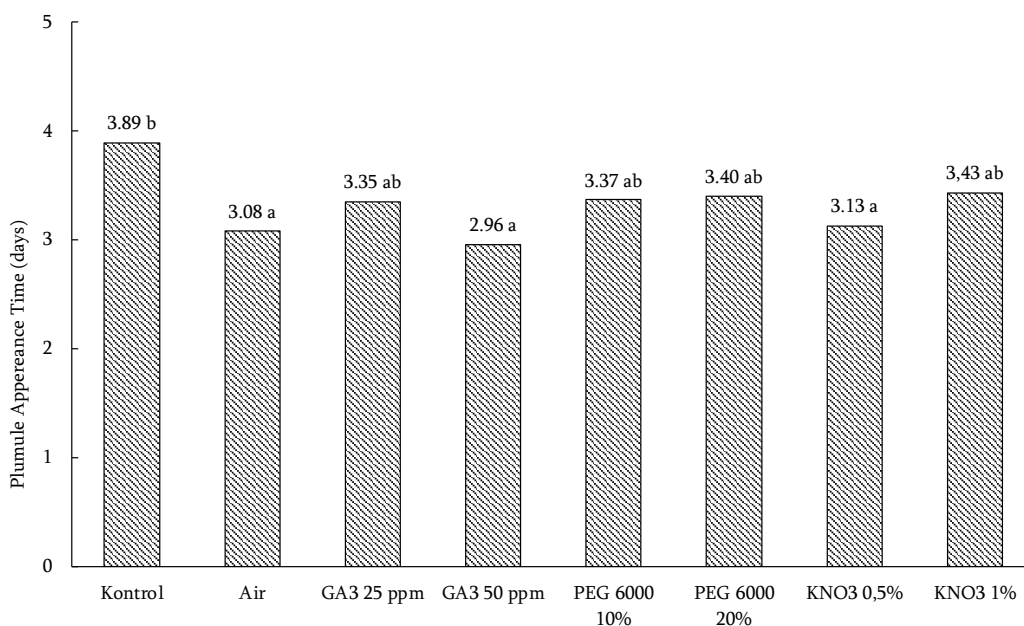


**Figure 4.** Effect of priming treatment of upland rice seeds on the appearance time of plumula sown in acid soil 14 days after sowing.

priming treatment on rice seeds improved germination under dry sowing conditions.

#### Speed of Germination (%NG day<sup>-1</sup>)

Figure 2 shows that the priming treatment significantly affected the speed of seed growth. The 50 ppm KNO<sub>3</sub> and GA<sub>3</sub> priming treatments obtained the highest values of 20.18% day<sup>-1</sup> and 19.71% day<sup>-1</sup>, respectively. These values were not different from other priming treatments but would be significant compared to no priming, which was only 14.32 % day<sup>-1</sup>. Bhatt et al [46] reported that priming with KNO<sub>3</sub> and GA<sub>3</sub> accelerated and uniformed Arabian desert seed germination. According to Javed et al. [42] priming KNO<sub>3</sub> increased vigor and formation of rice plant stands. Dhillon et al [23] also reported that

hormonal priming increased seed vigor and root biomass compared to those without treatment.

KNO<sub>3</sub> plays a significant role in germination due to its potassium and nitrogen content. Generally, potassium regulates ion balance within cells, influencing respiration and carbohydrate metabolism by activating the enzyme pyruvate kinase [47]. Nitrogen is also essential for plants' healthy and robust growth during the initial growth stage [32]. KNO<sub>3</sub> priming has a stimulatory effect on seed germination, particularly in light-sensitive seeds, by enhancing the role of gibberellins [48].

Priming significantly accelerates seed germination compared to non-primed seeds. In priming, gibberellin (GA<sub>3</sub>) plays a role in activating and synthesizing

**Table 1.** The effect of priming treatment on upland rice seeds sown in acidic soil media on the Normal Coleoptile Length (NCL), Normal Seedling Root Length (NSRL), Normal Seedling Fresh Weight (NSFW), and Normal Seedling Dry Weight (NSDW) 14 days after sowing

| Priming Treatment | NCL (cm) | NSRL (cm) | NSFW (g) | NSDW (g) |
|-------------------|----------|-----------|----------|----------|
| Control           | 20.05 a  | 9.98 a    | 5.31 a   | 0.907 a  |
| Water             | 21.00 a  | 10.49 a   | 5.60 a   | 0.966 a  |
| GA3 25 ppm        | 19.77 a  | 10.12 a   | 3.94 a   | 0.831 a  |
| GA3 50 ppm        | 21.98 a  | 9.95 a    | 5.75 a   | 0.939 a  |
| PEG-6000 10%      | 20.04 a  | 11.36 a   | 5.32 a   | 0.961 a  |
| PEG-6000 20%      | 20.63 a  | 10.13 a   | 5.85 a   | 0.957 a  |
| KNO3 0.5%         | 20.64 a  | 9.66 a    | 5.65 a   | 0.969 a  |
| KNO3 1%           | 20.59 a  | 10.56 a   | 6.13 a   | 0.960 a  |

hydrolytic enzymes such as lipase, amylase, and protease, which mobilize stored nutrients to be accessible by seeds [49]. In this experiment, the amount of GA<sub>3</sub> increased due to priming, resulting in enhanced germination in quantity and speed.

#### Vigor Index (%)

Figure 3 shows that the priming treatment significantly affected the seed vigor index. Priming treatment with GA<sub>3</sub> exhibited the highest value of 83.81%, but it was not significantly different from other treatments. However, a significant difference was observed compared to without priming, which was only 22.86%. Dhillon et al [23] on rice seeds primed with GA<sub>3</sub> 50 ppm during 24 hours with the highest germination percentage. GA<sub>3</sub> played a role in priming, stimulating synthesis, and producing hydrolases, especially  $\alpha$ -amylase, which provided energy during germination [30]. Gibberellins induced various enzymes required for seed germination, including amylase, protease, and glucanase [50].

#### Plumule Appearance Time (days)

In Figure 4, it was discovered that priming treatments accelerated the emergence time of the coleoptile in upland rice sown in acidic soil. Priming treatments with 50 ppm GA<sub>3</sub>, water, and 0.5% KNO<sub>3</sub> resulted in the fastest coleoptile emergence times at 2.96 days, 3.08 days, and 3.13 days, respectively. The non-primed treatment exhibited a delayed coleoptile emergence time, requiring 3.89 days. Dev et al [51] reported a significant increase in germination and early growth of grey-leaved-berries. The treatment consisted of GA<sub>3</sub> 250 ppm and KNO<sub>3</sub> (1%), resulting in the earliest seed germination ( $4.33 \pm 0.33$  days) compared to control  $11 \pm 0.33$  days after sowing.

#### Coleoptile length (cm), root length (cm), wet weight (g), and dry weight (g)

In Table 1, priming treatment did not affect coleoptile length, root length, fresh weight, and dry weight of upland rice sprouts sown on acid soils. The appearance of rice sprouts planted in acid soil, illustrated in Figure 1, showed that the treatment only affected the metabolic process of germination in overcoming the stress of an acidic environment. According to Dhillon et al [23] and

Kanjevac et al [52], no difference was observed in root length after being treated with KNO<sub>3</sub> or GA<sub>3</sub> in rice and wheat seedlings. Silva et al [53] stated that an Al content of 100  $\mu$ M caused root tip damage, this damage resulted in cell disorganization in the root cap cells and epidermis cells, causing disruption of nutrient uptake. The toxicity limit or critical content of Al in the soil was 3 me 100gr<sup>-1</sup> or 269.81 ppm or 0.027% Al content in the soil. Meanwhile, the soil in this research had an Al content of 0.44%, which was classified as having a higher aluminum content than the critical toxicity threshold in plants.

The use of PEG 6000 in priming on media with high aluminum content facilitated aluminum binding. PEG had a chemical structure ending in COOH, which produced H<sub>2</sub>O after reacting with AlCl<sub>3</sub>, causing PEG to bind with Al<sup>3+</sup> [54]. Consequently, aluminum stress did not affect seed germination. Priming with KNO<sub>3</sub> also positively affected abiotic stresses such as drought, salinity, high temperature, and aluminum toxicity [45][55][56][57].

Based on the research results, the viability and vigor of seeds treated with priming increased compared to untreatment. Viability is the ability of seeds to germinate normally under optimum environment conditions. Vigor is the ability of seeds to germinate normally under suboptimal environment conditions. Ultisol is a suboptimum environment seeding medium. High seed viability was obtained in the 0.5% KNO<sub>3</sub> priming treatment, namely (95.24%) while without treatment it was 82.86%. Priming treatment with GA<sub>3</sub> 50 ppm is the best priming treatment because it consistently increases seed viability and vigor with respective values of shown germination (92.38%), germination speed (19.71% day<sup>-1</sup>), vigor index (83.81%), and time of appearance of plumule (2.96 days).

## CONCLUSION

In conclusion, this research showed that priming treatment increased the germination of upland rice seeds planted on Ultisol. The results showed that the priming treatment increased the germination and the most effective treatment was priming GA<sub>3</sub> 50 ppm, each value

of showed germination (92.38%), germination speed (19.71% day<sup>-1</sup>), vigor index (83.81%), and time of appearance of plumule (2.96 day).

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