

# Enhancement of Okra (*Abelmoschus esculentus* L.) Germination through Seed Priming Techniques

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**Abstract.** The presence of a hard seed coat, coupled with various abiotic stresses during germination, can result in delayed and erratic crop establishment of okra (*Abelmoschus esculentus* L.). Seed priming offers an efficient and cost-effective method to improve the emergence of okra seeds. A laboratory experiment was conducted to assess the effect of various priming methods on germination parameters. The investigation was laid out in a Complete Randomized Design (CRD) with six levels of seed priming methods viz. priming with 200 ppm GA<sub>3</sub> solution, priming with 80% H<sub>2</sub>SO<sub>4</sub> solution, priming with 0.3% KNO<sub>3</sub> solution, priming with 5% PEG-6000 solution, priming with tap water, and control (without priming). The experiment was replicated four times. The effect of various priming methods on physiological and biometric parameters, i.e., germination percentage, mean days to germination, seed vigor index, root length, and shoot length, was investigated. The research results revealed that seed priming methods significantly influenced all the recorded parameters. Priming with GA<sub>3</sub> recorded a significantly higher germination percentage (73.75%), root length (114.5 mm), shoot length (85 mm), and seed vigor index (14719 mm). Furthermore, priming with GA<sub>3</sub> took significantly fewer mean days to germinate, requiring a mere 5.42 days. Priming with H<sub>2</sub>SO<sub>4</sub> was as effective as priming with GA<sub>3</sub> in terms of germination percentage, mean days to germination, seed vigor index, root length, and shoot length. The study concluded that priming with GA<sub>3</sub> and priming with H<sub>2</sub>SO<sub>4</sub> can be an effective method to expedite seed germination, enhance germination percentage, and increase the seed vigour of okra. In the absence of GA<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, hydropriming can be a felicitous alternative to combat poor crop emergence in an eco-friendly and cost-effective manner.

**Keywords:** GA<sub>3</sub> priming, priming, seed germination, seed vigor index

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## 1. Introduction

Okra (*Abelmoschus esculentus* L.) is ubiquitously cultivated in tropical, subtropical, and warm-temperature regions across South Asia, Brazil, and Africa [1], [2]. It is a vital warm-season vegetable in Nepal. Nepal produces 112,260 metric tons from 9,397 ha with an average productivity of 11.95 t ha<sup>-1</sup> [3]. While this versatile crop is primarily cultivated for its tender pods, it can also be used to add flavor to soups, salads, and curries [1], [2]. Even the fiber obtained from the okra stems has the potential to serve as a biocomposite [4]. Furthermore, okra has a high nutritional value. It constitutes rich reserves of calcium, iron, phosphorus, folates, pyridoxine, and

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vitamins C, B<sub>6</sub>, and K [5], [6]. In addition, okra has several health benefits. It has antioxidant properties since it contains beta carotene, lutein, and xanthine [7] – [9].

Okra seeds have an eccentric combination of hard seed coat and low permeability, which not only cause slow and erratic emergence of seeds, but also reduce crop yield. Seed hardness stands as the chief impediment for germination [10]. The hard seed coat impedes the uniform growth and developmental processes of the embryo, thereby impeding its capacity to absorb water, consequently hampering the initiation of seed germination [11]. Thus, cultivation of okra confronts a multitude of challenges, including diminished, delayed, and erratic seedling emergence [12], [13]. Besides the seed coat, environmental factors such as salt stress, low-temperature stress, and moisture stress can also hinder seed germination. In fact, slow and erratic seed germination is a harbinger of unsynchronized harvesting and diminished yield. There has been a perennial concern to address the challenges of seed germination in okra. Hence, interventions should be explored to develop economically viable and environmentally acceptable techniques to bolster seed germination and alleviate environmental stress.

The strategy of seed priming has emerged as a potent tactic in the quest to elevate seed germination of various agronomic and horticultural crops viz. rice, wheat, okra, carrot, celery, onion [14]–[17]. Seed priming entails the immersion of seeds in water or osmotic solutions for a prescribed duration, with subsequent drying before sowing [18], [19]. Various seed priming methods have been deployed, such as hydropriming (soaking in water) [14], osmotic-priming (soaking in osmotic solutions) [20], solid matrix priming (soaking in solid matrices) [21], halo priming (soaking in salt solutions) [22], and bio-priming (soaking in beneficial microorganisms) [23], each tailored to offer a strategic advantage [19], [24]. The control hydration can facilitate some of the pre-germination metabolic processes but are insufficient to permit radicle protrusion through the seed coat [25]. Seed priming enhances seedling vigor, accelerates germination, and mitigates issues arising from low-quality seed, delayed sowing, poor sowing techniques, inadequate soil moisture, salt stress, and adverse environmental conditions [26], [27]. The promotion of effective seedling growth and emergence has been documented through various techniques, including the use of water-soaked seed [14], priming with polyethylene glycol (PEG) [28], and the application of gibberellic acid (GA<sub>3</sub>) [29]. Although the effect of seed priming in other crops have been well-documented, there is lack of extensive research in crops such as okra. Therefore, the present study was carried out to determine the impact of different priming techniques on germination percentage, mean days to germination, seed vigor index, root length, and shoot length.

## 2. Materials and Methods

### 2.1. Description of the Experimental Setup

The experiment was conducted in the laboratory of Shree Khandadevi Secondary School, Chaunrideurali-2, Kavrepalanchowk district of Bagmati Province of Nepal (27°C 36' N and 85°C 51' E and 1400 masl). The okra cv. Arka Anamika was used for this investigation. The trail was laid out in a Complete Randomized Design (CRD) with six treatments and was replicated four times and was subjected to one-way ANOVA for analysis. Each treatment was carried out on a petri plate (12 mm diameter). A Whatman no. 1 filter paper was employed at the base of each petri plate. Twenty seeds were placed at the base of each petri plate. The seeds were arranged in two circles, with twelve seeds positioned in the outer circle and eight seeds placed in the inner circle. The germination chamber, petri plates, and experimental trays were surface sterilized with 99% ethanol to avoid contamination. The treatment details of the experiment are as follow:

1. GA<sub>3</sub> priming (T<sub>1</sub>): The seeds were primed in a 200 ppm GA<sub>3</sub> solution for 24 hours
2. Hydropriming (T<sub>2</sub>): The seeds were submerged in tap water for 24 hours
3. H<sub>2</sub>SO<sub>4</sub> priming (T<sub>3</sub>): The seeds were primed in an 80% H<sub>2</sub>SO<sub>4</sub> solution for 3 minutes
4. KNO<sub>3</sub> priming (T<sub>4</sub>): The seeds were primed by immersing in a 0.3% KNO<sub>3</sub> solution for 6 hours
5. PEG priming (T<sub>5</sub>): The seeds were primed in a 5% PEG-6000 solution for 24 hours
6. Control (T<sub>6</sub>): The control group of seeds received no priming treatment

After each seed priming methods, the seeds were thoroughly rinsed with distilled water and then subjected to drying until they reached their original moisture content at room temperature.

### 2.2. Parameters Under Study

#### 2.2.1. Final Germination Percentage (GP)

Seed germination was recorded at an interval of 12 hours. The seeds were counted as germinated when the radicle exhibited a protrusion measuring 5 mm or longer. The germination chamber was maintained at a temperature of 25±1°C with 85% relative humidity. Alternating daylight of 16 hours and darkness of 8 hours was maintained in the chamber. The final germination percentage was computed using the formula of [30].

$$GP = \frac{\text{Number of seeds germinated (n)}}{\text{Total number of seeds used (N)}} \times 100 \quad (1)$$

#### 2.2.2. Mean Germination Time (MGT):

The time taken for a seed lot to germinate can be computed using the mean germination time. However, the Mean Germination Time does not illustrate the information about the uniformity of

germination. It focuses on the day when most seed germination occurs. As the speed of seed germination increases, the mean germination time decreases. MGT was calculated by the formula given by [31].

$$MGT = \frac{\sum (Dn)}{\sum n} \tag{2}$$

where : D = number of days from the beginning of the germination test; n = number of newly germinated seeds on D-day

**2.2.3. Seedling Vigor Index (SVI):**

The vigor index of the seedling was calculated by following the formula of [32].

$$\text{Seedling Vigor Index} = (\text{average shoot length} + \text{average root length}) \times \text{GP} \tag{3}$$

where : GP = germination percentage

**2.2.4. Root and shoot length:**

The data on root and shoot length was collected by randomly selecting 10 seeds from each replicate at the end of the experiment. To calculate root length, the length from the hypocotyl to the longest root tip was measured using a precise ruler. The measurement of length from the base of the hypocotyl to the shoot tip was also done to compute shoot length.

**2.3. Statistical Analysis**

The recorded data were subjected to analysis of variance (ANOVA), and Duncan’s Multiple Range Test (DMRT) was done for mean comparison. Regarding the use of software, MS Word was used for word processing, MS Excel for the construction of graphs, and R-Studio v 4.3.1 for statistical analysis.

**3. Results and Discussion**

The experimental results elucidated significant variations in germination parameters across different seed priming treatments that underscore the pivotal role of seed priming in enhancing okra germination (Table 1).

**Table 1.** Effect of Different Seed Priming Methods on Various Germination Parameters

Treatments	Germination Percentage (%)	Mean days to germination (days)	Root length (mm)	Shoot length (mm)
GA <sub>3</sub> priming	73.75 <sup>a</sup>	5.42 <sup>e</sup>	114.50 <sup>a</sup>	85.00 <sup>a</sup>
Hydro priming	66.25 <sup>ab</sup>	6.50 <sup>d</sup>	98.13 <sup>b</sup>	69.37 <sup>b</sup>
H <sub>2</sub> SO <sub>4</sub> priming	68.75 <sup>ab</sup>	5.32 <sup>e</sup>	119.50 <sup>a</sup>	80.00 <sup>a</sup>
KNO <sub>3</sub> priming	60.00 <sup>b</sup>	9.05 <sup>b</sup>	80.00 <sup>c</sup>	60.50 <sup>c</sup>
PEG priming	46.25 <sup>c</sup>	7.30 <sup>c</sup>	85.00 <sup>c</sup>	55.50 <sup>cd</sup>
Control (No priming)	43.75 <sup>c</sup>	9.68 <sup>a</sup>	69.38 <sup>d</sup>	53.13 <sup>d</sup>
LSD (0.05)	11.56	0.53	5.06	5.06
SEm (±)	1.30	0.07	0.14	0.14
CV%	10.63	4.99	3.60	5.07
Mean	59.79	7.21	64.41	67.25

Note: treatment means followed by a common letter (s) are not significantly different among each other based on DMRT at 0.05 level of significance

### 3.1. Germination Percentage

All the seed priming treatments enhanced germination, even up to 73.75% (GA<sub>3</sub>) as compared to unsoaked seeds (43.75%). Hydropriming and H<sub>2</sub>SO<sub>4</sub> priming were as effective as GA<sub>3</sub> priming to enhance seed germination. The lowest germination was recorded in unprimed seed (43.75%), which was statistically at par only with PEG priming (46.25%). Moreover, a higher germination percentage in water soaked okra seeds compared to unsoaked seeds was reported by [28]. Furthermore, the observed increment in germination percentage through hydropriming aligns with the results reported by [33] for *Isatis indigotica* and [17] for wheat. Generally, there are three stages of seed germination viz. imbibition, lag phase, and emergence. Seed priming has the potential to extend the lag phase and produce pre-germination physiological and biochemical processes. Seed priming enhances such processes by increasing the activity of  $\alpha$  amylase, enhancing the content of soluble sugar and protein [34]. Better seed germination in primed seeds might be due to the elimination of germination inhibitors, the counteraction of ABA [35], the reduction of oxidative stress, and the enhancement of anti-oxidative and hydrolytic enzyme activities [36]. The reduction in oxidative stress is ascribed to the heightened enzymatic activities of antioxidants such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) as reported by Jiang et al. (2020). These enzymes capture free radicals and reactive oxygen species (ROS) before they damage membranes or other seed components, preventing lipid peroxidation during hydration. During seed germination, gibberellic acid stimulates the seed cells to produce mRNA molecules that comprise code for hydrolytic enzymes. In Arabidopsis, the release of embryonic GA<sub>3</sub> during seed germination elevates the expression of genes related to cell expansion and modification, which causes the seed cover to become weaker [37]. Moreover, GA<sub>3</sub> has been reported to induce radicle emergence by rupturing endosperm caps in tomatoes [38].

### 3.2. Mean Germination Time

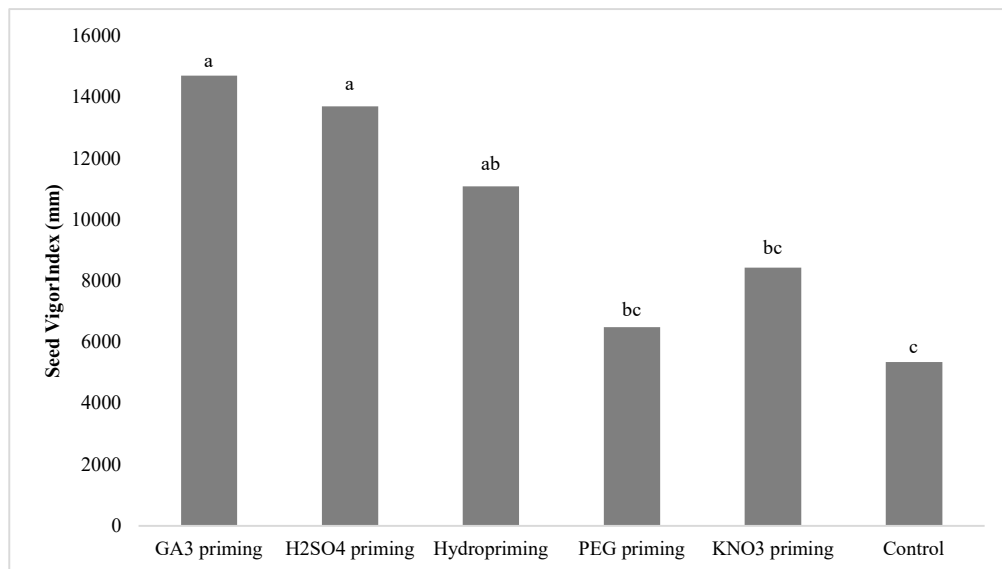
Statistical analysis revealed a significant effect of seed priming treatments on mean germination time at a 5% level of significance (Table 1). Mean germination time decreased significantly from 9.68 days (unprimed) to 5.42 days (priming with GA<sub>3</sub>). Priming with GA<sub>3</sub> and priming with H<sub>2</sub>SO<sub>4</sub> were equally effective in reducing mean germination time as compared to other priming agents. This was followed by hydropriming (6.5 days), priming with PEG (7.3 days), and KNO<sub>3</sub> (9.05 days). Unsoaked seeds took significantly longer days to attain mean germination time. Similarly, rapid seed germination as a result of both hydropriming and PEG-priming in comparison to the unsoaked seeds was reported by [28]. During germination, the high metabolism and rapid enzymatic activities of amylase, protease, and lipase may have provided readily available nutrients. This could have facilitated the primed seeds in completing germination quickly [39].

### 3.3. Root and Shoot Length

A significant effect was found in terms of root length due to various seed priming agents (Table 1). The longest root length was recorded in H<sub>2</sub>SO<sub>4</sub> (119.5 mm), which was statistically at par only with GA<sub>3</sub> priming (114.5 mm). A significantly shorter root length was recorded in unsoaked seeds (69.38 mm). Similarly, different seed priming agents recorded significant ( $p < 0.05$ ) variations in shoot length. GA<sub>3</sub> recorded the longest shoot length (85 mm) which was statistically at par with H<sub>2</sub>SO<sub>4</sub> priming (80 mm). Unprimed seed treatment and PEG priming recorded significantly shorter shoot lengths as compared to other treatments. The longer shoot length resulting from the priming treatments aligns with the results reported by [40] on maize. The notable increase in shoot length in the primed seeds may be due to meristematic growth, cell division, or its role in cell elongation. The longer seedling length in GA<sub>3</sub> priming was attributed to  $\alpha$ -amylase activity, which produces more reducing sugars [29]. The utilization of such sugars creates embryonic structures and improves subsequent seedling growth. Additionally, GA<sub>3</sub> could be involved in increasing protein level, aiding in RNA synthesis, and stimulating cell and internode expansion, potentially leading to improved growth and development.

### 3.4. Seed Vigor Index

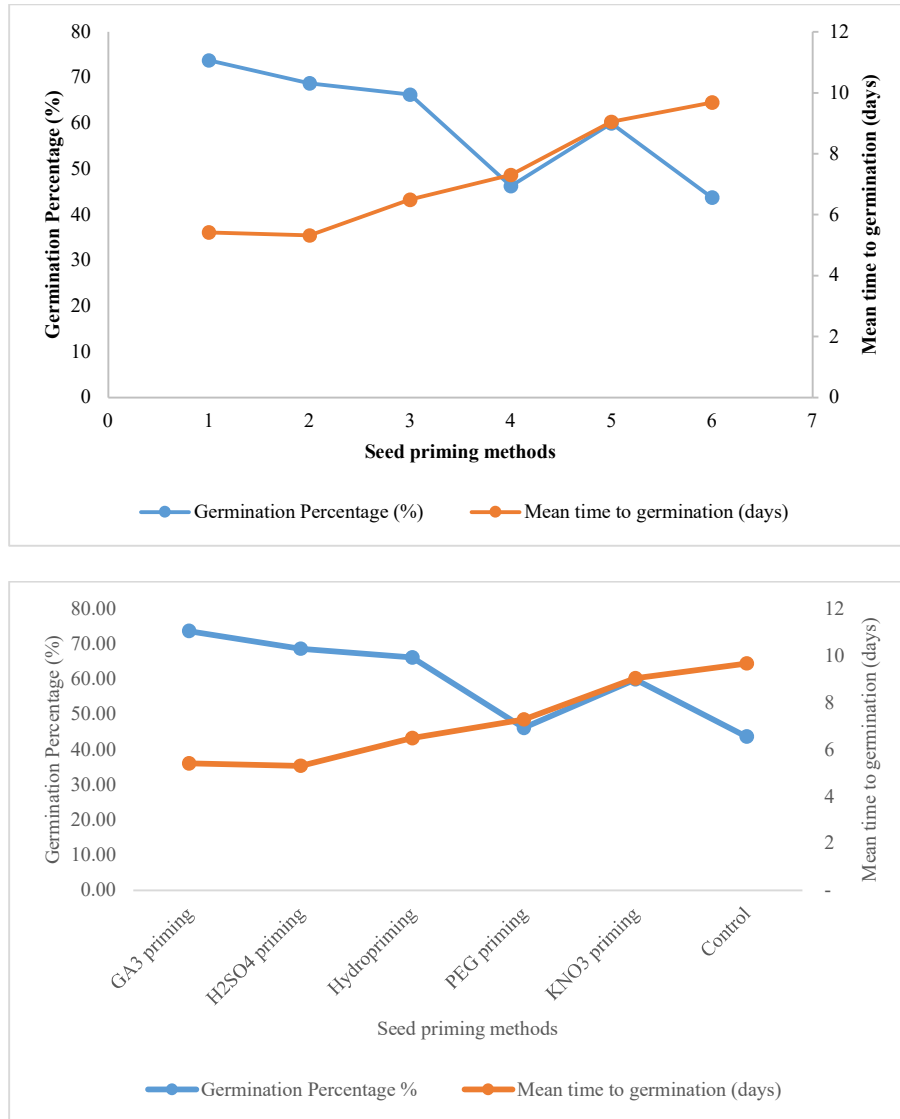
The seed vigor index varied significantly ( $p < 0.05$ ) with different seed priming treatments (Figure 1). The maximum seedling vigor index was recorded in priming with GA<sub>3</sub>, which was statistically at par with hydropriming and priming with H<sub>2</sub>SO<sub>4</sub>. The lowest seed vigor index was recorded in the control, which was statistically similar with the results obtained from PEG priming and KNO<sub>3</sub> priming. In contrary to our findings, higher seed vigor in PEG priming as compared to unsoaked okra seeds was reported by [16]. Moreover, a higher vigor index in hydropriming as compared to control has been reported by [28]. The higher seed vigor index in primed seeds could be attributed to elevated dehydrogenase and amylase enzymatic activity [34], [36].



**Figure 1.** Effect of Different Seed Priming Methods on Seed Vigor Index

### 3.5. Correlation Between Germination Parameters

There was a highly significant negative correlation ( $r = -0.69$ ) between germination percentage and mean germination time ( $p \leq 0.01$ ). Thus, the increase in germination percentage indicates a decrease in mean germination time and vice versa. Similar result was observed by [28]. The correlation between germination percentage and mean days to germination is demonstrated in Figure 2.



**Figure 2.** Germination Percentage and Mean Time to Germination as Influenced by Different Seed Priming Methods

### 4. Conclusion

This study underscores the need of seed priming to enhance okra seed germination parameters and seedling biometric attributes. Among the seed priming techniques tested, priming with GA<sub>3</sub> and priming with H<sub>2</sub>SO<sub>4</sub> proved to be the most effective in promoting germination percentage, reducing mean days to germination, increasing seed vigor index, and improving root and shoot

length of okra seedlings. Moreover, hydropriming offered pragmatic solutions for farmers with limited resources, albeit with slightly prolonged germination durations and slightly reduced germination percentage. Therefore, hydropriming is recommended as the next best alternative due to its simplicity, cost-effectiveness, safety, and its potential to enhance seed germination. In general, seed priming can be an efficient method to improve okra germination. However, the duration of hydropriming can be altered and the concentration of other priming agents can be varied for further research.

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