Dissimilarities of Shallot (*Allium ascalonicum* L.) Seedlings Growth and Quality through Priming with Moringa Leaf Extract

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Abstract

Seed priming is one method for increasing the growth and quality of seedlings. This study aims to evaluate moringa leaf extract as a priming agent and its effect on the seedling growth and quality of shallot from true seed. This study used a randomized block design with five replications. Priming treatment consisted of unprimed, hydropriming, and three levels of Moringa leaf extract concentration, including 12.5%, 25%, and 37.5%. A one-way analysis of variance was used to perform statistical analysis, with a p-value of 0.05 considered significant. In addition, the mean comparison was performed by Tukey multiple comparison test. According to the research, seed priming with moringa leaf extract at 37.5% resulted in the best effects on the parameter seedling emergence index (1.20), plant height (31.12 cm), number of leaves (4.80), pseudo stem diameter (3.22 mm), total root length (329.70 mm), number of root tips (94.60), root volume (4.64 mm^3), fresh weight seedling (2.77 g), dry weight seedling (0.19 g) and seedling quality index (0.0126), while compared to other treatments.

Keywords: *Allium ascalonicum* L., botanical seed, leaf extract, *Moringa oleifera*

Introduction

One species of plant that is extensively cultivated in Southeast Asia and several African nations is shallot. Because they are consumed daily by every family, shallots are a crucial horticultural product. For example, in Indonesia, the average weekly consumption of shallots is 15.87 g per capita (8). In addition, multivitamins, minerals, and antioxidants are a few components of shallots. As a result, this plant is used as a supplement to lower the risk of cancer, manage diabetes, enhance heart health, boost immunity, and prevent obesity, in addition to being used as a spice (27).

Indonesia's shallot production in 2021 reached 2,004,590 tons with a harvest area of 194,570 hectares, with Central Java serving as the major producing region. Shallots are also an Indonesian export commodity, with fresh and processed volumes reaching 47,955 tons (12). However, due to the rising population, numerous processed forms, and growing domestic demand for shallots, there can occasionally be a shortage. Therefore, shallots are listed as one of the "seven vital commodities" because they significantly impact inflation in the event of a shortage (15).

As happened in July 2022, shallot bulb supplies for seedlings became scarce and difficult to find, resulting in a very high price spike ranging from Rp48,000 to Rp50,000 per kg, and it is expected to soar even higher (4). So naturally, the high prices are driving up the cost of production. Additionally, dormancy requires bulbs used as seeds to be left for three months. True shallot seeds (TSS) might be a workaround to solve this issue.

True shallot seeds are more efficient because they are only required in small quantities, specifically 4-6 kg/ha, as opposed to 1-1.5 tons/ha for seeds from bulbs. Because TSS is free of diseases typically found in seed bulbs, using it as seeds in shallot production is more effective and healthier. Shallot seeds have an excellent yield potential when used as planting material. Numerous TSS studies have been conducted with varying degrees of success. TSS treated with various planting methods on sub-optimal land had productivity of 11.67 to 17.48 t/ha. Moreover, using various sowing methods, TSS can achieve productivity between 11.79 and 15.89 t/ha (24 and 25).

The use of seeds as planting material also ran into several difficulties, including uneven seed growth and poor seedling quality. The quality of the cultivated seeds and seedlings is a crucial indicator of successful output; hence, seed preparation is an important step. Shallot seeds are susceptible to diminished germination, which will lessen their capacity for growth. Handling seed storage leads to a rapid reduction in quality, which is reflected in weak field growth. Only 56.25% of the shallot seeds undergoing a germination test could retain germination throughout a 7-month storage period (30). In addition, the percentage of seedlings that grew after transplanting just ranged from 83% to 91% (26). Therefore, it is vital to increase the growth and quality of seedlings.

Seed priming is one of many techniques used to improve the quality and growth rate of seeds in plants. Seed priming is a low-cost and efficient hydration strategy to promote seed germination. The physiological processes of hydration and controlled drying that the seeds undergo during priming improve and accelerate pre-germinative metabolic processes for quick germination. The usage of fertilizers is reduced, crop yields are increased with uniform seed germination, systemic plant resistance is induced, and the seed priming technique is economical and environmentally beneficial (21). Moringa leaf extract is one of the seed priming agents that can be utilized. Moringa leaves contain many phytohormones that can stimulate plant growth, making them ideal for use as a priming agent to replace chemical substances, which are inexpensive and easy to collect (18), particularly in Indonesia. Several previous studies have shown the positive impact of using Moringa leaf extract as a priming agent. Research conducted by (13) showed that moringa leaf extract was able to increase the speed of seed germination and the vigor of wheat seedlings. As a result, this study will add to our understanding of using moringa leaf extract as a priming agent in true shallot seed.

Material and Methods

The experiment was conducted at the Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar. Plants are placed in the screen house at a temperature of 34.27±4.72°C and humidity of 60.33±14.22%. This research started in September until November 2022.

The moringa leaf extract (MLE) preparation refers to (17) with some modification. Moringa leaves are purchased from local Makassar markets and imported directly from Gowa Regency in South Sulawesi. The first step is to pick moringa leaves that are clean and healthy. Next, Moringa leaves that have been selected, separated from the stalk, and thoroughly washed under running tap water. After that, it was ground in a blender with distilled water in a 1:10 (v/w) ratio. Next, Moringa leaves that have been smoothed, squeezed with a soft cloth, and filtered with filter paper. The extract is then stored in a sterile bottle in the fridge.

This Testing for seedling growth is done by growing seeds in pots. Previously, plastic pots (height = 10 cm; bottom diameter = 9 cm; and top diameter = 12 cm) were provided, which were filled with soil and chicken manure in a 1:1 (w/w) ratio. Five seeds were sown in each pot at a depth of 0.5 cm. Then, the pot was watered to keep the soil moist. The experiment lasted for 40 days.

Parameters observed included seedling emergence index, plant height (cm), number of leaves, pseudo stem diameter (mm), total root length (mm), root volume (mm^3), fresh seedling weight (g), dry seedling weight (g), and seed quality index. The seedling emergence index was calculated by a formula referring to (6) as follows:

$$SEI = \frac{a}{t1} + \frac{b}{t2} + \ldots + \frac{n}{tn}$$

Remarks:
a, b, n : number of seed that grow
t1, t2, tn : observation day

While according to (9), the seed quality index can be calculated using the Dixon Quality Index (DQI) mathematical model as follows:

$$DQI = \frac{DW}{(\frac{PH}{LDW}+\frac{SD}{RDW})}$$

Remarks:
DW : dry weight of seedling
PH : plant height
SD : stem diameter (pseudo stem)
LDW : leaf dry weight
RDW : root dry weight

Data were analyzed with RStudio software. A one-way analysis of variance (ANOVA) was used to perform statistical analysis, with a p-value of 0.05 considered significant. The mean comparison was performed by Tukey multiple comparison test.

Results and Discussion

1. Seedling Emergence Index

Priming treatment with moringa leaf extract (MLE) of true shallot seed significantly affected the seedling emergence index, resulting in a higher index (Table 1). Among the observations of the seedling emergence index, priming with a 37.5% concentration of MLE has the highest seedling emergence index (1.20), which is statistically same with 25% MLE (1.16). However, it significantly has a different effect with unprimed seed (0.80), hydropriming (0.95), and 12.5% MLE (12.5%).

Establishing a seedling is critical for optimizing field production of any crop plant. Seed priming is an effective technology for promoting rapid and uniform emergence and high vigor, leading to better stand establishment, and yielding (23). Fast and uniform emergence and vigorous seedlings are required for solid growth (11). Many methods can be used, one of which uses plant extracts as priming agents. Moringa is a tropical plant that can be used for extracts from its leaves to be used as a priming agent. Several studies conducted by Khan *et al.* (14) and Basra *et al.* (5) utilize moringa leaf extract (MLE) as a priming agent. Seeds treated with MLE as a priming agent had a higher seedling emergence index. The increasing seedling emergence index is due to the priming process that triggers the activation of seed metabolism so that when rehydration is carried out, it will accelerate the growth of the radicle. The results of the research conducted by Yasmeen *et al.* (29) found that wheat seed priming with MLE was able to speed up the emergence time of the seeds and higher seedling emergence index, rather than the other type of priming agents such as CaCl2, hydropriming, and on-farm priming.

Table 1. Seedling emergence index of shallot in seed priming treatment

Treatment	Seedling emergence index
Unprimed	0.80 ± 0.005^{a}
Hydropriming	0.95 ± 0.031^{b}
12.5% MLE	1.04 ± 0.043^{bc}

25% MLE	1.16 ± 0.018[cd]
37.5% MLE	1.20 ± 0.015[d]
Tukey (0.05)	0.12

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test. Data are presented as mean values ± standard error

2. Aerial Part

The treatments for priming with MLE significantly influenced morphological traits in the aerial parts of seedlings (Table 2). The highest seedling (31.12 cm) was found in priming with MLE at 37.5% concentration and statically the same with 12.5% MLE (30.74 cm) and 25% MLE (30.94 cm). Moreover, the minimum seedling length was recorded from unprimed (26.46 cm) and hydropriming (27.54 cm), which were statistically alike. Similar with plant height, different MLE priming concentrations significantly affected the number of leaves and pseudostem diameter. The treatment priming with 37.5% MLE produced the most leaves (4.80), which differed from the other treatments except for the unprimed seed (3.40). The observation on the pseudostem has a similar result to the number of leaves in that priming with 37.5% MLE has a wider pseudostem diameter (3.22 mm), which is similar with other treatments except for unprimed seed (2.50 mm).

The growth of seedlings treated with priming treatment with MLE also gave significant results compared to unprimed seeds. Plant height, number of leaves, and pseudo stem diameter, respectively, were longer, more numerous, and broader. This significant growth is undoubtedly strongly influenced by the content of phytohormones in the MLE. Many studies conducted by Yap *et al.* (28), Alkuywati *et al.* (3), and Ali *et al.* (2) found several phytohormones in MLE, such as gibberellin acid, zeatin, and indole acetic acid. It is known that phytohormones can regulate the growth and development of the plant. Gibberellin is a plant growth regulator that stimulates shoot growth. Gibberellin promotes plant elongation at the molecular level by regulating cell growth (7)

Table 2. Seedling height, number of leaves, and pseudo stem diameter of shallot seedling in seed priming treatment

Treatment	Seedling height (cm)	Number of leaves	Pseudostem diameter (mm)
Unprimed	26.46 ± 0.97[a]	3.40 ± 0.244[a]	2.50 ± 0.07[a]
Hydropriming	27.54 ± 0.78[ab]	3.60 ± 0.244[ab]	2.58 ± 0.13[ab]
12.5% MLE	30.74 ± 1.89[bc]	3.80 ± 0.200[ab]	2.70 ± 0.10[ab]
25% MLE	30.94 ± 1.87[bc]	4.20 ± 0.374[ab]	3.00 ± 0.25[ab]
37.5% MLE	31.12 ± 2.19[c]	4.80 ± 0.200[b]	3.22 ± 0.15[b]
Tukey (0.05)	3.46	1.21	0.71

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test. Data are presented as mean values ± standard error

3. Above ground parts

Various concentrations of MLE as a priming agent significantly affected the underground parts of seedlings (Table 3). The most extended root length was recorded in priming with MLE at 37.5% concentration (329.70 mm) and statistically similar with 25% MLE (301.92 mm) and 12.5% MLE (291.97 mm). The shorter root length was recorded in unprimed seed (223.44 mm). The number of tip roots was significantly influenced by priming treatment with MLE. Most root tips were counted in priming seed with 37.5% MLE (94.60), significantly different from the other treatment except 25% MLE (82.20). The least number of root tips was counted in unprimed seed (32.40), similar with hydropriming (62.20). In line with that, priming with MLE also strongly influences the observation of root volume. An enormous root volume was recorded in priming with MLE at 37.5% concentration (4.64 mm3) and similar with 25% (4.10 mm3) and 12.5% MLE (3.98 mm3). The smallest root volume was recorded in unprimed seed (2.51 mm3), while hydropriming (2.79 mm3), which were alike.

The root length, number of root tips, and volume were significantly more extended, numerous, and prominent than in the unprimed and water-primed treatments. It is inextricably linked to the role of phytohormones and the nutritional content of the extract, which promotes root growth. IAA is one of many auxins that play essential roles in plant growth. IAA can promote lateral and adventitious root development (19).

Table 3. Total root length, number of tip roots, and root volume of shallot seedling in seed priming treatment

Treatment	Total root length (mm)	Number of root tips	Root volume (mm^3)
Unprimed	223.44 ± 17.507[a]	37.40 ± 1.860[a]	2.51 ± 0.274[a]
Hydropriming	233.71 ± 16.996[a]	62.20 ± 2.437[ab]	2.79 ± 0.313[ab]
12.5% MLE	291.97 ± 12.19[ab]	81.40 ± 3.529[ab]	3.98 ± 0.196[bc]
25% MLE	301.92 ± 24.51[ab]	82.20 ± 3.954[bc]	4.10 ± 0.347[bc]
37.5% MLE	329.70 ± 23.98[b]	94.60 ± 11.552[c]	4.64 ± 0.342[c]
Tukey (0.05)	87.93	26.38	1.41

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test. Data are presented as mean values ± standard error

4. Seedling Biomass and Quality

There was a significant difference in the fresh and dry weight of seedlings, also the quality of seedlings in the priming treatment with MLE (Table 4). The heaviest seedling was recorded in priming with MLE at 37.5% concentration (2.77 g) and was same with other treatments, except for the unprimed seed (1.71 g). Hydropriming (2.21 g), 12.5% MLE (2.43 g), and 25% MLE (2.50 g) have a non-significant effect compared to the unprimed seed. Like fresh weight, dry weight seedling observation shows that seed priming with MLE at 37.5% concentration has the heaviest dry weight (0,19 g) and is significantly different from unprimed. The lightest dry-weight seedling was recorded in unprimed seed (0.13 g) and was similar with hydropriming (0.16 g) and 12.5% MLE (0.16 g) based on statistical analysis. Afterward, priming the seed with MLE significantly affects the quality of the seedling. Priming the seed with 37.5% MLE has the highest quality (0.0127) and is significantly different from unprimed (0.0075) and hydropriming (0.0084).

Seed quality and germinability significantly affect the success of producing healthy, well-growing seedlings (22). Firmly established seedlings can compete for resources and interact better with biotic stresses, and they typically have a higher yield (10). The fresh and dry weights of seedlings treated with priming with MLE were recorded as heavier than seedlings that were unprimed. The findings by Khan *et al.* (14) also found that priming with MLE increased rice seedlings' fresh and dry weight, even under stress conditions. In addition, Al Khazan (1) found that under optimal and lead stress conditions, the seedling of the fenugreek plant with 5% MLE has a heavier fresh and dry weight than the control. The existence of better growth can indicate that the formation of assimilate and other physiological processes is running better on priming with MLE than other treatments. Several studies have proven that priming with MLE significantly impacts the physiological process of plants. Research conducted by Rehman et al. (20) and Mahboob et al. (16) found that priming seeds with MLE in maize plants increased the net assimilation rates and chlorophyll a and b content.

Table 4. Fresh weight seedling, dry weight seedling and seedling quality index of shallot seedling in seed priming treatment

Treatment	Fresh weight seedling (g)	Dry weight seedling (g)	Seedling quality index
Unprimed	1.71 ± 0.144[a]	0.13 ± 0.005[a]	0.0076 ± 0.0002[a]
Hydropriming	2.21 ± 0.242[ab]	0.16 ± 0.016[ab]	0.0084 ± 0.0007[ab]
12.5% MLE	2.43 ± 0.200[ab]	0.16 ± 0.016[ab]	0.0090 ± 0.0005[abc]
25% MLE	2.50 ± 0.251[ab]	0.18 ± 0.018[ab]	0.0116 ± 0.0011[bc]
37.5% MLE	2.77 ± 0.177[b]	0.19 ± 0.014[b]	0.0126 ± 0.0011[c]
Tukey (0.05)	0.87	0.06	0.0038

Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test. Data are presented as mean values ± standard error

5. Correlation Among Parameters

As can be seen from (Figure 1) the different types of correlations between the different effects of growth on seedling quality index. All of parameters appear to be positively correlated with others. Positive correlations are statistically significant, whereas others are not. Correlation values between parameters have positive or negative values. The range of correlation values obtained ranges from 0.85 to 1.00. This value can describe a positive relationship, which means the value of a parameter will increase along with the increase in the value of other parameters.

The most significant correlations are found between pseudo stem diameter and seedling quality index, number of root tips and fresh weight seedling, and root volume and total root length. This means that increasing the value of one of these parameters creates an increase in the value of the parameter that it is significantly correlated. Plant growth covering both above and below ground must be optimized in order to improve the quality of shallot seedlings.

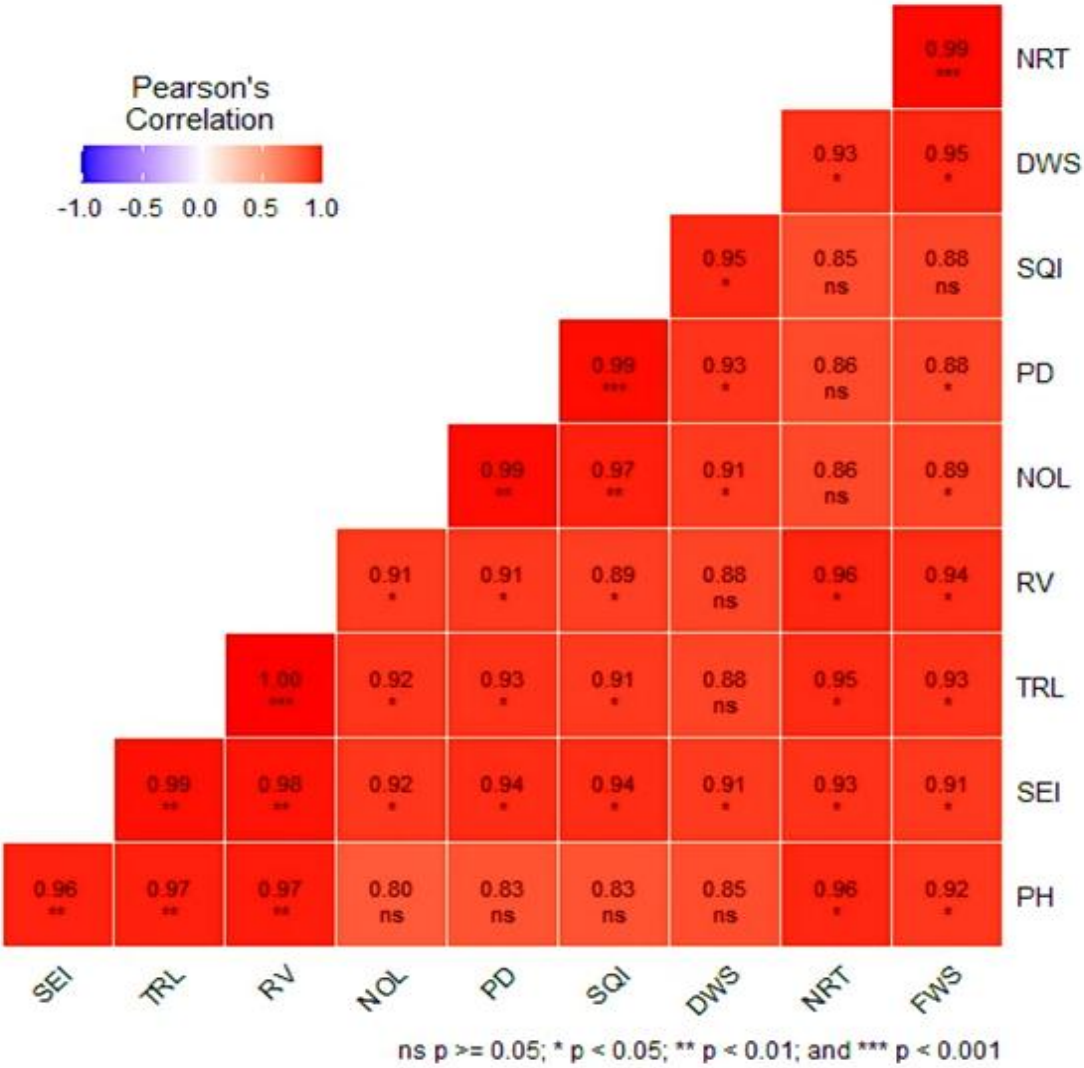

Figure 1. Correlation Graph

Conclusion

From the outcome of our investigation, the seed priming process can be used to boost the growth of shallot seedlings grown from seeds. Moringa leaf extract (MLE) is one seed priming agent that can be used. All observations on the parameters revealed that seeds priming with MLE at 37.5% grew significantly faster than unprimed seeds, even seeds that were primed with water. Furthermore, the effect of seed priming also directly improves the quality of seeds, which is one of the critical factors in producing plants that produce optimally. Furthermore, it can be tested on the performance of seedlings primed with

MLE to evaluate the growth and yield for future research.

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Conflict of interest

The authors have no conflict of interest.

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