

## Germination performance and seedling characteristics of chili pepper after seed priming with leaf extract of *Moringa oleifera*

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**Abstract.** Germination is the most critical stage of the plant life cycle since it is a principal component of seedling establishment and survival. The germination of chili pepper (*Capsicum annuum*) seeds is typically slow and non-uniform under favorable and unfavorable conditions. To overcome these problems, seed priming with moringa leaf extract is a viable option. The objective of this study was to investigate the effect of moringa leaf extract priming on germination performance and seedling characteristics of chili pepper. A completely randomized design was applied with five replications. Different concentrations of moringa leaf extract (1:30, 1:20, and 1:10) as priming solution were evaluated along with priming with distilled water (hydro priming), whereas non-primed seed was taken as control. The results revealed that hydro priming and moringa leaf extract priming effectively increased final germination percentage, germination rate index, germination index, vigor index, shoot length, and decreasing mean germination time. Conversely, the treatments had no significant effect on root length, while moringa leaf extract-primed seeds significantly increased shoot fresh weight. Furthermore, priming with moringa leaf extract at 1:20 had slightly better results than hydro priming, since it yielded higher germination index (363.60) and germination rate index (13.74), although it was at par with other concentrations. Additionally, it significantly produced the highest root fresh weight (13.56 mg) and lower coefficient of variation of germination time (14.61) than the control. Based on these findings, priming with moringa leaf extract in amount of 1:20 can be suggested for improving germination and seedling growth of chili pepper.

**Key words:** *Capsicum annuum*, biostimulant, germination index, moringa leaf extract, vigor index.

### INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in the world. It belongs to the Solanaceae family and is a warm-season annual crop (Samarah et al., 2020). Pepper fruit is mostly consumed as a fresh vegetable or dried as a spice (Lemos et al., 2019). It is noteworthy that the fruit is an excellent source of

vitamins (A, C, E), phenolic acids, flavonoids, carotenoids, and capsaicinoids, which are essential for human health (Abou-Sreya et al., 2021).

The most critical stage of the plant life cycle is germination (Diel et al., 2019). Seed germination will affect the establishment of crops, which are accordingly related to ultimate crop yield and quality (Boter et al., 2019). It begins with water uptake and is completed when the radicle protrudes outside of the seed coat (Wolny et al., 2018). It has been reported that the germination of chili pepper is slow and non-uniform under favorable as well as unfavorable conditions (Yadav et al., 2011; Barchenger & Bosland, 2016). In order to overcome these issues, the seed priming technique has been widely used by farmers (Chen et al., 2021).

According to Yadav et al. (2011), seed priming is a pre-sowing seed treatment in which seeds are hydrated and induce pre-germination metabolic activities without allowing radicle emergence. Then, the seeds are dried back to their initial moisture content. It was also observed that seed priming has possibility to reduce germination time, increase seed vigor, and enhance germination uniformity (Espanany et al., 2016; Tu et al., 2022).

The process of seed priming can be divided into three phases (Ruttanaruangboworn et al., 2017). In phase I, seeds imbibe water to activate enzymes. Moreover, phase II includes the degradation of food reserve, reorganization of cell membrane, and biosynthesis of starch to promote root protrusion and seedling growth later in the next phase. This phase stops after re-drying the seeds. Finally, phase III occurs when the radicle protrusion can be seen, and afterward, root and seedling growth continues.

Numerous studies have been conducted to evaluate the effect of seed priming with natural growth-promoting substances as a sustainable approach (Masondo et al., 2018; Neto et al., 2020; Mutlu-Durak & Kutman, 2021). Priming with *Moringa oleifera* leaf extract (MLE) has gained attention as a promising method to improve germination performance due to having a higher amount of minerals, cytokinin, GA<sub>3</sub>, ascorbic acid, and more (Bibi et al., 2016; El Sheikha et al., 2022). Previously, seed priming with MLE reduced germination time and enhanced final germination percentage (Nouman et al., 2012; Hala & Nabila, 2017). Other reports by Basra et al. (2011) showed that seed priming with MLE was effective to improve seedling growth.

The studies on the priming of seeds with plant extracts are rare in pepper (Pérez et al., 2021). To the best of our knowledge, plant hormones and nutrients are prevalent priming solutions for chili pepper seeds (Aloui et al., 2014; Quintero et al., 2018; Cano-González et al., 2021). They are relatively expensive for poor farmers (Afzal et al., 2012). The use of MLE which possesses considerable amounts of hormones and nutrients can be a cheaper alternative priming agent, but most studies dealing with MLE priming were mainly focused on cereal seed crops (Phiri, 2010; Ahmed et al., 2021). The MLE effect on germination and seedling characteristics of chili pepper remains unknown. Therefore, the objective of this study is to investigate the effect of MLE priming on germination performance and seedling characteristics of chili pepper.

## MATERIALS AND METHODS

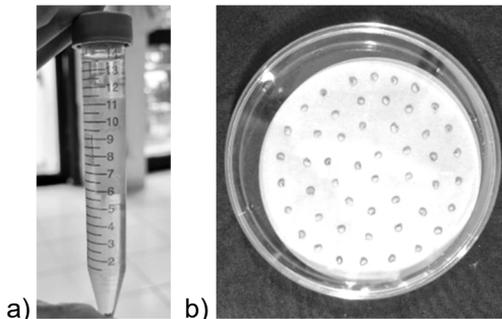
### Experimental Design

The experiment was carried out under laboratory conditions at the Faculty of Agriculture, Universitas Padjadjaran, Indonesia. A completely randomized design was

performed with five replications. Chili pepper seeds of the commercial cultivar ‘Tanjung’ were subjected to five priming treatments. It comprised of priming with distilled water (hydro priming), priming with moringa leaf extract (MLE) diluted with distilled water for 30, 20, and 10 times (1:30; 1:20; and 1:10 respectively), and the non-primed seed was considered as a control treatment.

### MLE Preparation

Fresh, healthy, and mature leaves of moringa were harvested, cleaned with tap water, and stored overnight in refrigerator. The extraction process followed the method of El Sheikha et al. (2022). It was conducted by mixing moringa leaves (30 g) with distilled water (300 mL) in a home blender, and then the mixture was sieved through cheese cloth and Whatman No. 1. Afterwards, the extract was centrifuged at 8,000 rpm for 15 min. The supernatant was collected and diluted with distilled water to achieve the required concentration. MLE used in this study (Fig. 1, a) contains beneficial substances such as P (98.93 mg L<sup>-1</sup>), K s(410.30 mg L<sup>-1</sup>), Ca (147.23 mg L<sup>-1</sup>), flavonoid (151.96 mg L<sup>-1</sup>), vitamin C (14.09 mg L<sup>-1</sup>), cytokinin (12.00 mg L<sup>-1</sup>), and GA<sub>3</sub> (23.00 mg L<sup>-1</sup>).



**Figure 1.** Moringa leaf extract (MLE) applied to germination of chili pepper (a) and germination test of chili pepper seeds in Petri dishes (b).

### Seed Priming and Germination Test

The chili pepper seeds were surface sterilized with 1% sodium hypochlorite for 30 s, washed with distilled water, and shade dried. The seeds then were soaked in priming solutions for 24 h and dried back for 48 h at room temperature (26 °C) to reach their original weight. For germination test, 50 seeds per treatment per replication were placed in a 15 cm Petri dish, which contained double layers of Whatman No. 1 filter papers moistened with 5 mL of distilled water (Fig. 1, b). Petri dishes were incubated for 14 d at 26/27 °C day/night temperature and exposed to white LED light at 100 μmol m<sup>-2</sup> s<sup>-1</sup> (12 h photoperiod). Germination was observed daily in which the criterion of germination was 2 mm of radicle protrusion.

### Data Collection

Final germination percentage (FGP) and mean germination time (MGT) were determined according to Wu et al. (2019). Germination rate index (GRI) and germination index (GI) were analysed using the formula proposed by Shah et al. (2021). Meanwhile, coefficient of variation of the germination time (CV<sub>t</sub>) and vigor index (VI) were measured according to Ranal & Santana (2006) and Guragain et al. (2021) respectively. Those parameters were calculated using the following equations:

$$FGP = \frac{NGS}{NTS} \times 100 \quad (1)$$

$$MGT = \frac{\sum (N1T1 + N2T2 + \dots + NiTi)}{\sum (N1 + N2 \dots + Ni)} \quad (2)$$

$$GRI = \frac{N1}{T1} + \frac{N2}{T2} + \dots + \frac{Ni}{Ti} \quad (3)$$

$$GI = (10 \times N1) + (9 \times N2) + \dots + (1 \times Ni) \quad (4)$$

$$CV_t = \frac{S_t}{MGT} \times 100 \quad (5)$$

$$VI = \frac{\text{Seedling length (cm)} \times FGP (\%)}{100} \quad (6)$$

where *NGS* is the number of germinated seeds at the end of experiment (14 d after incubation), *NTS* is the number of tested seeds, *N<sub>i</sub>* is the number of seeds germinated in the *i<sup>th</sup>* time, *T<sub>i</sub>* is the time taken for seeds to germinate at *i<sup>th</sup>*, *S<sub>t</sub>* is standard deviation of the germination time, *MGT* is mean germination time, and *FGP* is final germination percentage.

Moreover, seedling characteristics were analysed at 14 d after incubation. The measurements consisted of root length, shoot length, root fresh weight, shoot fresh weight, and vigor index. Twenty uniform and normal seedlings from each replication were selected and averaged to measure those parameters.

### Statistical Analysis

Data were collected and statistically analysed using analysis of variance (ANOVA) with SPSS v21 software. The differences in treatments were assessed by Duncan's multiple range test (*P* < 0.05).

## RESULTS AND DISCUSSION

### Effect of Priming on Seed Germination

The presented results revealed that seed priming treatment notably affected the final germination percentage (FGP) (Table 1). Hydro priming and MLE priming showed the same noticeable increment regarding FGP, which increased by up to 7.60% compared to that of non-primed seeds (control). These results are in accordance with Hala & Nabila (2017) that seeds primed with different concentrations of MLE enhanced FGP of sweet pepper due to the role of phytohormones, amino acids, and mineral elements in MLE, which positively influenced this parameter. The percentage of increase in FGP reported by these authors was higher by about 53.52% than our study. Priming treatment promotes the mobilization of seed reserve from endosperm to embryo, resulting in better performance of germination (Majda et al., 2019). Higher FGP in primed seeds also may be attributed to the early metabolic processes during the hydration (Mir et al., 2021).

In the current study, germination rate index (GRI) was significantly influenced by seed priming treatment (Table 1). Compared with the control, all primed seeds exhibited remarkably higher GRI. The highest GRI was observed in MLE 1:20 (13.74), with an enhancement of 11.71%, but it was statistically at par with MLE 1:30 (13.58) and MLE 1:10 (13.56). Previously, seed priming improved the speed of germination, which was the fastest noted in MLE-primed seeds (Yasmeen et al., 2013). Better result in germination rate by MLE application was related to the improvement in metabolic activity (Nouman et al., 2012). Afzal et al. (2012) pointed out that MLE priming induced

the activity of amylase enzymes in seeds, hydrolysing starch into smaller molecules for the growth and development of embryo. Besides, the presence of calcium (Ca) in MLE also acts as an enzyme cofactor and facilitated faster germination (Gunasekar et al., 2017). During germination, Ca modulates the activity of kinase enzymes and certain phosphatase involved in signal translation (Karim et al., 2020). Fast seed germination is considered an essential attribute marking a quick transition to the growth stage in the plant life cycle (De Ron et al., 2016). Seed priming in most horticultural crops has primarily displayed an increase in germination rate (Tu et al., 2022).

According to Table 1, seed priming treatment showed a significant effect on germination index (GI). Priming of seeds with MLE 1:20 displayed the maximum GI (363.60), being higher by about 7.83% as compared to the control (337.20). However, it was statistically similar to other MLE concentrations. According to Hassanein & Al-Soqeer (2017), GI emphasizes both germination percentage and its speed. Afzal et al. (2012) confirmed that MLE priming advanced the germination of maize seeds, providing higher GI values. In MLE-primed seeds, numerous nutrients and vitamins possessed in MLE are transferred to embryo during phase II of seed priming, which ultimately improved GI. Seed germination also is influenced by plant hormones (Vysokova et al., 2019). Gibberellic acid (GA), a growth hormone found in MLE, plays a marked role in stimulating seed germination (Gunasekar et al., 2017). GA triggers the synthesis, activation, and secretion of hydrolytic enzymes, realising reducing sugars and amino acids, which are important for embryo growth (Vieira et al., 2002).

**Table 1.** Effect of priming chili pepper seeds with moringa leaf extract (MLE) on final germination percentage (FGP), germination rate index (GRI), germination index (GI), mean germination time (MGT), coefficient of variation of germination time (CV<sub>t</sub>), and vigor index (VI)

Treatments	FGP (%)	GRI	GI	MGT (day)	CV <sub>t</sub> (%)	VI
No priming (control)	90.71 a	12.30 a	337.20 a	4.09 b	19.12 b	5.80 a
Hydro priming	96.64 b	13.11 b	349.60 b	3.81 a	16.05 ab	6.20 b
MLE priming (1:30)	95.98 b	13.58 bc	360.00 bc	3.74 a	15.93 ab	6.59 b
MLE priming (1:20)	96.37 b	13.74 c	363.60 c	3.73 a	14.61 a	6.54 b
MLE priming (1:10)	97.60 b	13.56 bc	361.00 bc	3.75 a	13.33 a	6.45 b
Critical Value	3.66	0.53	11.49	0.13	3.67	0.37

<sup>1</sup> Mean values followed by different letters on the same column indicate significant differences according to Duncan's multiple range test ( $P < 0.05$ ).

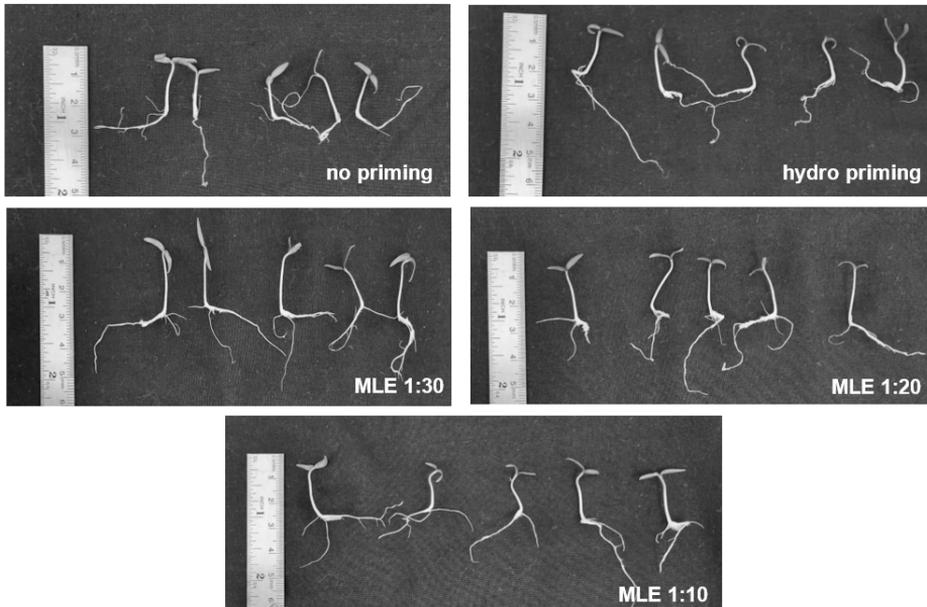
Moreover, mean germination time (MGT) responded differently to seed priming treatment, as presented in Table 1. MGT denotes the day at which most seeds have germinated (Talská et al., 2020). Hydro priming and MLE priming at all concentrations tested took less time to germinate, in a range from 3.73 to 3.81 days. These treatments reduced MGT by up to 8.80% as compared to the control. The primed seeds have the ability to accomplish germination in a short time (Nazari et al., 2017). Gilberio et al. (2014) supported these results, reporting that hydro priming and MLE priming were recorded as the most effective treatment in decreasing MGT. During the pre-germination stage, a solution-retention effect occurs in primed seeds and subsequently affects vital metabolic processes before radicle protrusion (Makhaye et al., 2021). Seed priming induces RNA activity and improves adenosine triphosphate (ATP) production, which helps the seeds to germinate (Ahmed et al., 2021).

Data depicts that the impact of seed priming was significant on the coefficient of variation of germination time ( $CV_t$ ) in this study (Table 1). Seeds primed with MLE 1:20 (14.61%) and MLE 1:10 (13.33%) appreciably decreased  $CV_t$  by up to 30.28% in comparison with the control. However, they were statistically the same with MLE 1:30 (15.93%) and hydro priming (16.05%) treatments.  $CV_t$  interprets the uniformity or variability of germination, in which lower  $CV_t$  values express higher uniformity (Ranal & Santana, 2006). Nonetheless, the relevant study regarding MLE effect on  $CV_t$  is still limited. A lower  $CV_t$  of wheat seeds has been previously documented due to MLE priming compared to the control (Ahmed et al., 2021) and the percentage of decrease was lower by about 25.49% from our study. El-Katony et al. (2020) described a reduction in  $CV_t$  by using another biostimulant such as algae extract. They also proved that the highest  $CV_t$  was obtained in non-primed seeds. Uniform seed germination often resulted in a more uniform seedling establishment and healthier plant growth (Wu et al., 2019). In order to achieve uniform harvests, the factors that contribute to the variations in seed germination must be eliminated (Hayashi et al., 2020).

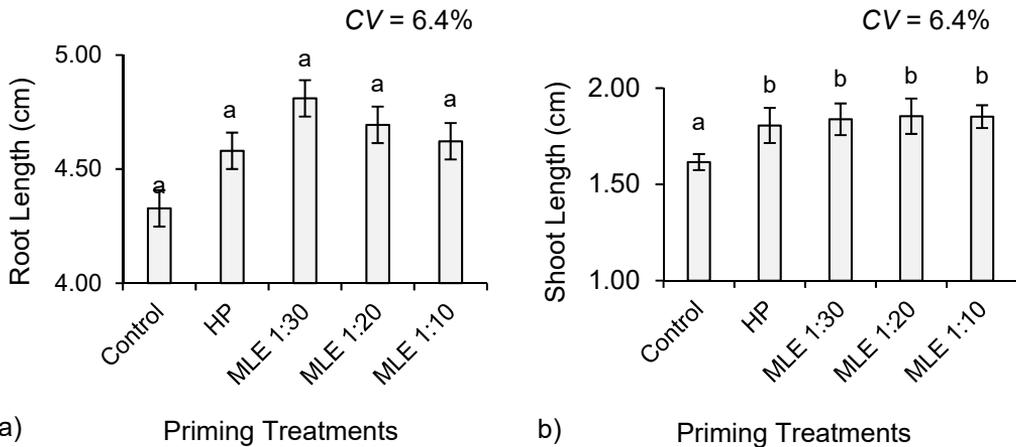
According to Table 1, the priming of seeds varied substantially on the vigor index (VI). A similar enhancement in VI was observed by hydro priming and MLE priming, being higher than in non-primed seeds. These treatments improved VI by up to 13.62%. Basra et al. (2011) mentioned that seed priming with MLE caused most of the N and Ca in MLE appeared to be partitioned to embryo, resulting in higher VI. Similarly, the high contents of Ca and other minerals in MLE might be responsible for increasing VI (Yasmeen et al., 2013). In addition, the hydration state of primed seeds is controlled, and subsequently, seeds can avoid the endoderm to break and produce pre-germination metabolism, which improves the seedling vigor and growth potential of seedlings (Chen et al., 2021). VI determines the potential for uniform and rapid germination as well as development of normal seedlings (Damalas et al., 2019). Vigorous growth together with earlier crop establishment can minimize weed competition, increasing water and nutrient absorption (Karim et al., 2020).

### **Effect of Priming on Seedling Growth**

Fig. 2 represent the characteristics of seedling in the current study. No significant difference was observed in root length among the treatments, ranging from 4.33 to 4.81 cm (Fig. 3, a). In contrast, the treatment had a significant impact on shoot length (Fig. 3, b). The longest shoot was achieved by hydro priming and priming with MLE, which ranged from 1.80 to 1.85 cm and increased by up to 14.91%. Meanwhile, shoot length was significantly lower in the control (1.61 cm). The results in this study are consistent with the findings of Phiri & Mbewe (2010), who reported that the application of MLE as seed priming agents negatively altered the root length of bean and groundnut seedlings. Likewise, MLE-primed seeds had no significant effect on root length of sorghum and wheat (Phiri, 2010). Furthermore, an increase in shoot length by MLE priming has been previously reported by Sarmin (2014). This stimulatory effect of MLE might be linked to the cellular proliferation in shoot apical meristem after priming with this extract (Noor et al., 2016).



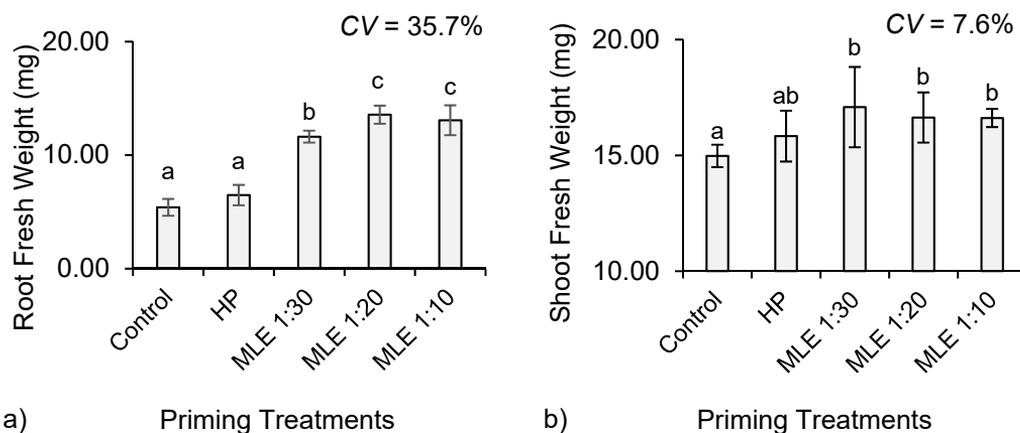
**Figure 2.** Effect of priming chili pepper seeds with moringa leaf extract (MLE) on seedling characteristics of chili pepper at 14 d after incubation.



**Figure 3.** Effect of priming chili pepper seeds with moringa leaf extract (MLE) on root length (a) and shoot length (b) of seedlings (control = no priming, HP = hydro priming, MLE = moringa leaf extract priming). Mean values followed by different letters indicate significant differences according to Duncan’s multiple range test ( $P < 0.05$ ). Critical value for root and shoot length is 0.35 and 0.10 respectively. *CV* = coefficient of variation.

Seeds primed with MLE 1:20 (13.56 mg) and MLE 1:10 (13.07 mg) significantly increased root fresh weight by up to 151.11% (Fig. 4, a). Among the treatments, the lowest root fresh weight was recorded in the control (5.40 mg) and hydro priming (6.48 mg). In similar, a significant change was detected in shoot fresh weight of chili pepper seedlings (Fig. 4, b). Seeds primed with MLE 1:30 (17.08 mg), MLE 1:20

(16.63 mg), and MLE 1:10 (16.61 mg) yielded heavier shoot in comparison with the control (14.98 mg). They increased by up to 14.02% from the control but statistically similar to hydro-primed seeds (15.83 mg).



**Figure 4.** Effect of priming chili pepper seeds with moringa leaf extract (MLE) on root fresh weight (a) and shoot fresh weight (b) of seedlings (control = no priming, HP = hydro priming, MLE = moringa leaf extract priming). Mean values followed by different letters indicate significant differences according to Duncan’s multiple range test ( $P < 0.05$ ). Critical value for root and shoot fresh weight is 1.18 and 1.42 respectively.  $CV$  = coefficient of variation.

In other words, the present study clearly indicated that seedlings fresh weight of pepper was positively affected by MLE treatment. The same trend was observed in rice seedlings due to MLE application, (Khan et al., 2022). Priming treatment repairs membrane damage, decreases physical barriers of the endosperm, enhances immature embryo growth, and leaches germination inhibitors to promote root growth (Chen et al., 2021). In terms of root weight, the data were in line with Yasmeen et al. (2013), who demonstrated the effect of seed priming with MLE on the growth of wheat seedlings. They revealed that MLE-primed seeds showed higher root fresh weight compared to control treatment. Similarly, fenugreek seeds primed with MLE were considerably effective in improving shoot fresh weight under non-stressed conditions (Al Khazan, 2020). These beneficial effects of MLE can be attributed to the presence of zeatin, a naturally-occurring cytokinin, which is a growth-promoting substance (Iqbal et al., 2015). Cytokinin plays a major role to promote cell division in root and shoot systems (Azzam et al., 2022). In the present study, seed priming treatments are effective for improving germination process, yielding more uniform germination, and producing better seedling characteristics of chili pepper under laboratory conditions. The use of MLE as seed priming agent, particularly at the concentration of 1:20, had slightly better results than hydro priming, which was expressed by higher GI, GRI, and root fresh weight. These positive results are in agreement with Yousof et al. (2017) observations that seeds primed with MLE at moderate concentrations displayed better germination parameters and seedling characteristics. Seeds primed with MLE solution trigger several biochemical changes, including enzyme activation, starch hydrolysis as well as dormancy breaking (Hala & Nabila, 2017). It also activates the synthesis of GA and

proteins in cell wall for radicle protrusion, besides promoting antioxidant mechanism, as a protection against DNA damage (Gunasekar et al., 2017). Moreover, MLE contains the beneficial substances such as zeatin, ascorbic acid, Ca, and K, which accelerate seed germination and seedling development (Yasmeen et al., 2013).

## CONCLUSIONS

In conclusion, hydro priming and moringa leaf extract (MLE) priming effectively improved the final germination percentage, germination rate index, germination index, shoot length, as well as vigor index of chili pepper seeds and reduced mean germination time. Meanwhile, the treatments did not show any significant effect on root length. Seeds primed with MLE increased shoot fresh weight compared to non-primed seeds (control). Furthermore, MLE priming at the concentration of 1:20 had slightly better effects than hydro priming, since it yielded higher values of germination index (363.60) and germination rate index (13.74), although it was statistically similar to other MLE treatments. It also produced the highest root fresh weight (13.56 mg) and more uniform seedlings compared to the control, which was reflected by lower coefficient of variation of germination time (14.61). Future studies may be needed to understand how MLE priming affects the biochemical changes of chili pepper seeds during germination and seedling growth, also the MLE impact on the chili growth and final yield.

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