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Effect of Aeration and KNO_3 in Seed Priming on The Germination of Tomato (*Solanum lycopersicum*) Seeds

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Abstract. *Seed priming by soaking in KNO_3 has been used to enhance seed performance and crop establishment in tomatoes. However, seeds that are immersed in a solution for a long time may be deprived of oxygen during critical phases of imbibition. Aeration during priming is a solution to solve this problem. Thus, this study aims to determine the effect of aeration and KNO_3 in seed priming on the germination of tomato seeds. The seeds used in this study were tomato seeds var. Palupi. This study used seven treatments i.e. non-primed, KNO_3 moistened paper, KNO_3 soaking, KNO_3 aeration, water moistened paper, water soaking, and water aeration. The study was arranged in a completely randomized design (CRD). All data were statistically analyzed using one-way ANOVA and followed by means analysis using Fisher's LSD (Least Significant Difference) test at $P \leq 0.05$. The result showed that all priming treatments gave a better performance in FGP (Final Germination Percentage), MGT (Mean Germination Time), and GRI (Germination Rate Index) compared to the non-primed. The best treatments, however, were observed in the aeration method in either KNO_3 or water in all parameters observed. KNO_3 gave a slightly better performance than water in all parameters, although there was no statistical difference between the two treatments.*
Keywords: *aeration, germination, KNO_3, seed priming, tomato*

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a globally grown species with great economic importance. In Indonesia, tomato is the fifth most important vegetable crop in terms of cultivated area with 50,000 – 60,000 ha harvested each year (Badan Pusat Statistik, 2020). Tomatoes were first introduced to Europe from America in the early sixteenth century (Baldina *et al*., 2016). Tomato offers significant nutritional value that gives a beneficial effect on human health. Tomatoes are well-known for being a good source of lycopene, an antioxidant that has anticarcinogenic properties. They also provide a high content of minerals and vitamins, such as iron, calcium, potassium, vitamin B, vitamin A, ascorbic acid, and tocopherols (Kheyrodin & Kheyrodin, 2017).

In tomatoes, the emergence of the seed is a vital stage because the rest of the plant life is directly dependent on the rate of germination (Vivek & Duraisamy, 2017). The seed germination

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process is initiated by water uptake (imbibition) which is known as phase I. The seed will then experience a lag phase (phase II) when water potential is in balance with the surroundings. In this phase, metabolic activities surges, such as the activation of enzymes, hormones, antioxidants, and amino acids, as well as DNA repair. Phase III occurs as the radicle starts to elongate and drives the increase of fresh weight. Waterworth *et al.* (2019) identified repair mechanisms and the DNA damage response (DDR) as key factors that control germination and determine a seed's germination potential..

The seed priming technique can start some of the metabolic processes during phase II to occur without a radicle protrusion. The strategy of seed priming is to perform a controlled water uptake by the seeds during the rapid water imbibition phase and up to the end of phase II (Bewley, 1997). Liu *et al.* (2002) reported that priming improves the anti-oxidative metabolites activities, such as superoxide dismutase (SOD) and peroxidase during seed germination. Priming also aids plants in accelerating cell division, transporting stored proteins, and increasing germination speed (de Castro *et al*., 2000), reduce membrane permeability, and control tissue water contents (Farooq *et al*., 2008). Priming activates DNA repair pathways and ROS scavenging systems (which impart seed repair response) and aids in genome integrity preservation (Paparella *et al*., 2015). Hence, seed priming is used to enhance seed performance, such as germination rate and uniformity which determine the crop establishment (Job *et al*., 2000).

Seed priming techniques are commonly classified into several categories based on the priming substances used, such as hydro-priming (water), osmo-priming (low water potential), halo-priming (salt solutions), hormo-priming (hormones), solid matrix-priming, bio-priming (bio-agent), nutrio-priming (minerals) and thermo-priming (temperature) (Dutta, 2018). Potassium Nitrate (KNO_3) is one of the agents that is commonly used in halo-priming or osmo-priming. Nitrate from KNO_3 helps with the synthesis of nitric oxide (NO) (Hendricks & Taylorson, 1974). NO helps to stimulate germination by breaking a seed dormancy through interactions with ethylene production, phytochrome signaling pathways, and reactive oxygen species (ROS) (Šírová *et al*., 2011). Some studies reported that the use of KNO_3 is effective in improving germination in tomatoes (Frett *et al*., 1991; Govinden-Soulange & Levantard, 2008; Lara *et al*., 2014; Zhang *et al*., 2012). Ali *et al.* (2020) also revealed that seed priming with 0.75% of KNO_3 for 24 hours at 25°C gave a significant increase in the final emergence of tomato seeds.

The most commonly used method in tomato seed priming is soaking. However, seeds that are immersed in a solution for a long time may be deprived of oxygen during critical phases of imbibition (Nakorn & Kaewsorn, 2021). Aeration is among the factors affecting seed priming, although the effect of aeration varies with the species (Raj & Raj, 2019). Aeration increases the oxygen content in the solution will enhance the germination process (Akers & Holley, 1986).

Moistened paper method can be used to solve the lack of oxygen during priming. However, this method is not feasible for industrial scale. As an alternative, seed priming can be done by supplying oxygen directly into the priming solution while the seeds are being soaked. Kaewsorn & Chatbanyong (2022) reported that the aeration of papaya seed in KNO_3 solution for 24 hours during seed priming gave out better results in the mean germination time (MGT) than the one without aeration. Thus, this study aims to investigated the effect of aeration and KNO_3 during seed priming on the germination of tomato seeds.

2. Material and Methods

This study was conducted at the Seed Processing Laboratory of the Department of Agricultural Production, State Polytechnic of Jember. The seeds used in this study were tomato seeds var. Palupi, which is an open-pollinated variety and specialized for lowland to medium land. This variety has good storability and resistance to bacterial wilts. This variety is quite popular among the farmers since it has more affordable price. The initial seed germination percentage stated on the packaging is 85%, which still has some room to be improved.

The aerator was home-built from an electric air pump, rubber tubes, and plastic bottles. The air pump and the plastic bottle were connected with rubber tubes. One end of the rubber tube was attached to the air pump outlet whilst the other end was put into the bottle through a hole on the cap and then sealed with glue. To create air flows, one more hole was made on each bottle cap. This tool was all set up on a plastic rack.

There were two solutions tested for the seed priming, namely 0.75% of KNO_3 and Reverse-Osmosis (RO) water. The priming method included moistened paper, soaking, and aeration. Thus, asides from the control (non-primed), there were six treatments investigated in this study, which were: KNO_3 moistened paper, KNO_3 soaking, KNO_3 aeration, water moistened paper, water soaking, and water aeration. The slow imbibition on moistened paper was conducted by putting the seeds on a germination paper that has been moistened with the solutions. The soaking treatments were done by putting the seeds in plastic bottles filled with 50 ml solutions. For the aeration treatments, the seed aerator was used. The seeds were put inside the plastic bottles filled with 50 ml of solutions and let to be aerated. The seeds were primed with respective treatments for 24 hours and placed under 12 hours light period per day at 25 – 30 ºC. All treatments were replicated four times. The seeds were then slowly air-dried in an open space for 3 days. Dried primed seeds were then germinated on top of the paper and placed inside a plastic thinwall box, with 100 seeds for each replication. The seeds were kept under 12 hours light period per day at 25 – 30 ºC for 6 days.

The observation parameters included Radicle Emergence (RE), Final Germination Percentage (FGP), Mean Germination Time (MGT), and Germination Rate Index (GRI). FGP only reflects the final percentage of germination. The higher the FGP value, the better a seed population's germination (Scott *et al*., 1984). Germination in this definition based on ISTA Rules is the emergence of 2 mm of radicles. FGP is calculated with this Formula 1.

$$FGP = \frac{no.of\ seeds\ germinated}{no.of\ seed\ population} \times 100\% \quad (1)$$

MGT is defined as the mean time taken for the seeds to germinate. The lower the MGT, the faster a seed population germinates (Orchard, 1977). MGT is calculated with this Formula 2.

$$MGT = \frac{\sum f.x}{\sum f} \quad (2)$$

f = number of seeds germinated
x = day

The GRI displays the germination rate for each day of the germination phase. Higher GRI values indicate faster and greater germination (Esechie, 1994). GRI is calculated with this Formula 3.

$$GRI = \frac{G1}{1} + \frac{G2}{2} + \cdots + \frac{Gx}{x} \quad (3)$$

G1 = Germination percentage (%) on the first day after sowing
G2 = Germination percentage (%) on the second day after sowing
Gx = Germination percentage (%) on the x day after sowing

The experiment was arranged in a completely randomized design (CRD). All data were statistically analyzed with Microsoft Excel software using one-way ANOVA and followed by means analysis using Fisher's LSD (Least Significant Difference) test at $P \leq 0.05$.

3. Results and Discussion

In this study, aeration as a new method of solution uptake in tomato seed priming was investigated and compared to the more common methods, such as soaking and slow imbibition on moistened paper, as well as investigating KNO_3 as the priming agent. The result presented in Table 1 shows that the best treatments for FGP were aeration, both in KNO_3 and water, with 92.50 % and 92.25% respectively. The lowest percentage of final germination percentage (FGP) was observed in non-primed seeds. This means that all priming treatments gave better FGP than non-primed seeds. Following this finding, Several studies have shown that seed priming can improve seed germination and germination potential when compared to non-primed seeds. Many germination-related processes have been linked to seed priming, including increased energy metabolism, early reserve mobilization, embryo expansion, and endosperm weakening (Pandita *et al*., 2007).

Table 1. Effect of solution uptake methods and KNO_3 during tomato seed priming on FGP, MGT, and GRI

Treatments	FGP (%)	MGT (day)	GRI
Non-primed	88.50 ± 0.87 a	3.78 ± 0.10 d	25.37 ± 0.82 a
KNO_3 Moistened Paper	90.50 ± 0.65 ab	2.63 ± 0.08 b	43.82 ± 0.48 d
KNO_3 Soaking	92.00 ± 1.83 ab	3.29 ± 0.10 c	31.47 ± 0.94 b
KNO_3 Aeration	92.50 ± 0.96 b	2.39 ± 0.02 a	45.15 ± 0.60 d
Water Moistened Paper	90.50 ± 1.55 ab	2.65 ± 0.06 b	39.03 ± 0.58 c
Water Soaking	89.50 ± 0.65 ab	3.13 ± 0.07 c	31.75 ± 0.75 b
Water Aeration	92.25 ± 1.38 b	2.34 ± 0.07 a	45.25 ± 0.23 d
LSD	3.54	0.22	1.96

Remarks:
Means within each column followed by different letters are significantly different according to Fisher's Least Significant Difference test at $P \leq 0.05$.

Similar results were also observed in mean germination time (MGT) and germination rate index (GRI) where control resulted in the lowest performance, and aeration for both KNO_3 and water performed as the best treatment. In MGT, non-primed seeds needed 3.78 days to germinate on average, whereas the two aeration treatments resulted in 2.39 and 2.34 days. In GRI, non-primed seeds resulted in 25.37 seeds germinating per day on average, whereas the aeration treatments gave 45.15 and 45.25 seeds per day. Accordingly, several studies have also mentioned the decrease of MGT as a result of using KNO_3 as a priming agent (Kaewsorn & Chatbanyong, 2022; Mauromicale & Cavallaro, 1997; Nakorn & Kaewsorn, 2021). The difference between treatments was also quite distinct for these parameters and was visually seen especially in the early days after germination (Figure 1).

The radicle emergence of each treatment was observed 3 times a day and presented in Figure 2. With this graph, the speed of germination of each treatment was easily recognized. It is implied that although the final germination of each treatment is almost similar, some treatments resulted in more accelerated germination than the others. In agreement with MGT, non-primed seeds were the slowest to germinate, followed by soaking treatments. Both moistened paper and aeration treatments implied to give faster germination than the other treatments. The reasons for faster germination appears to be the completion of pre-germinative metabolic activities, which may have resulted in status seeds being ready for radicle protrusion when compared to non-primed seeds (Mukherjee, 2018).

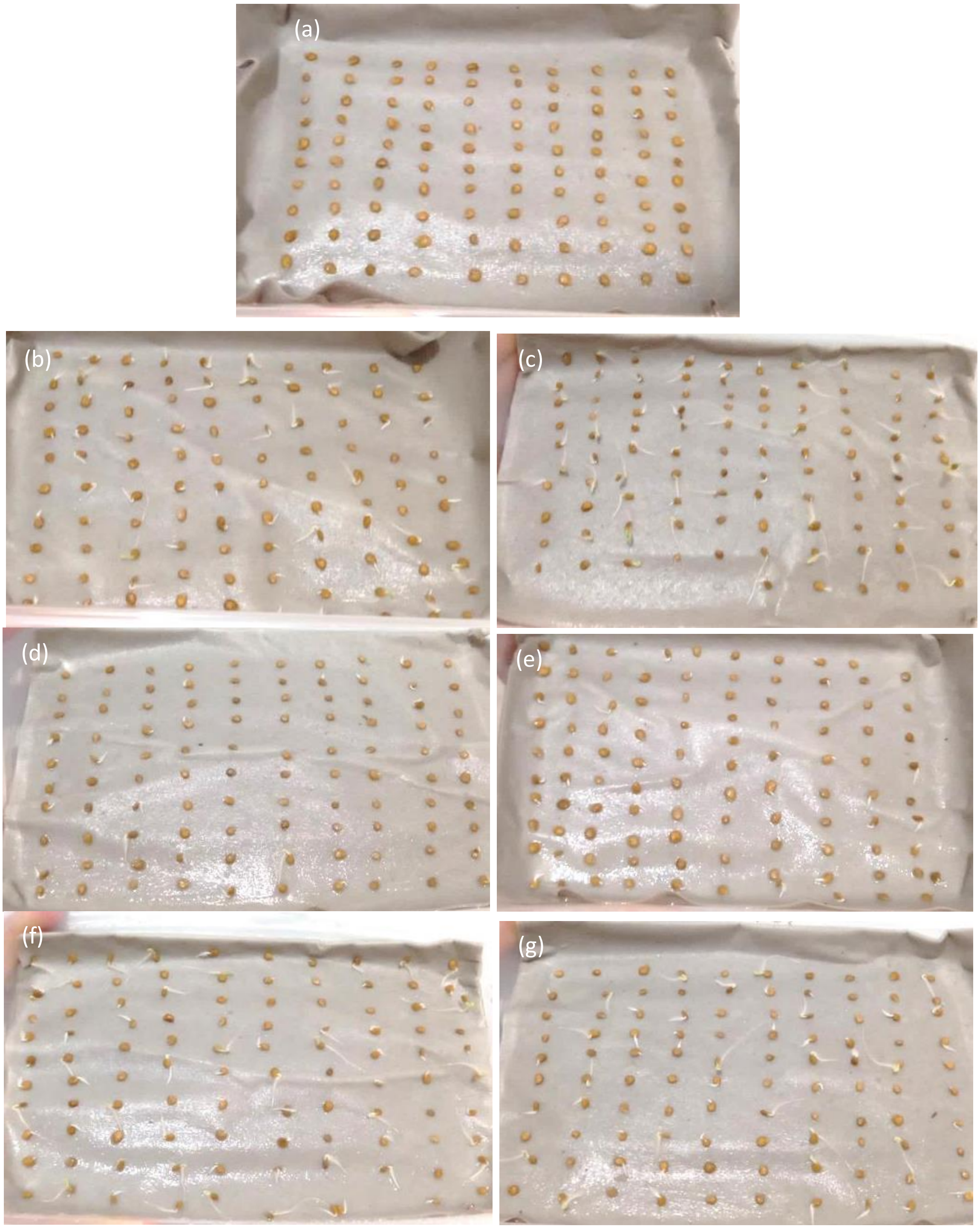

Figure 1. Tomato seeds at 48 hours after germination with different treatments: (a) non-primed, (b) KNO_3 moistened paper, (c) water moistened paper, (d) KNO_3 soaking, (e) water soaking, (f) KNO_3 aeration, (g) water aeration

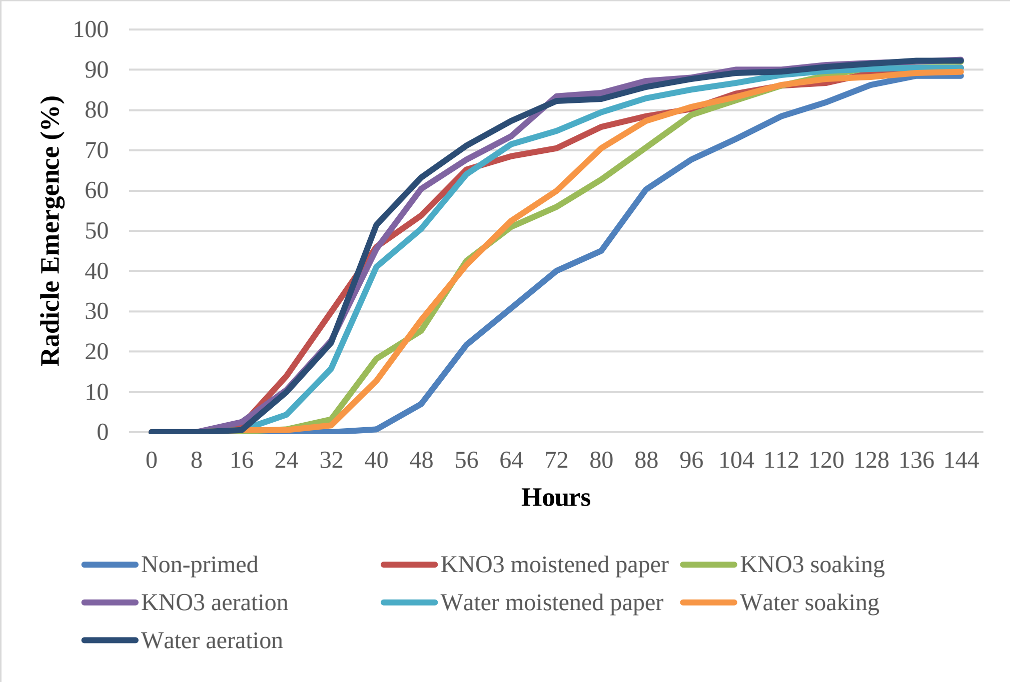

Figure 2. Radicle emergence of tomato seed with different treatments observed every 8 hours for 6 days

The single effect of the solution uptake method was also investigated and shown in Table 2 below. In terms of FGP, the three methods were not significantly different. The MGT and GRI, however, implied otherwise. In both MGT and GRI, aeration was observed to give the best performance, followed by moistened paper and soaking at the last. This indicates that tomato seed performance is better when provided with oxygen during priming. Similarly, Kaewsorn & Chatbanyong (2022) stated that priming with aeration in the solution resulted in better speed of germination as well as germination percentage compared to non-aerated priming. Seeds that are immersed in a solution for a long time may be deprived of oxygen during critical phases of imbibition, which may lead to the accumulation of toxic compounds produced during the onset of anaerobic metabolism during priming (Nakorn & Kaewsorn, 2021). On the other hand, although moistened paper also gave better results than non-primed and soaking, it may not be applicable for large batches of seed.

Table 2. Effect of solution uptake methods during tomato seed priming on FGP, MGT, and GRI

Treatments	FGP	MGT	GRI
Soaking	90.75 ± 1.01 a	3.21 ± 0.06 c	31.61 ± 0.56 a
Moistened paper	90.50 ± 0.78 a	2.64 ± 0.04 b	41.43 ± 0.97 b
Aeration	92.50 ± 0.76 a	2.39 ± 0.02 a	45.20 ± 0.30 c
LSD	2.52	0.22	1.97

Remarks:
Means within each column followed by different letters are significantly different according to Fisher’s Least Significant Difference test at $P \leq 0.05$.

The performance of KNO_3 alone was shown in Table 3. The result implied that, although KNO_3 gave a slightly better performance than water in all parameters, there was no statistical difference between the two treatments in this research design. In other studies, however, the use of KNO_3 as a priming agent was established to give better seed germination in tomatoes (Ali *et al*., 2020; Lara *et al*., 2014; Mauromicale & Cavallaro, 1997). KNO_3 is reported to be related to the activity of nitrate reductase which promotes faster germination (Lara *et al*., 2014). KNO_3 also plays a role in the formation of protoplasm and new cells and membrane permeability which leads to the activations of protein synthesis and carbohydrate metabolism enzymes (Preece & Read, 2005). Furthermore, KNO_3 also protects cells from oxidative injury (Waqas *et al*., 2019).

Table 3. Effect of KNO_3 during tomato seed priming on FGP, MGT, and GRI

Treatment	FGP	MGT	GRI
Water	90.83 $\pm$ 0.75 a	2.72 $\pm$ 0.10 a	38.68 $\pm$ 1.69 a
KNO_3	91.67 $\pm$ 0.70 a	2.77 $\pm$ 0.12 a	40.15 $\pm$ 1.89 a
LSD	2.12	0.32	5.26

Remarks:
Means within each column followed by different letters are significantly different according to Fisher's Least Significant Difference test at $P \leq 0.05$.

4. Conclusion

In conclusion, for tomato seed priming, aeration treatment resulted in better Final Germination Percentage, Mean Germination Time, and Germination Rate Index compared to moistened paper and soaking. It is also revealed that, in this study, KNO_3 gave a slightly better performance than water in all parameters, although there was no statistical difference between the two treatments. However, it is suggested that more studies regarding the field performance of primed seeds, as well as biochemical attributes will help to further understand and sew up the result of this study.

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