



Research article

## Effects of $\text{KNO}_3$ concentration and aeration during seed priming on seed quality of wax gourd (*Benincasa hispida* [Thunb.] Cogn.)

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### Abstract

Wax gourd seed has an extremely low germination rate and a high dormancy level. A laboratory experiment studied the effects of  $\text{KNO}_3$  concentration and aeration during seed priming to enhance the rate of germination and the germination percentage of wax gourd seed. A  $3 \times 2$  factorial experiments in completely randomized design including non-primed seeds (control) was implemented, using factor A as the  $\text{KNO}_3$  concentration (0%, 3% or 5%) and factor B as either non-aeration or aeration. Seeds were soaked in a  $\text{KNO}_3$  solution for 72 hr at  $30 \pm 2^\circ\text{C}$ ; then, the seed moisture content was reduced to approximately 7%. Priming treatments were applied in four replicates of 50 seeds per replicate. The effects of the priming treatments were evaluated based on a comparative analysis of a standard germination test, days to emergence (DTE) and mean germination time (MGT) among the treatments. The results indicated that seed priming with 3% or 5%  $\text{KNO}_3$  solution with aeration had the greatest mean ( $\pm \text{SD}$ ) germination rates ( $91.00 \pm 3.83\%$  and  $95.00 \pm 2.58\%$ , respectively), the lowest DTE ( $3.63 \pm 0.28$  d and  $3.70 \pm 0.32$  d, respectively) and the lowest MGT ( $10.60 \pm 0.60$  d and  $10.42 \pm 0.29$  d, respectively) compared to those of non-primed seeds ( $40.00 \pm 7.12\%$  germination,  $6.59 \pm 0.38$  d DTE and  $13.67 \pm 0.30$  d MGT). Therefore, priming wax gourd seed with 3% or 5%  $\text{KNO}_3$  solution with aeration can be used to increase the speed of seed germination while simultaneously increasing the germination percentage.

### Introduction

The wax gourd (*Benincasa hispida* [Thunb.] Cogn.), which belongs to the family Cucurbitaceae, is cultivated in tropical Asia for its immature and mature fruits (Rifai and Reyes,

1993). Also known as winter melon and Chinese water melon, wax gourd is an important fruit vegetable crop that is valued for its long storage life, unique flavour, diverse culinary uses and regional economic importance (Shobha, 2016). The fruit is a viable source of nutrients, including organic acids, natural sugars, amino acids, vitamins and mineral elements (Zaini et al., 2011). The seeds are characterised by a high linoleic acid content (63.10–70.64%) (Farooq et al., 2011). Wax gourd

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is propagated by seed, but previous studies revealed that seed germination is extremely low, with seed dormancy being one of the reasons for low germination. Wax gourd seeds are characterized by the presence of a hard seed coat and inhibitor (abscisic acid) (Rungcharoenthong, 2002; Nerson, 2007; Rahman et al., 2014; Shobha, 2016). Rungcharoenthong (2002) reported that wax gourd seed reached the developmental stage of physiological maturity at 55 d after anthesis. At this stage, the seed germination was approximately 8%, dormant seed accounted for 80% and the seed moisture content was 50%. However, the germination percentage after 12 mth increased to 71.5% as a consequence of decreasing seed abscisic acid content and the loss of seed dormancy. Shobha (2016) reported that field emergence is a persistent problem in wax gourd, even in seed lots with high germinability, due to a thick seed coat. Seed priming could be used to improve seed germination, seed emergence from the soil and stand establishment.

Seed priming is a technique used to improve seed germination and vigor, where the seeds imbibe water under controlled conditions to initiate the early events of germination while avoiding radicle emergence, followed by drying the seed to its initial moisture content before planting (McDonald, 2000). Seed priming improved the germination percentage, uniformity, seedling growth and yield of several vegetable crops (Bradford et al., 1990). Various seed priming techniques have been developed, including hydropriming (imbibing seeds in water) or osmopriming (imbibing seeds in aerated or non-aerated osmotic solution); these osmotic solutions have low water potential and thus, control the amount of water absorbed by the seed—for example, potassium nitrate ( $\text{KNO}_3$ ) (Bewley et al., 2013).  $\text{KNO}_3$  is utilized as a dormancy-breaking chemical agent according to the International Rules for Seed Testing (International Seed Testing Association, 2018). Furthermore,  $\text{KNO}_3$  is relatively cheap compared to other seed treatments and is widely available in the urban markets of Thailand, where wax gourd is a popular crop. There have been several reports of attempts to break the dormancy of the wax gourd seed. Boonchoo et al. (2006) reported that the germination percentage of wax gourd seeds placed on a paper towel saturated with 0.2%  $\text{KNO}_3$  significantly increased from 35% to 66% compared to that of non-treated seeds. Additionally,  $\text{KNO}_3$  improved seed vigor as measured by the germination index and seedling growth rate. Rahman et al. (2014) reported that wax gourd seeds imbibing 0.4%  $\text{KNO}_3$  solution for 24 hr achieved the maximum germination percentage (54%) and vigor index (1.59), compared with non-treated seeds (40.88% and 1.12, respectively).

Several factors affect seed priming techniques, such as seed quality, method, priming technique, soaking duration, temperature, aeration and time (Waqas et al., 2019). Of these factors, aeration significantly affects seed respiration, viability, emergence and germination (Heydecker et al., 1973; Bujalski and Nienow, 1991). There have been some reports comparing the use of aeration and no aeration during osmopriming with a  $\text{KNO}_3$  solution in cucurbit crops. Nascimento (2003) reported that muskmelon (*Cucurbita melo* L.) seeds osmoprimed in a -1.29 MPa  $\text{KNO}_3$  solution with aeration during different periods (3 d, 6 d, 9 d) had better seed germination rates and seedling development at 17°C and 25°C than osmopriming without aeration and non-priming. The effectiveness of aeration use in seed priming was demonstrated by Farooq et al. (2007), who reported enhanced germination percentages with an aerated  $\text{KNO}_3$  solution treatment of melon seed compared to non-aerated  $\text{KNO}_3$  solution. However, the effects of hydropriming wax gourd seed with aeration or non-aeration have only been reported by Sopa (2005), who found that hydropriming at 30°C with aeration for 7 hr following 24 hr incubation at 20°C improved the germination percentage and the speed of germination. There have been many investigations on the effects of seed priming with  $\text{KNO}_3$  solution without aeration in various cucurbit crops, such as watermelon (Barbosa et al., 2016), cucumber (Oliveira and Steiner, 2017) and melon (Oliveira et al., 2019), all of which showed enhanced seed germination, speed of germination, seedling emergence and vigor index, whereas there have been few reports investigating the effects of priming with aeration in cucurbit crops, such as melon (Farooq et al., 2007). Furthermore, there have been no published reports regarding osmopriming wax gourd seed using  $\text{KNO}_3$  and aeration methods to enhance seed germination and vigor.

Seed priming is a low-cost technique of soaking seeds in a solution containing  $\text{KNO}_3$  for specified time after which the seeds are redried and sown. The primed seeds produce a more rapid and uniform seed germination and subsequent better seedling establishment in many crops. The dry primed seeds can be stored for commercial distribution to end users, and when germination is subsequently reinitiated upon cultivation it is more rapid and homogeneous. The commercial utilization and availability of primed seeds are beneficial to vegetable seedling businesses where a high germination percentage and seedling uniformity are needed for commercial success. Thus, optimization of the seed priming technique is important, especially at the commercial scale. Rapid and uniform seed germination and seedling emergence are important factors for successful stand establishment and crop production.

An additional benefit of seed priming is that farmers, seed businesses or seedling businesses can conduct this approach, as it is considered a low-cost and simple practice. To assist with better understanding the priming technique applied to wax gourd seed, the current research used priming solutions of  $\text{KNO}_3$  in different concentrations, to investigate the need for aeration of the soaking solution and to clarify the correlation between the  $\text{KNO}_3$  concentration and aeration method. Thus, the objective of this research was to investigate the effects of the  $\text{KNO}_3$  concentration with or without aeration on the seed germination and seedling uniformity of wax gourd.

## Materials and Methods

### Seed material

Seeds of wax gourd cv. Pra-Lak were obtained from Thai Seed & Agriculture Co., Ltd (Bangkok, Thailand). The seeds were harvested and processed in December 2019. The initial seed quality measurements were: 7% seed moisture content, 35% germination, 4% abnormal seedlings, 60% fresh non-germinated seeds and 1% dead seeds. Seeds were kept in aluminum foil zip-lock bags at  $20 \pm 2^\circ\text{C}$  and  $40 \pm 2\%$  relative humidity (RH) for approximately 1 mth before starting the priming treatments.

### Method of seed priming

The experiment was conducted in the Seed Technology Laboratory, Department of Horticulture, Bangkok, Thailand, from January to February 2020. The experimental design used was a  $3 \times 2$  factorial experiments in completely randomized design with non-primed seeds (control) and four replicates. Factor A was the  $\text{KNO}_3$  concentration of 0%, 3% or 5%. Factor B was non-aeration or aeration. Each sample of 200 seeds was placed in a 250 mL Erlenmeyer flask containing 150 mL of either the solution of 0% (reverse osmosis [RO] water; hydropriming), 3% or 5%  $\text{KNO}_3$  (osmopriming) under natural light with non-aeration or continuous aeration sourced from an aquarium air pump for 72 hr at  $30 \pm 2^\circ\text{C}$ . The solutions were changed daily. After soaking in the priming solution for 72 hr, seeds were removed and rinsed with RO water and dried in an electric desiccator cabinet at  $30 \pm 2^\circ\text{C}$  and  $40 \pm 2\%$  RH until the seeds reached a moisture content of 7%. A standard germination test, days to emergence (DTE) and mean germination time (MGT) were carried out immediately after drying based on four replicates with 50 seeds per replicate.

### Germination

Each sample (50 seeds per replicate) was germinated on moist paper towels using the between-paper method and kept in the germinator under alternating temperature at  $20\text{--}30^\circ\text{C}$  ( $20^\circ\text{C}$  in darkness for 16 hr and  $30^\circ\text{C}$  under light for 8 hr) (International Seed Testing Association, 2018). The first and final counts were recorded at 4 d and 15 d, respectively, after testing, (Sopa, 2005). The percentage of normal seedlings was recorded. Normal seedlings consisted of those seedlings with a fully developed primary root, hypocotyl and two cotyledons. The percentage of abnormal seedling, fresh ungerminated seed and dead seed were recorded and used to calculate the final count.

### Days to emergence

The number of emerged radicles (2 mm radicle length) was counted each day from the day of first count until the day of final count (15 d after testing). The DTE was calculated using Equation 1 (Dhillon, 1995):

$$\text{DTE} = \sum nT / \sum n \quad (1)$$

where T is the number of days counted from the beginning of the test and n is the number of seeds with emerged radicles on day T.

### Mean germination time

The number of normal seedlings was counted daily from the day of first count until the day of the final count (15 d after testing). The MGT was calculated using Equation 2 (Ellis and Roberts, 1980):

$$\text{MGT} = \sum nd / \sum n \quad (2)$$

where d is the number of days counted from the beginning of the test and n is the number of normal seedlings that germinated on day d.

### Statistical analysis

All data were subjected to analysis of variance following by mean comparisons using Duncan's multiple range test. The analyses were done using the R program version 4.1.1 (R Core Team, 2021).

## Results and Discussion

The main effects of  $\text{KNO}_3$  concentration and aeration, as well as their interactions, were significant ( $p < 0.05$ ) for all traits measured (Tables 1 and 2).

### Germination

The mean germination percentage of wax gourd seeds after priming in different  $\text{KNO}_3$  concentrations and aeration methods is shown in Table 1. Priming seeds with 5%  $\text{KNO}_3$  solution achieved the greatest germination percentage ( $87.75 \pm 9.04\%$ ), but there were no significant differences between the 5% and 3% ( $84.25 \pm 8.03\%$  germination)  $\text{KNO}_3$  priming solutions. Seeds primed with 0%  $\text{KNO}_3$  solution (hydropriming) and non-priming had the lowest germination rates ( $53.00 \pm 8.14\%$  and  $40.00 \pm 7.12\%$ , respectively). Seeds primed using osmopriming with 3% or 5%  $\text{KNO}_3$  solution had greater germination percentages than for non-primed seeds, which demonstrated that the priming

treatments enhanced germination as reported by Farooq et al. (2007). Varier et al. (2010) reported similar results where priming promoted seed germination at three seedling developmental stages (imbibition, germination and growth). They also found that during seed imbibition, water uptake promoted protein synthesis and respiratory activities through messenger ribonucleic acid, where the second stage (lag phase) of the triphasic pattern of seed imbibition was related to the initiation of different physiological activities associated with germination, such as protein synthesis, mitochondrial synthesis and alteration of soluble sugars. Similarly, Farooq et al. (2007) reported that osmopriming of 'Ravi' melon seeds in aerated 1%, 2% or 3%  $\text{KNO}_3$  solution for 24 hr improved the germination rate, uniformity and early seedling growth. Likewise, Oliveira et al. (2019) reported that osmopriming of 'Gaúcho Casca de Carvalho Comprido' melon seeds with 5%  $\text{KNO}_3$  solution for 22 hr produced the highest seed germination and growth rate of seedlings. In the current study, osmopriming of wax gourd seeds with 3% or 5%  $\text{KNO}_3$  solution produced

**Table 1** Effects of priming using different  $\text{KNO}_3$  concentrations and aeration methods on germination, days to emergence (DTE) and mean germination time (MGT) of wax gourd seeds

Factors	Germination (%)	DTE (d)	MGT (d)
Concentration of $\text{KNO}_3$ (A)			
0%	53.00 $\pm$ 8.14 <sup>b</sup>	6.59 $\pm$ 0.86 <sup>a</sup>	13.93 $\pm$ 0.29 <sup>a</sup>
3%	84.25 $\pm$ 8.03 <sup>a</sup>	4.65 $\pm$ 1.12 <sup>b</sup>	11.13 $\pm$ 0.72 <sup>b</sup>
5%	87.75 $\pm$ 9.04 <sup>a</sup>	4.45 $\pm$ 0.84 <sup>b</sup>	11.07 $\pm$ 0.73 <sup>b</sup>
F test ( $p$ -value)	6.55 $\times$ 10 $^{-13}$	9.15 $\times$ 10 $^{-13}$	9.62 $\times$ 10 $^{-15}$
Aeration (B)			
Non-aeration	68.67 $\pm$ 16.41 <sup>b</sup>	6.07 $\pm$ 1.00 <sup>a</sup>	12.50 $\pm$ 1.22 <sup>a</sup>
Aeration	81.33 $\pm$ 17.63 <sup>a</sup>	4.39 $\pm$ 1.10 <sup>b</sup>	11.58 $\pm$ 1.62 <sup>b</sup>
F test ( $p$ -value)	1.62 $\times$ 10 $^{-5}$	6.87 $\times$ 10 $^{-12}$	6.72 $\times$ 10 $^{-7}$

Mean  $\pm$  SD in a column within each main effect followed by different lowercase letters are significantly ( $p < 0.05$ ) different.

**Table 2** Mean germination percentage, days to emergence (DTE) and mean germination time (MGT) of wax gourd seeds either non-primed or primed with  $\text{KNO}_3$  solutions of different concentrations and with or without aeration

Factor	Germination (%)	DTE (d)	MGT (d)
Concentration of $\text{KNO}_3$ (A)	Aeration (B)		
Non-primed seeds (control)	Non-aeration	40.00 $\pm$ 7.12 <sup>d</sup>	6.59 $\pm$ 0.38 <sup>b</sup>
	Aeration	48.00 $\pm$ 8.33 <sup>d</sup>	7.34 $\pm$ 0.40 <sup>a</sup>
0%	Non-aeration	58.00 $\pm$ 4.32 <sup>c</sup>	5.84 $\pm$ 0.26 <sup>c</sup>
	Aeration	77.50 $\pm$ 3.79 <sup>b</sup>	5.68 $\pm$ 0.22 <sup>c</sup>
3%	Non-aeration	91.00 $\pm$ 3.83 <sup>a</sup>	3.63 $\pm$ 0.28 <sup>c</sup>
	Aeration	80.50 $\pm$ 6.61 <sup>b</sup>	5.20 $\pm$ 0.21 <sup>d</sup>
5%	Non-aeration	95.00 $\pm$ 2.58 <sup>a</sup>	3.70 $\pm$ 0.32 <sup>c</sup>
	Aeration	11.71 $\pm$ 0.19 <sup>b</sup>	10.42 $\pm$ 0.29 <sup>c</sup>
F test ( $p$ -value)	0.01	0.01	0.03
CV (%)	7.95	5.59	2.64

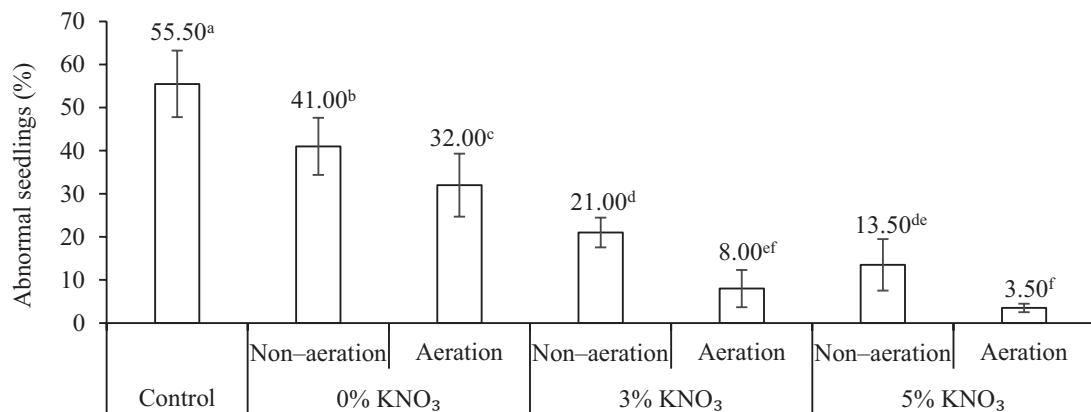
Mean  $\pm$  SD in a column superscripted by different lowercase letters are significantly ( $p < 0.05$ ) different.

greater germination percentages than for hydroprimed seeds (Table 1). Similarly, Nath and Deka (2015) stated that seeds of 'amar' angled luffa (*Luffa acutangula*) soaked in a  $\text{KNO}_3$  solution of 0.2% for 24 hr produced a greater percentage of germination than hydroprimed seeds. However, Oliveira and Steiner (2017) reported that osmoprimering of cucumber seeds with 0.2%  $\text{KNO}_3$  solution for 6 hr had little effect on the germination capacity compared to hydroprimed seeds. Because  $\text{KNO}_3$  is a chemical agent that is used for breaking seed dormancy (International Seed Testing Association, 2018), it increases the ambient oxygen concentrations by making less oxygen available for the citric acid cycle or Krebs cycle (Hilton and Thomas, 1986). Nitrates function in seed germination through reduction to ammonium ions as occurs in the nutrition of plants (Bethke et al., 2007b).  $\text{KNO}_3$  is related to the activity of enzyme nitrate reductase in the production of nitrite/nitric oxide, which acts to break dormancy and promote faster germination (Lara et al., 2014). Furthermore,  $\text{KNO}_3$  plays a role in the formation of protoplasm and new cells, as well as encouraging plant elongation (Preece and Read, 2005). It is plausible that the positive effect of  $\text{KNO}_3$  might be due to its role in influencing the permeability of the membranes which ultimately leads to activation of the enzymes involved in protein synthesis and carbohydrate metabolism (Preece and Read, 2005). Therefore, osmoprimering through the delayed water entry into the seed reduces reactive oxygen species accumulation and thus protects the cell from oxidative injury (Waqas et al., 2019).

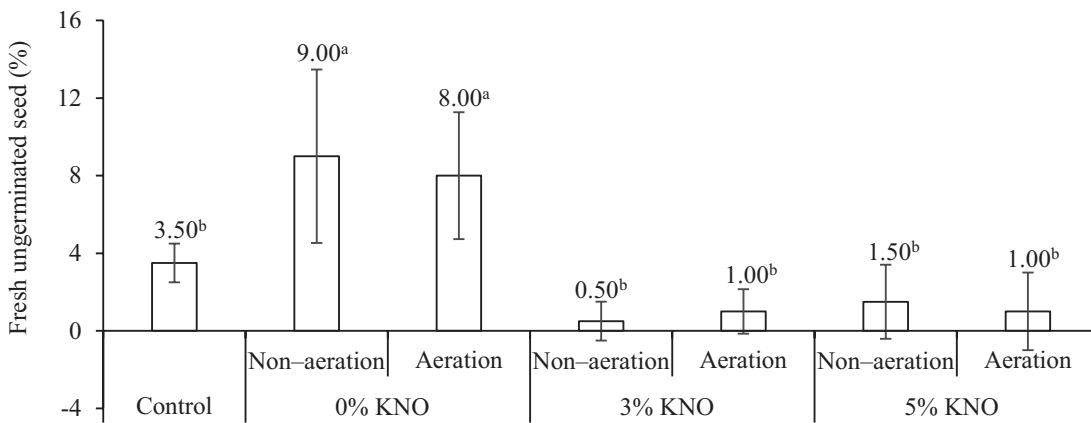
The wax gourd seeds primed in  $\text{KNO}_3$  solutions of different concentrations with aeration had the greatest germination ( $81.33 \pm 17.63\%$ ), followed by seeds primed in different concentrations without aeration ( $68.67 \pm 16.41\%$ ), whereas non-primed seeds had the lowest germination ( $40.00 \pm 7.12\%$ ), as shown in Table 1. These results indicated that wax gourd seeds need aeration during hydropriming (0%  $\text{KNO}_3$  solution) or osmoprimering (0%, 3% or 5%  $\text{KNO}_3$  solution). Appropriate aeration of the osmotic solution is essential to the survival of living seeds immersed in a solution and to support seed germination after priming, as the aeration system supports seed respiration, especially in an osmoticum solution (Bujalski and Nienow, 1991). However, the response to aeration during priming varies by species (Vanangamudi et al., 2010). Similarly, Sopa (2005) reported that hydropriming of wax gourd seeds at 30°C with continuous aeration for 7 hr following 24 hr incubation at 20°C achieved a greater germination percentage and a faster speed of germination than from hydropriming without aeration.

There was an interaction between the concentration of  $\text{KNO}_3$  and aeration that impacted the percentage of seed germination, as there were different effects with the different concentrations of  $\text{KNO}_3$  with or without aeration. The data were reanalyzed by concentration in the solution and aeration presence/absence resulting in a significant interaction among factors and means, as presented in Table 2. Seeds primed with 5%  $\text{KNO}_3$  solution with aeration achieved the greatest germination ( $95.00 \pm 2.58\%$ ), although this germination result was not significantly different from that of seeds primed with 3%  $\text{KNO}_3$  solution with aeration ( $91.00 \pm 3.83\%$ ). However, significant differences were detected for the effect of using aeration versus not using aeration. Seeds primed in 3% or 5%  $\text{KNO}_3$  solution without aeration had germination rates of  $77.50 \pm 3.79\%$  and  $80.50 \pm 6.61\%$ , respectively. Primed seeds using hydropriming or osmoprimering achieved a greater germination percentage than from non-primed seeds, except for hydropriming without aeration. Seeds hydroprimed without aeration had the lowest germination, which was not significantly different from that of non-primed seeds. Seeds immersed in RO water without aeration for 72 hr may be deprived of oxygen during the critical phases I and II of water imbibition. The lack of oxygen may result in the accumulation of toxic compounds produced during the onset of anaerobic metabolism during priming. Khalil et al. (2001) reported that priming soybean seeds for a period exceeding 48 hr without aeration reduced germination and may have had harmful effects on the seeds. Dezfuli et al. (2008) reported that priming with PEG 6000 at -1.2 MPa for 96 hr without aeration was harmful and reduced the percentage of maize seed germination. Thus, priming of wax gourd seeds in 3% or 5%  $\text{KNO}_3$  solution with aeration for 72 hr may have led the increased germination percentage compared to that of non-primed seeds.

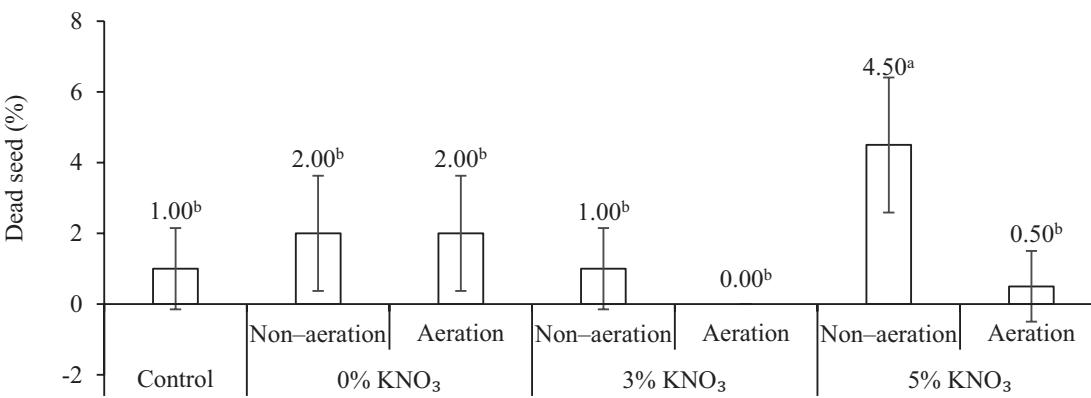
There were significant interactions between the  $\text{KNO}_3$  concentration and aeration during priming on abnormal seedlings, fresh ungerminated seeds and dead seeds of wax gourd (Figs. 1–3). Non-primed seeds (control) had the highest abnormal seedlings ( $55.50 \pm 7.72\%$ ), as shown in Fig. 1, with a short hypocotyl, the cotyledons still enclosed in the seed coat and a short root. These abnormalities probably were caused by slow radicle penetration and slow seedling development, which resulted in the lowest germination ( $40.00 \pm 7.12\%$ ), as shown in Table 1. These features indicated that some wax gourd seeds were released from dormancy after storage in a cool room ( $20 \pm 2^\circ\text{C}$  and  $40 \pm 2\%$  RH) for 1 mth before the priming treatments. Similarly, Ganar et al. (2004) reported that freshly harvested seeds of wax gourd have a relatively high dormancy level that is gradually broken during subsequent dry seed storage (after-ripening).



**Fig. 1** Abnormal seedling of wax gourd seeds after either without priming (control) or priming in different concentrations of KNO<sub>3</sub> and with or without aeration (error bars = ± SD), where mean values on bar graph with different lowercase superscripts are significantly ( $p < 0.05$ ) different



**Fig. 2** Fresh ungerminated seed of wax gourd after either without priming (control) or priming in different concentrations of KNO<sub>3</sub> and with or without aeration (error bars = ± SD), where mean values on bar graph with different lowercase superscripts are significantly ( $p < 0.05$ ) different



**Fig. 3** Dead seed of wax gourd after either without priming (control) or priming in different concentrations of KNO<sub>3</sub> and with or without aeration (error bars = ± SD), where mean values on bar graph with different lowercase superscripts are significantly ( $p < 0.05$ ) different

The seeds hydroprimed with aeration and without aeration yielded the highest percentage of fresh ungerminated seeds ( $8.00 \pm 3.27$  to  $9.00 \pm 4.76\%$ ; Fig. 2). Furthermore, seeds primed with 5%  $\text{KNO}_3$  solution without aeration had the highest percentage of dead seeds ( $4.50 \pm 1.91\%$ ; Fig. 3). However, the overall amount of fresh ungerminated seeds and dead seeds was low in each treatment.

#### Days to emergence

The DTE values of the wax gourd seeds primed with different concentrations of  $\text{KNO}_3$  with aeration are presented in Table 1. The DTE means for the main effects of the  $\text{KNO}_3$  concentration and aeration were significantly different. Seeds primed with 3% or 5%  $\text{KNO}_3$  solution achieved the shortest DTE values ( $4.65 \pm 1.12$  d and  $4.45 \pm 0.84$  d, respectively) followed by seeds primed in 0%  $\text{KNO}_3$  solution (hydropriming) and non-primed seeds ( $6.59 \pm 0.86$  d and  $6.59 \pm 0.86$  d, respectively). These results followed the same pattern as those of the germination results (Table 1). The radicle protrusion of primed seeds occurred earlier than that of non-primed seeds, resulting in a lower (earlier) DTE value. These results were expected as water absorption and radicle emergence are enhanced due to the enhancement of the pre-germination metabolic processes induced by seed priming (Mansour et al., 2019). Similarly, Farooq et al. (2007) reported that osmoprimeing of 'Ravi' melon seeds in aerated 1%, 2% or 3%  $\text{KNO}_3$  solution for 24 hr achieved greater germination rates than for non-primed seeds.

Primed seeds of wax gourd with aeration had the shortest DTE ( $4.39 \pm 1.10$  d), followed by primed with non-aeration ( $6.07 \pm 1.00$  d), whereas non-primed seeds had the longest DTE ( $6.59 \pm 0.38$  d), as shown in Table 1. Therefore, primed seeds of wax gourd using aeration had a shorter DTE than priming without aeration and non-primed seeds, indicating that wax gourd seeds need oxygen during priming treatment. Similarly, Sopa (2005) reported that hydroprimed and aerated seeds of wax gourd had a faster germination rate than non-aerated seeds.

There was a significant effect of the treatments and their interaction on seed germination parameters. The interaction between the  $\text{KNO}_3$  concentration and aeration on the DTE is shown in Table 2. Seeds primed in 3% or 5%  $\text{KNO}_3$  solution with aeration had the shortest DTE values ( $3.63 \pm 0.28$  d and  $3.70 \pm 0.32$  d, respectively), whereas seeds primed in 0%  $\text{KNO}_3$  solution (hydropriming) with non-aeration had the longest DTE ( $7.34 \pm 0.40$  d). Priming is the most effective

physiological method for improving seed performance and for obtaining faster and synchronized germination (Bradford, 1986). Aeration of the soaking solution improves water uptake and decreases the time needed for seed priming (Ozbingol et al., 1998; Nascimento, 2003). Priming with aeration promotes faster seed germination and accelerates seedling growth in many crops (Nascimento, 2003; Demir and Okcu, 2004). On the other hand, soaking seeds in an aerated solution for an extended time had deleterious effects on seed germination due to increased metabolism and salt penetration in seeds (Nascimento, 2003). Because of oxygen's low solubility (50%) and mobility (10%), the relative oxygen availability in seeds decreased by 5% following soaking (Bujalski et al., 1989). Therefore, an aeration system that supports seed respiration is needed, especially in an osmoticum solution (Bujalski and Nienow, 1991).

#### Mean germination time

The MGT values of the wax gourd seeds primed with different concentrations of  $\text{KNO}_3$  with aeration are shown in Table 1. Priming of wax gourd seeds in 3% or 5%  $\text{KNO}_3$  solution produced the shortest MGT ( $11.13 \pm 0.72$  d and  $11.07 \pm 0.73$  d, respectively), whereas seeds primed in 0%  $\text{KNO}_3$  solution and non-primed seeds had the highest MGT values ( $13.93 \pm 0.29$  d and  $13.67 \pm 0.30$  d, respectively), indicating that osmoprimeing with  $\text{KNO}_3$  achieved more rapid germination than hydroprimed and non-primed seeds. Similarly, Farooq et al. (2007) reported that osmoprimeing of 'Ravi' melon seeds in aerated 1%, 2% or 3%  $\text{KNO}_3$  solution for 24 hr achieved greater germination rates than for non-primed seeds. Jett et al. (1996) stated that controlled seed hydration in osmoprimeing treatments preserves the plasma membrane and causes quicker germination. Furthermore, osmoprimeing with  $\text{KNO}_3$  produced higher results than hydropriming for all tested parameters. Osmoprimeing is technically and financially more feasible compared to hydropriming, because osmoprimeed seed can achieve quicker germination with a lower cost and better water conservation, thus providing a promising alternative for farmers (Moradi and Younesi, 2009). These findings also agreed with Kubala et al. (2015), who found that rapeseed primed with an osmotic solution may have improved germination performance through metabolic activation involving the synthesis of proteins, nucleic acids and enzymes as a result of increasing water uptake, respiratory activity and reserve mobilization. The primed seeds of wax gourd with aeration had the shortest MGT ( $11.58 \pm 1.62$  d), followed by seeds primed without aeration

( $12.50 \pm 1.22$  d), as shown in Table 1. There was a similar trend to that for DTE (Table 1). Therefore, primed seeds using aeration had a shorter MGT than non-aerated and non-primed seeds.

The interaction between the  $\text{KNO}_3$  concentration and aeration on the MGT of wax gourd seeds after priming is shown in Table 2. Seeds primed in 3% or 5%  $\text{KNO}_3$  solution with aeration had the shortest MGT values ( $10.60 \pm 0.60$  d and  $10.42 \pm 0.29$  d, respectively), whereas non-primed seeds or seeds primed in 0%  $\text{KNO}_3$  solution (hydropriming) with non-aeration or with aeration had the highest MGT values ( $13.67 \pm 0.30$  d,  $14.13 \pm 0.24$  d and  $13.72 \pm 0.13$  d, respectively). After sowing, the primed seeds were able to take up further moisture rapidly and exhibited a reduced duration of phase II of imbibition relatively quickly from hydration to radicle emergence and growth. This considerably reduced the time from planting to seedling emergence and improved the uniformity of emergence (Bewley et al., 2013).  $\text{KNO}_3$  is an osmoticum of low water potential (Bewley et al., 2013) and is the most widely used chemical for promoting seed germination in many species, including *Brassica* spp., tomato (*Solanum lycopersicum* L.), celery (*Apium graveolens* L.) and *Capsicum* spp. (International Seed Testing Association, 2018). It can overcome the dormancy of wax gourd seed (Boonchoo et al., 2006; Rahman et al., 2014).  $\text{KNO}_3$  increases the ambient oxygen concentrations by making oxygen less available for the citric acid cycle (Hilton and Thomas, 1986).  $\text{KNO}_3$  may also be helpful for reactivating the metabolic processes of seeds (Bethke et al., 2007a). Nitrate ( $\text{NO}_3^-$ ) functions in supporting the nutrition required for seed germination by reducing ammonium ions (Bethke et al., 2007a). It acts as an oxidization substrate in a metabolic regulatory process involving nicotinamide adenine dinucleotide with an extra phosphate (NADH) and its relative nicotinamide adenine dinucleotide phosphate (NADP) in the pentose pathway of glucose metabolism (Hendricks and Taylorson, 1974).

The effects of seed priming depend on the crop species. Seed priming is safe, effective and easily adopted by farmers. Furthermore, seed priming can be widely used by seed producers, farmers and gardeners to improve seed performance in terms of germination speed, final germination rate, seedling vigor and uniformity. From the above results, it can be concluded that the priming of wax gourd seed with a 3% or 5%  $\text{KNO}_3$  solution with aeration is an affordable and effective method for increasing the seed germination speed and percentage. Therefore, priming of wax gourd seed with a 3%  $\text{KNO}_3$  solution with aeration for 72 hr can be recommended

as the most effective treatment. Such an easy-to-use, cost-saving and practical pre-treatment technique can be utilized by farmers and plant growers to help increase crop quality and ultimately achieve a higher yield.

## Conflict of Interests

The authors declare that there are no conflicts of interests.

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