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Research Article

Seed invigoration using ultrafine bubble water to increase the vigor of true shallot seed (*Allium ascalonicum* L.)

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ABSTRACT

True shallot seeds are preferable to tubers as planting materials because of their advantages in storage longevity, lower management cost, and lower seed-borne disease risk. However, environmentally limited production and seed deterioration in uncontrolled storage have limited its application. Pre-sowing hydration invigoration accelerates and synchronizes the germination of deteriorated seeds. Ultra-fine bubble water (UFBW) contains reactive oxygen species, which can advance the pre-germinative metabolism of the seed. This study examined true shallot seed invigoration using UFBW. The experiment was conducted in a two-factor of randomized complete block design. The first factor was initial seed vigor based on the vigor index with six levels, i.e., 24, 15, 13, 12, 10, and 9%. The second factor was the invigoration treatment of UFBW (8 and 23 ppm dissolved oxygen concentrations) with two durations (24 and 48 h), 50 ppm GA_3, and 3% KNO_3 (24 h). UFBW with 23 ppm invigoration for 48 h improved seed vigor based on all vigor parameters. The GA_3 50 ppm treatment is the most effective in increasing seed vigor.

Keywords: dissolved oxygen; pre-sowing hydration; reactive oxygen species; seed viability; seed vigor

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INTRODUCTION

Shallot is one of Indonesia's promising horticultural products, with national production reaching 1.82 million tonnes in 2020 and enabling it to enter the export market with 6,800 tonnes export volume p.a in 2015-2019. However, harvest area expansion primarily influenced the shallot's national productivity development strategy (5.71% per year). In contrast, crop productivity remained stagnant with declining trends (-0.53% per year) in the 2015-2019 periods (Indonesian Ministry of Agriculture Information System and Data Center, 2020)).

True shallot seeds (TSS) are botanical seeds produced for cross-pollination (Devi et al., 2015). It has recently been adapted as a planting material by substituting bulbs to improve the productivity of shallot crops. TSS has considerable advantages over vegetative bulbs, such as a longer storage period (2-3 years), lower management costs, more uniform field performance, and lower seed-borne disease risk (Van Den Brink & Basuki, 2012; Rosliani et al., 2016). To date, the application of TSS as planting material has remained low because of several issues in seed production and cultivation techniques. TSS production in Indonesia only occurs on a small scale once a year due to environmentally induced constraints such as high humidity and monthly rainfall, which also increase plant disease risk. Low seed set issues owing to limited pollinator availability also appear in TSS production (Sembiring et al., 2019). In the cultivation technique, early generation TSS (G_0)

is typically used by farmers to produce mini-bulbs (from G_1 to G_6) and then sold to another farmer as planting material. This phenomenon shows that TSS utilization on the farmer scale only occurs in 2–4 years intervals, assuming two planting periods in a year. The physiological quality of shallot seeds is known to deteriorate easily during storage for longer than 18 months in uncontrolled temperature and humidity storage facilities (Selvi & Saraswathy, 2018). Supported by this fact, market-circulating TSS is likely to decrease viability and vigor when needed.

Seed invigoration using a pre-sowing hydration technique accelerates and synchronizes the germination of deteriorated seeds. This technique can be performed by soaking the seed in a solution for a certain period and then drying it until a specific germination-favorable moisture content is reached. Seed immersion can accelerate seed imbibition and promote seed metabolism, resulting in uniform seed germination (Farooq et al., 2019). The immersion duration should be accounted for so that the total water imbibed to the seed and its rate do not lead to seed moisture content reaching the germination-favorable level (Hay et al., 2022).

An ultra-fine bubble (UFB) is a micro-to-nano-size bubble that lasts longer in water than a macro bubble and potentially provides the special characteristic of UFB-containing water (UFBW) (Tanaka et al., 2020). UFBW is known for its ability to enhance seeds' physiological and metabolic activities. Its reactive oxygen species (ROS) content is essential in seed signaling pathways related to many important activities, such as the gibberellic acid and abscisic acid ratio, cell synthesis, and respiration during germination (Rosental et al., 2014). Various studies have indicated that UFBW positively affects seed physiological quality. Liu et al. (2013) showed germination rate improvement 15–25% higher on barley seed immersed with UFBW. Iijima et al. (2020) studied soybean seed invigoration using UFBW and revealed a positive effect on the seed germination rate and seedling size under unfavorable conditions. Experiments on *Falcataria mollucana* by Siregar et al. (2021) and *Albizia chinensis* by Sudrajat et al. (2022) showed seed immersion for 30 min in UFBW increased deteriorated seed's viability from 11% to 50%. The application of UFBW for true shallot seed invigoration has remained unexplored. Thus, this study was conducted to investigate UFBW invigoration efficiency to enhance deteriorated seed vigor and viability.

MATERIALS AND METHODS

This research was conducted from January to June 2022 at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, and Biosystems Environmental Engineering Laboratory, Department of Agriculture Engineering and Biosystems, IPB University. A randomized complete block design with two factors was used in this study, with the initial vigor level based on the vigor index enabled as the first factor, consisting of six levels (24%, 15%, 13%, 12%, 10%, and 9%). The second factor was the invigoration treatment, which included UFBW, GA_3, and KNO_3 (Table 1). Untreated seeds were used as controls. Invigoration using 3% KNO_3 and 50 ppm GA_3 was used for comparison based on Muruli et al. (2016), as it was proven to significantly improve onion seeds (*Allium cepa*) quality. Each combination of treatments was performed in triplicate. Replication was assigned as a block.

Table 1. Treatment applied to true shallot seed as invigoration.

Invigoration treatment	Description
T1	Control (untreated)
T2	UFB DO 8 ppm 24 h immersion
T3	UFB DO 8 ppm 48 h immersion
T4	UFB DO 23 ppm 24 h immersion
T5	UFB DO 23 ppm 48 h immersion
T6	3% KNO_3 24 h immersion
T7	50 ppm GA_3 24 h immersion

True shallot seed of Lokananta variety (expired label January 2023) was used in this study. Several levels of seed vigor used in this research were achieved through accelerated aging. Briefly, four grams of seeds (initial moisture content of ±7.5%) were uniformly distributed on a 60 mesh wire screen in a thin-walled plastic box containing sterile distilled water. The boxes were placed in an oven at 41±1 °C for 20–80 h at 20 h intervals to create different vigor levels (Kamanga et al., 2021). After aging, the seeds were air-dried for 4–6 h until they reached their initial moisture contents. Non-aged seeds were assigned to the highest vigor level.

A UFB water generator (FZ1N-10, IDEC) produced UFBW from distilled water poured into a water container connected to the device. UFB water with DO 8 ppm was made using regular atmospheric air injection, whereas UFB water with DO 23 ppm was created by injecting O_2 gas. The device was operated with 15 L distilled water for 45 min to achieve the desired DO. Invigoration was accomplished by immersing the seeds in UFBW solution with 8 ppm and 23 ppm DO, 3% KNO_3 solution, and 50 ppm GA_3 solution at a ratio of 1 g of seed in 200 mL of solution. The seeds were then incubated in a room at 20±1 °C, as shown in Table 1. An aerator was applied on 3% KNO_3 and 50 ppm GA_3 invigoration to prevent decay. Finally, the immersed seeds were air-dried for 6-8 h until they reached the initial weight at room temperature (22-25 °C).

The germination test was carried out with a total of 150 seeds in triplicate (50 seeds per replicate) following the ISTA (2018) rules, using the top-of-paper method with CD paper as a substrate in a controlled temperature (20±2 °C). Germination percentage (GP) was determined by calculating the total normal seedling percentage at the first count (6 days after sowing; DAS) and the final count (12 DAS). The vigor index (VI) defined normal seedlings percentage in the first count (6 DAS). Normal seedlings were characterized by bright green, undamaged cotyledons showing a visible, sharp curve (knee), and intact long radicles (Elias et al., 2012). Radicle emergence (RE) described the total percentage of seeds with ≥2 mm radicle length 72 h after sowing at 20 °C (Kamanga et al., 2021). The speed of germination (SG) (%/etmal) was determined by calculating normal seedlings percentage per etmal (24 h) until the final count period (Copeland & McDonald, 2001). The T_{50} (day; d) described the time needed for half of the total seeds to produce normal seedlings and was calculated according to (Farooq et al., 2005).

The data obtained from the parameters in every treatment were subjected to a one-way analysis of variance (ANOVA) with a 95% confidence level. Significantly effective treatment (p-value <0.05) means were compared using Duncan's multiple range test (DMRT) at a 5% probability level. SAS® OnDemand for Academics software was used to analyze the data statistically.

RESULTS AND DISCUSSION

ANOVA demonstrated that the interaction between invigoration treatment and initial seed vigor significantly influenced VI, SG, and T50 but did not significantly influence GP and RE. Replication as a block significantly affected RE, SG, and T_{50} parameters with higher mean parameter values in the second and third replicate. Such effect was most likely due to the temperature and RH conditions of germination in replicates 2 and 3 at 20.2 ± 1.8 °C and 68.2 ± 10.1%, while replicate 1 was lower at 19.2 ± 1.3 °C and 60.2 ± 5.6%, respectively. This phenomenon allows for variations in germination compared to optimum conditions. However, both locations' temperature is still acceptable by ISTA (2018) standards of 20 ± 2 °C.

All invigoration treatments significantly improved the vigor index compared to the control at all vigor levels (Table 2). The difference in the percentage of seed vigor improvement showed the interaction of invigoration treatments with the initial seed vigor level. The vigor index improvement response increased as initial seed quality decreased. Seeds with lower initial vigor (9-13%) treated with 50 ppm GA_3 improved VI (70-82%) more than those treated with 3% KNO_3 (52-67%). Invigoration using 50 ppm GA_3 and 3% KNO_3 treatments increased the vigor index of seeds with initial vigor levels of 15% and 24%, but statistically insignificant.

Table 2. Seed vigor index (%) of TSS from seed initial vigor level and invigoration treatments.

Invigoration treatment	Seed initial vigor level (%)					
	24	15	13	12	10	9
Control (untreated)	24kl	15lm	13lm	12lm	10lm	9m
UFB DO 8 ppm 24 h	38hij	34ijk	46g-j	49f-i	55efg	45g-j
UFB DO 8 ppm 48 h	52fgh	52fgh	38hij	51fgh	37h-k	34ijk
UFB DO 23 ppm 24 h	43g-j	36ijk	62def	52fgh	40hij	45g-j
UFB DO 23 ppm 48 h	42g-j	44g-j	44g-j	31jk	51fgh	44g-j
3% KNO_3 24 h	68b-e	71a-d	67b-e	67cde	52fgh	55efg
50 ppm GA_3 24 h	81abc	72a-d	84a	72a-d	82ab	70a-d

Note: Values in each row and column followed by the same letter are not significantly different based on DMRT test at 5%.

Invigoration with 8 and 23 ppm DO UFBW for 24, and 48 h increased the vigor index at all seed vigor levels (Table 2). The response of seeds with low initial vigor (9–13%) to UFBW invigoration showed the same improvement as the 3% KNO_3 treatment. Seeds with higher initial vigor levels responded differently to 50 ppm GA_3 and 3% KNO_3 treatments than UFBW. Invigoration with UFBW on seeds with low initial vigor levels gives comparable improvement to the seeds' vigor index with higher initial vigor levels. Seeds with 13% initial vigor in the 23 ppm UFBW treatment for 24 h had the highest VI value (62%) compared to other vigor levels in the same treatment.

Invigoration significantly increased SG at all seed vigor levels (Table 3). Treatment with 50 ppm GA_3 increased and produced uniform SG value for all vigor levels. The extent of the increase stagnated in the KNO_3 treatment for seed with initial vigor level of 9–13%. The SG of seeds with high initial vigor levels (15% and 24%) was 14% higher than that of the control treated with 23 ppm UFBW for 48 h. Application of UFBW for invigoration significantly accelerated the germination of deteriorated seeds (initial vigor level of 9–13%) compared to the control.

Table 3. Speed of germination (%/day) of TSS from seed initial vigor level and invigoration treatments.

Invigoration treatment	Seed initial vigor level (%)					
	24	15	13	12	10	9
Control (untreated)	11.7l	13.0g-j	12.9hij	12.2jkl	12.9hij	11.9kl
UFB DO 8 ppm 24 h	13.0f-j	13.1e-i	12.8h-k	13.1e-i	12.7ijk	13.0g-j
UFB DO 8 ppm 48 h	12.8h-k	13.9b-g	13.2e-i	14.1b-e	13.9b-g	13.5d-i
UFB DO 23 ppm 24 h	13.1f-j	13.1e-i	13.7c-h	13.2e-i	13.5d-i	13.7c-h
UFB DO 23 ppm 48 h	13.9b-g	14.1b-e	13.3d-i	14.0b-f	13.6d-i	13.0f-j
3% KNO_3 24 h	14.7ab	14.1b-e	13.9b-g	14.2bcd	13.6d-i	13.6d-i
50 ppm GA_3 24 h	15.3a	15.2a	14.3bcd	15.2a	15.1a	14.6abc

Note: Values in each row and column followed with the same letter are not significantly different based on DMRT test at 5%.

Table 4. Seed's T_{50} (d) of TSS from seed initial vigor level and invigoration treatments.

Invigoration treatment	Seed initial vigor level (%)					
	24	15	13	12	10	9
Control (untreated)	7.5a	6.7cd	6.6cde	6.9bc	6.7cd	7.1b
UFB DO 8 ppm 24 h	6.6c-f	6.5c-i	6.6c-f	6.6c-g	6.8cd	6.5c-i
UFB DO 8 ppm 48 h	6.7cd	6.5c-j	6.3e-m	6.3f-m	6.3f-m	6.4d-l
UFB DO 23 ppm 24 h	6.5c-h	6.4d-j	6.1k-o	6.5c-i	6.4d-j	6.2g-n
UFB DO 23 ppm 48 h	6.2h-n	6.1k-p	6.1i-n	6.1j-n	5.9m-q	6.1k-p
3% KNO_3 24 h	6.0l-q	6.0m-q	6.0m-q	5.9m-q	6.0m-q	6.0m-q
50 ppm GA_3 24 h	5.7op	5.8opq	6.1k-p	5.9n-q	5.6q	5.7opq

Note: Values in each row and column followed with the same letter are not significantly different based on DMRT test at 5%.

Seeds' T_{50} was successfully shortened by invigorating the seeds with 50 ppm GA_3 24 h (Table 4). The T_{50} values shortening was similar for high initial vigor seeds but varied for seeds with lower initial vigor (9–13%) for all invigoration treatments. Compared with the control, the 50 ppm GA_3 and 3% KNO_3 solutions made the T_{50} shorter at all vigor levels.

Both treatments cut the T_{50} one day faster than the control (7 d), resulting in a relatively uniform T50 (5.6–6.1 d). Seeds with 13% initial vigor responded best to the 50 ppm GA_3 invigoration and had the fastest T_{50} (5.6 d) compared with the other treatments. Invigoration using 23 ppm UFBW for 48 h showed a uniform T_{50} (5.9–6.1 days) and significantly differed from the control at all seed vigor levels. The UFBW 8 ppm invigoration for 24 h shortened the T_{50} of seed germination with the lowest vigor level (6.5 d), uniform with the undeteriorated seed (6.6 d). UFBW 8 ppm treatment of 48 hours was responded by undeteriorated seeds (faster from 7.5 d to 6.7 d) and deteriorated seeds (initial vigor level of 9–12%) to 6.3 and 6.4 d, respectively. Increasing DO to 23 ppm for 24 h immersion accelerated the T_{50} of undeteriorated and deteriorated seeds (initial vigor of 13%) by one day and 0.5 d, respectively, compared to the control. In general, invigoration techniques did not significantly affect RE, except for the 23 ppm UFBW invigoration for 24 h, which significantly reduced the percentage of RE from 26.2% to 41.7% in the control (Table 5). The GP value increased only in the seed treated with 50 ppm GA_3 and 3% KNO_3.

Table 5. Germination percentage (GP) and RE (%) of TSS from seed initial vigor level and invigoration treatments.

Invigoration treatment	GP	Radicle emergence
Control (untreated)	90.8bc	41.7a
UFB DO 8 ppm 24 h	91.9ab	37.2a
UFB DO 8 ppm 48 h	89.8c	37.2a
UFB DO 23 ppm 24 h	91.7abc	26.2b
UFB DO 23 ppm 48 h	89.9bc	37.8a
3% KNO_3 24 h	93.2a	42.8a
50 ppm GA_3 24 h	93.3a	41.2a

Note: Values in each column that followed with the same letter are not significantly different based on DMRT test at 5%.

Accelerated seed aging using high temperature and humidity was conducted to create seed lots with different viability. This technique resulted in seeds with viability dynamically varied over several aging durations applied in this study. Such those variability also occurred in other seeds, such as in experiments on *Bombax ceiba* seeds (Zheng & Ma, 2014), which demonstrated an increase in germination index after exposure to 45°C and 100% RH for 1 d and then gradually decreased with a longer duration of exposure. Khan et al., (2003) showed that humidifying *Celosia argentea* seeds with high temperature and humidity (45°C and 100%, respectively) increased germination.

The invigoration techniques significantly increased the germination of low-vigor TSS seeds to nearly the same as high-vigor seeds (Table 5). Invigoration with GA_3 (hormone priming) promotes significant improvement than the other treatments. GA_3 effect on germination was also found in the degraded seeds of several commodities such as wheat (Ardebili et al., 2019), onion (Muruli et al., 2016), papaya (Sehrawat et al., 2010), and *Gmelina arborea* (Siregar et al., 2020). Exogenous application of GA_3 to seeds improves seed germination by increasing the GA:ABA ratio of seeds (Ucarli, 2021), amplifying the hydrolytic enzyme α-amylase activity (Yu et al., 2016), and accelerating seed storage protein metabolism (Subedi and Bhattarai, 2003). Such pre-germination treatment improves seed metabolism, leading to better seed germination.

KNO_3 invigoration induces seedling resistance to stresses, including salinity, drought, and soil acidity (Steiner et al., 2018; Ali et al., 2021; Khan et al., 2022). Nitrate ions induce the gene translation of ABA catabolism enzymes and suppress ABA-inhibiting role germination (Duermeyer et al., 2018). Other effects, such as cell membrane repair, occurred in KNO_3 priming subjected to leek (*Allium fistulosum*) seeds. That treatment increased the antioxidant activity and reduced the H_2O_2 concentration to unhazardous levels (Dong et al., 2014). Invigoration of onion seeds with 0.5% KNO_3 and 50 ppm GA_3

with a shelf life of 1-3 years resulted in seedling length close to the undeteriorated seeds (Brar et al., 2020).

Seed vigor improvement was observed with the UFBW invigoration like on seeds of *Albizia chinensis* (Sudrajat et al., 2022) and *Gmelina arborea* (Siregar et al., 2020). UFBW promotes germination through its capability to produce ROS (Terasaka et al., 2021). Exogenous ROS induces internal ROS production in seeds to a certain extent (Mahakham et al., 2017). ROS influence on seed germination was determined by its concentration inside the seeds. Beneficial outcomes arise when ROS concentration is within the oxidative window range (Bailly et al., 2008). The ROS at these status plays a role in seed metabolic signaling pathways and alters seed GA and ABA equilibrium. ROS's function in cell wall loosening also affects the embryonic axis' cell elongation and division (Muller et al., 2009). ROS production and eradication equilibration by antioxidants must be considered so that ROS does not become destructive. ROS function transformation is also regulated by environmental conditions, where the favorable effect arises in optimum germination environment, while stressful one induced adverse consequences (Gomes & Garcia, 2013).

ROS is hypothesized to be a link between the effect of invigoration duration and oxygen concentration within UFBW. Increased ROS production occurs in seeds exposed to hypoxic conditions (Rajashekar & Baek, 2014). This phenomenon shifts the effects of ROS and triggers seed deterioration. The experimental finding demonstrated that immersing in 23 ppm DO UFBW for 48 h shortened the T_{50} of the seeds compared to the 8 ppm DO UFBW treatment for 24 h. Water absorption in the pre-sowing hydration technique tends to be uncontrolled and is regulated by seed water potential. Seeds with low water content absorb more water to reach equilibrium water content. A water-saturated environment triggers hypoxia that limits cellular respiration. This condition may not occur in seeds with a low water content when they are soaked for a certain period until they reach its equilibrium with the environment. A higher oxygen supply in UFB water with higher DO minimizes the chance of seeds experiencing hypoxia due to flooding.

CONCLUSIONS

Invigoration with 50 ppm GA_3 and 3% KNO_3 effectively increased the vigor of TSS, presented by the increase in VI, SG, and shorter time of T_{50} at all vigor levels. Seeds with low initial vigor (9-15% vigor index) responded better to the invigoration treatment and brought their VI, SG, and T_{50} values nearly identical to seeds with high initial vigor (24%). Applying 23 ppm UFBW for 48 h increased seed vigor with effectiveness that closely mimicked the 3% KNO_3 invigoration with an increase in the average VI value of seeds with low initial vigor (9-13% with various improvements). It was also detected at the SG and T_{50} parameters. All initial vigor levels of seeds' average SG and T50 increased with varying percentages. Applying cheaper and simpler UFBW invigoration could be an alternative substitute for GA_3 and KNO_3.

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