Hindawi
International Journal of Agronomy
Volume 2023, Article ID 3459377, 10 pages
https://doi.org/10.1155/2023/3459377

*Research Article*

# Effect of Dissolved Oxygen and Chemical Scarification on *Andrographis paniculata* Seed Germination in Macrobubble Conditions

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Received 22 December 2022; Revised 4 April 2023; Accepted 31 May 2023; Published 13 June 2023

Academic Editor: Othmane Merah

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*Andrographis paniculata* is used in Thai traditional medicine. This plant contains a bitter compound called andrographolide, which is highly effective in the prevention of many diseases. It is an effective treatment for infectious diseases and has a prophylactic effect owing to its powerful immunity-boosting benefits. Recently, it has been widely used to treat COVID-19. However, commercial planting of *A. paniculata* is performed by seeding, which leads to seed germination problems. The seed germination is relatively low and not efficient under normal conditions for various reasons, such as a combined dormancy of physical and innate nature, the diversity of the seeds in different lots, and the fact that the germination duration was not uniform in the same lot. An easily applied and inexpensive method for farmers to develop mass plantings to stimulate germination is by using macrobubble conditions by aerating seeds in sterile water in collaboration with chemical scarification, which is the idea of creating a hard seed coat that causes seed dormancy to break while root germination occurs at 25°C. Germination was completed after 16 days. The dissolved oxygen (DO) concentrations in this environment were 5, 6, 7, 8, and 9 mg·L$^{-1}$. The oxygen intensity of 9 mg·L$^{-1}$ showed the highest germination percentage (26.33%). It was found to be optimal for macrobubble conditions. Seedlings were treated with chemicals (PEG, NaCl, $H_2SO_4$, KCl, $KNO_3$, $NaHClO_3$, and $GA_3$) after soaking in macrobubbles with optimum DO. The results showed that $NaHClO_3$ conc. (30 min) showed a generation percentage reaching 92%, which could greatly promote up to 3.63 folds compared with the control in the macrobubble aeration system.

## 1. Introduction

*Andrographis paniculata*, also known as Fah Talai Jone, is a popular traditional Thai medicine. This plant belongs to the family Acanthaceae and is widely used in medicinal and pharmaceutical applications. It is generally known as "the King of Bitters," and its secondary metabolite, andrographolide (AG), has various medical applications [1, 2]. In Asia, America, and Africa, *A. paniculata* has been used for centuries to cure a variety of diseases, such as diabetes, high blood pressure, cancer, ulcers, leprosy, dysentery, dyspepsia, flatulence, and malaria [2]. Andrographolide is a major active constituent of this plant, and it has a lot of different biological activities. Some of these activities are anti-inflammatory, antibacterial, antitumor, antidiabetic, antimalarial, hepatoprotective, and antiviral for HIV [3, 4]. COVID-19 has recently emerged as a global health threat with a rapid global spread and high mortality rates. Therefore, it is critical to identify new treatments as soon as possible. According to the integrative medicine, some herbs can help cure COVID-19 when used in combination with traditional therapies. Thailand is affected by epidemics, and infected patients are being treated with herbs. For example, some patients are administered a different dose of *A. paniculata* than those prescribed for fever and sore throats [5]. The COVID-19 therapeutic potential of these plants was

chosen. Integrating Thai traditional medicine concepts with modern COVID-19 treatment mechanisms would almost certainly result in a more effective clinical treatment [6]. Because of its importance in the treatment of a variety of ailments, *A. paniculata* is in high demand owing to its potent immune-boosting abilities [7]. However, seed germination remains a significant issue because this plant has such a wide variety. The seeds were very small and dormant between 5 and 6 months after dispersal, germinating at an extremely low rate. Despite germination issues, *A. paniculata* is commonly propagated through seeds, which may indicate the presence of physical and innate dormancy, a usual survival strategy of plants for the effective spread on this planet [8, 9].

One fundamental issue with the production of this plant from seeds is its destitute seed germination execution. Under normal conditions, the germination percentage and germination rate of this seed are generally poor [8, 10]. The dormancy of *A. paniculata* seeds is primarily ascribed to the hard seed coat. The seed coat secures the embryo and its environment from water and any outside dangers. It is a physical obstruction that actuates the seed dormancy [9]. The seeds of *A. paniculata* contain mainly alkaloids, saponins, and monounsaturated fatty acids. Seed storage is a big problem in countries with high temperatures and humidity. This can cause seeds to age quickly, which can make them less viable [11]. Seed dormancy can take the form of physical, morphological, physiological, morphophysiological, or a combination of physiological and physical both [12]. Seed dormancy is an internal state that prevents seeds from germinating, even under ideal temperature and gaseous and hydric conditions [13]. Dormancy is a feature of plant seeds that prevents germination and must be overcome by exogenous stimuli [14].

For breaking seed dormancy, scarifications using physical techniques or chemical agents can be applied to overcome dormancy. According to reports, sunflower seed priming had a substantial impact on increasing germination percent, germination speed, and seedling dry weight and had the opposite effect in drought conditions, producing anomalous seedling decrement [15]. Several crops, including maize, wheat, rice, and canola, can benefit from seed priming treatments that improve seed germination and establishment [16, 17]. Some plant seed dormancy is susceptible to chemical agents, for example, potassium nitrate ($KNO_3$), plant growth regulators, gibberellic acid ($GA_3$), and osmotic solutions such as polyethylene glycol (PEG) and salt solutions such as sodium chloride (NaCl), sulphuric acid ($H_2SO_4$), potassium chloride (KCl), and sodium hypochlorite ($NaHClO_3$) [9, 18, 19]. From early research, seed germination of Kalmegh (*A. paniculata*) was enhanced by PEG and NaCl [19]. *A. paniculata* seeds were soaked in $GA_3$ at 100 ppm for 4 hours, which showed noticeably better seed germination and field emergence [11]. The *A. paniculata* seeds showed the highest germination percentage of 57.20 at 20°C while utilizing the top of a paper substrate after being treated with 0.5% potassium nitrate ($KNO_3$) for 24 hours [20]. Seed germination of *A. paniculata* was observed to be enhanced by the 25% sulphuric acid ($H_2SO_4$) pretreatment before planting [21]. Soaking the seeds in 1 and 2% KCl for 10 min was also quite effective in promoting seed germination of *A. paniculata* [9]. The study of Kumari et al. [18] found that almost all of the seeds germinated; $KNO_3$ and $GA_3$ made the seeds germinate faster, and the seeds germinated quickly in the presence of $NaHClO_3$.

There are insufficient scientific reports available on the seed quality and germination of *A. paniculata*. As a result, the current study is required to generate data on the standardization of germination tests to improve germination [18]. Germination starts with the seed absorbing water, known as imbibition, and ends with the elongation of the embryonic axis. It is a complicated procedure in which the seed must physically recover from maturation drying, restart a sustained intensity of metabolism, complete the necessary cellular activities required for the embryo to emerge, and prepare for subsequent seedling development. Seeds require moisture, a suitable temperature, and an aerobic environment to germinate [22]. In *A. paniculata*, a temperature of 25°C was optimal for seed germination [23]. Some of the dissolved oxygen (DO) in the water is caused by stream turbulence, which occurs when air is trapped by moving water, resulting in the dissolution of oxygen into the water [24, 25]. A high DO concentration has been maintained and enhanced using macrobubbles [26].

This study hypotheses that chemical and macrobubble aeration in a water system saturated with dissolved oxygen and moisture can activate seed germination. Our purpose was to find the basic system by using a normal air pump connected with a bubble air stone. It was the equipment that cost the lowest for breaking dormancy and improving the germination for commercial production of *A. paniculata* in small farming.

## 2. Materials and Methods

*2.1. A. paniculata Seeds and Chemicals.* *A. paniculata* seeds were collected in October 2021 from the Experimental Garden of Walailak University (8°38′42.7″N 99°54′04.4″E). The seeds were dehydrated at 25 ± 2°C for a week and kept in a zipper bag under ambient temperature control at 4°C until the experiment was conducted in November–December 2021. These seeds were selected using a light microscope, and the large seeds with a dark brown color were selected. All of the chemicals utilized in the treatments were of analytical grade.

*2.1.1. Generation of Macrobubble Water for the Germination Experiment.* The macrobubble system was set up in an Erlenmeyer flask with a capacity of 500 mL, filled with 300 mL of water, which was autoclaved for sterilisation at 121°C for 15 min (Autoclave SX-700, Tomy Seiko Co., Ltd., Japan). Air was pumped into the water by a pump (Twin Air Pump Magic-8800, A.S. Union UNION Co., Ltd., Thailand) and circulated through a perforated stone with continuous aeration to generate macrobubbles at 25°C to obtain "water-containing macrobubbles." After the seed was germinated,

the seedling will be moved to a Petri dish that is filled with 10 mL of autoclaved water and the water was changed every day until completion (Figure 1).

*2.1.2. Oxygen Concentration for the Seed Germination Test.* *A. paniculata* seeds' germination was carried out using a completely randomised design (CRD). Germination tests were performed in three replicates with five seed groups. Each sample consisted of 100 seeds. The seeds were first disinfected in 10% sodium hypochlorite for 30 seconds and then washed with sterile autoclaved water three times before being immersed in macrobubble water. One flask was filled with autoclaved water as the control, and the others were filled with different concentrations (6, 7, 8, and 9 mg·$L^{-1}$) of DO under macrobubble conditions.

*2.1.3. Study of Seed Germination after Chemical Treatment.* Eight parallel groups were prepared for each seed type to evaluate the effects of seven chemical treatments on daily germination, which were estimated by the percentage of germinated seed number to the total number of macrobubble conditions with optimum DO. The chemical scarification method for breaking seed dormancy was based on soaking seeds with seven different chemical agents: 2% (v/v) sulphuric acid ($H_2SO_4$) for 10 min, 2% (w/v) potassium chlorite (KCl) for 10 min, 10% polyethylene glycol (PEG6000) for 24 h, 25 mM sodium chloride (NaCl) for 24 h, 150 mM potassium nitrate ($KNO_3$) for 30 min, conc. sodium hypochlorite ($NaHClO_3$) for 30 min, and 200 ppm gibberellic acid ($GA_3$) for 30 min. Among all groups, the control group was treated without any chemicals. Each group had three replicates and each with 100 seeds. They were germinated in macrobubble water using a randomised laboratory design. After germination, the seedlings were separated and submerged in 10 mL of autoclaved water in a nonsterilised Petri dish. The dishes were kept under natural light conditions at 25°C. During the test, every 24 h, the macrobubble water and autoclaved water were replaced.

*2.1.4. Observations.* Seed germination was recorded daily, and seven germination parameters were evaluated. The formulas of calculating the parameters for (1a) (germination percentage: GP) and (1b) (germination energy: GE) followed Kumar et al. [23], (1c) (germination index: GI) and (2) (germination rate index: GRI) followed Al-Mudaris [27], (3) (mean germination time: MnGT) followed Ellis [28], mean germination time is a measure of the rate and time-spread of germination, it is an index of germination speed [29] and the final germination time was estimated by the following formula (4) (maximum generation time: MxGT), while seedling secondary roots (SSR) and cotyledons (SR) were calculated from the mean of the number of seedlings that generate secondary roots and cotyledon, respectively [7].

$$\mathbf{GP} = \frac{\textbf{Number of germinated seeds}}{\textbf{Number of seeds from all replications}} \times \mathbf{100}, \tag{1a}$$

$$\mathbf{GE} = \frac{\textbf{(1/4)of maximum number of da ily germinated seed}}{\textbf{Total number of seeds from all replications}} \times \mathbf{100}, \tag{1b}$$

$$\mathbf{GI} = (\mathbf{16} \times \mathbf{n1}) + (\mathbf{15} \times \mathbf{n2}) + \ldots. + (\mathbf{1} \times \mathbf{n16}), \tag{1c}$$

where $n1$, $n2$,. . .$n16$ = No. of germinated seeds on the 1st, 2nd and following days to the 16th day, 16, 15,. . ., and 1 = weights of the number of germinated seeds on the 1st, 2nd and following days:

$$\mathbf{GRI} = \frac{\mathbf{G1}}{\mathbf{1}} + \frac{\mathbf{G2}}{\mathbf{2}} + \ldots. + \frac{\mathbf{Gi}}{\mathbf{I}}, \tag{2}$$

where $G1$ = the germination percentage on 1st day, $G2$ = the germination percentage at 2nd day, and $Gi$ = the germination percentage at $i$ day.

$$\mathbf{MnGT} = \sum \frac{(\mathbf{n} \times \mathbf{d})}{\mathbf{N}}, \tag{3}$$

where $n$ = No. of germinated seeds on each day, $d$ = No. of days from the beginning of the test, and $N$ = total No. of germinated seeds at the termination of the experiment.

$$\mathbf{MxGT} = \frac{\sum (f\mathbf{x})}{\sum (\mathbf{x})}, \tag{4}$$

where $X$ = No. of new germinated seeds on each day and $f$ = No. of day after seeds germinated.

*2.2. Data Analysis.* Analysis of variance (ANOVA) was performed on the results. The comparison was followed Duncan's multiple range test (DMRT) and the differences were reported at $P < 0.05$.

## 3. Results and Discussion

*3.1. Effect of Different DO Concentrations on Germination Percentage and Seed Germination Energy.* Reducing the germination time and increasing the germination percentage of *A. paniculata* seeds are important goals in commercial cultivation. In addition to being essential for plant establishment in both natural and agricultural settings, seed germination is a critical stage in the life cycle of seeds and plants [30]. The seeds quickly recover physiologically from maturation drying during germination, resume a sustained

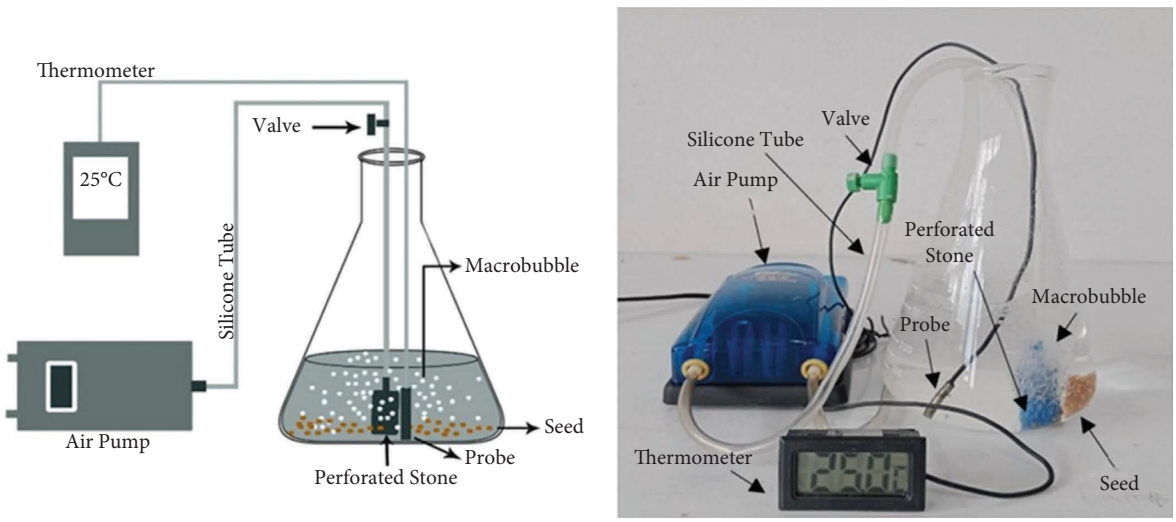


Figure 1: Macrobubble generation was achieved by connecting an air pump to a perforated stone, which was filled with autoclaved water and into which *A. paniculata* seeds were prepared for germination in a volumetric flask.

level of metabolism, finish crucial cellular processes that enable the embryo to emerge, and become ready for following seedling growth [31]. According to a study by Liu et al. [32], nanobubble (NB) water can produce exogenous reactive oxygen species (ROS) offering physiological promotion and oxidation effects of seed. However, stimulating seed germination in aerated water requires consideration of optimal ROS concentration because in high density of bubble was beyond their toxic threshold, and negative effects were shown on hypocotyl elongation and chlorophyll formation. In order to confirm whether water containing macrobubble can enhance physiological processes, a germination test is a suitable procedure. Using distilled water and water that had macrobubble created from each batch of distilled water, comparison experiments were conducted. We proved, to the best of our knowledge, that air macrobubbles encourage seed germination as well.

One hundred seeds were recorded by the maximum generation time within 16 days of seed culture. Following seed germination in under macrobubble aeration with the dissolved oxygen (DO) 6.0, 7.0, 8.0, and 9.0 mg.L$^{-1}$ (Figures 2(a)–2(e)). The control is nonmacrobubble aeration that DO 5.0 mg·L$^{-1}$. After germination at stage i*i* in volumetric flask, seedlings were transferred to Petri dishes for growth to stages ii–iv (Figure 2(f)). Figure 2(e) shows the highest amount of seed germination in dissolved oxygen 9.0 mg·L$^{-1}$ while Figure 2(g) shows that the number of secondary seedling root (SSR) growth was accelerated by DO 9 and 8 mg·L$^{-1}$, making no significant difference followed by DO 7, 6, and the control, respectively. Figure 2(h) presents the number of seedlings with cotyledons (SC) under different kinds of DO concentration for germination. The cotyledon numbers increase with exposure to most of the tested DO 7.0 mg·L$^{-1}$ with no significant difference with DO 8.0. Treatment DO 7.0 mg·L$^{-1}$ SC 5.5 seedlings is the first treatment generation, followed by treatment DO 8.0 mg·L$^{-1}$ SC 5.0, the second seedling, respectively (Table 1). So, this result indicates that increasing of the macrobubble aeration tended to increase the amount of seed germinations and the number of seeds that occur secondary root (SSR) increased correspondingly, while number of seedling cotyledon (SC) formation was not directly proportional to the macrobubble concentration, but the growth of cotyledons depends on the germination period by pregerminated seeds produce cotyledons before later-germinated seeds.

After 16 days of submersion in macrobubble, the highest germination percentage and germination energy of DO 9.0 mg·L$^{-1}$ reached 26.33 and 6.58, respectively, as presented in Table 1, followed by DO 8, 7, 6, and control. Thus, DO 9.0 mg·L$^{-1}$ significantly promoted seed germination compared to other treatments that had a higher germination percentage (GP) and germination energy (GE) than immersion seed in distilled water without aeration or control at 11.3 folds. As supported by the study of Chauhan et al. [7], seed germination can occur in any substrate that allows for enough aeration. More detailed findings on fine bubbles have been reported in previous studies. It has been found that fine bubbles can add air and oxygen to the water needed for respiration to oxidize starch, fat, and other food reserves and induce metabolic activity in seeds [33]. Corresponds to the study of Purwanto et al. [34] that allows ultrafine bubble to be infused into liquids for extended periods of time that the application of water containing ultrafine bubbles has a positive effect on seed germination rate. Ultrafine bubble water treatment can also improve the germination of seeds with poor physiological quality [35]. Soaking seeds in bubbled water is one form of seed priming. Seed preparation by priming is an ancient, simple, and effective technique for improving germination rate and speed, achieving uniform plant stands, and improving yields in a variety of environmental conditions to improve low seed viability and vigor [36].

Four levels of dissolvable oxygen consistently promoted higher seed germination than the control treatment (Figure 3). After 3 days of submersion, seed germination started 3 folds faster than in the control treatment, which started

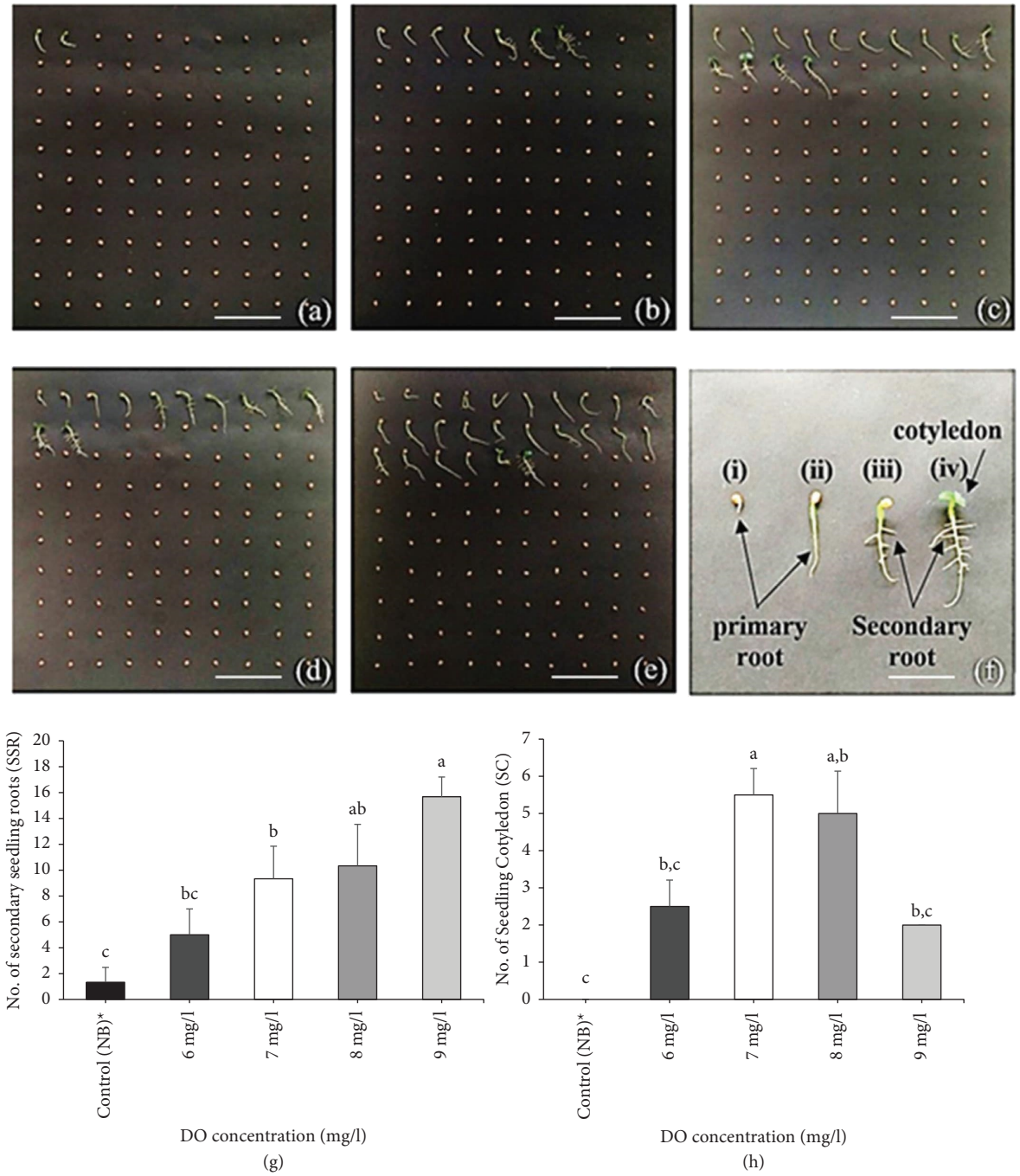


Figure 2: Effect of seed germination on difference dissolved oxygen concentration of macrobubble aeration. One hundred *A. paniculata* seeds were shown for representative of (a)–(e). (a) Nonmacrobubble aeration with dissolved oxygen (DO) 5.0 mg·L$^{-1}$ as control, (b) DO 6.0 mg·L$^{-1}$, (c) DO 7.0 mg·L$^{-1}$, (d) DO 8.0 mg·L$^{-1}$, and (e) DO 9.0 mg·L$^{-1}$. (f) The stage of seed germination for i, ii, iii, and iv that show primary roots, secondary roots, and cotyledon. (g) The number of seedlings that occur secondary roots after germination. (h) The number of seedlings that occur cotyledon (bar = 1 cm).

germination after 9 days of submersion. After a week of submersion, germination percentage (GP) and germination energy (GE) (Figures 3(a) and 3(b)) were stable, and regeneration began to decline at DO 6, 7, and 8 mg·L$^{-1}$, but at 9 mg·L$^{-1}$, it was still increasing. According to the study of Vashisth and Nagarajan [37], the higher molecular mobility of the bulk and hydration water fractions, and the increased activity of germination-related enzymes (amylase, dehydrogenase, and protease), including the early hydration of the membrane, may all contribute to the earlier germination. Corresponding to the study in barley seeds, fine bubbles can promote seed bioactivity and activate germination-related enzymes, and high dissolved oxygen is important to increase the germination rate [38, 39]. Moreover, fine bubbles significantly improved the germination rate of Chinese celery and sweet corn seeds compared

Table 1: Comparison parameter of means and variation for germination of dissolved oxygen concentration treatment.

| Dissolved oxygen (DO) | Germination percentage (GP ± SD) | Germination energy (GE ± SD) | No. of secondary seedling roots (SSR ± SD) | No. of seedling cotyledon (SC ± SD) |
|---|---|---|---|---|
| Control (NB)* | $2.33^{d} \pm 0.58$ | $0.58^{d} \pm 0.14$ | $1.33^{c} \pm 1.15$ | $0.00^{c} \pm 0.00$ |
| 6 mg·L$^{-1}$ | $6.67^{c} \pm 0.58$ | $1.67^{c} \pm 0.14$ | $5.00^{bc} \pm 2.00$ | $2.50^{bc} \pm 0.71$ |
| 7 mg·L$^{-1}$ | $12.00^{b} \pm 2.00$ | $3.00^{b} \pm 0.50$ | $9.33^{b} \pm 2.52$ | $5.50^{a} \pm 0.71$ |
| 8 mg·L$^{-1}$ | $14.33^{b} \pm 2.08$ | $3.58^{b} \pm 0.52$ | $10.33^{ab} \pm 3.21$ | $5.00^{ab} \pm 1.41$ |
| 9 mg·L$^{-1}$ | $26.33^{a} \pm 1.53$ | $6.58^{a} \pm 0.38$ | $15.67^{a} \pm 1.53$ | $2.00^{bc} \pm 0.00$ |

*Control was conditional on nonmacrobubble aeration with dissolved oxygen (5.0 mg·L$^{-1}$).

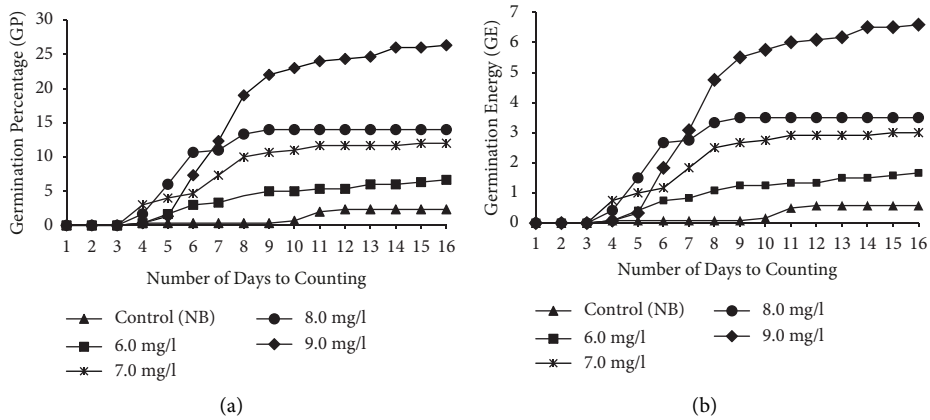


Figure 3: Effect of the dissolved oxygen on germination percentage (a) and germination energy (b).

with conventional distilled water. In addition, fine bubbles also extended the root length of sweet corn seedlings [40]. However, although in this experiment, the germination percentage of *A. paniculata* was highest at a dissolved oxygen concentration of 9 mg·L$^{-1}$, further studies are needed at higher DO concentrations to determine the optimum germination percentage.

*3.2. Effect of Chemical Scarification for Seed Germination in Macrobubble Aeration.* One hundred *A. paniculata* seeds were soaked in solution to investigate the effects of chemical treatments, namely, $GA_3$, $NaHClO_3$, $H_2SO_4$, $KNO_3$, KCl, PEG, and NaCl. Seeds respond differently to chemical treatments. The highest seed germination was observed with $NaHClO_3$, followed by $H_2SO_4$ and control, respectively, as shown in Figures 4(a), 4(c), and 4(d). But the number of seed germinations was reduced less than control by $KNO_3$, KCL, $GA_3$, PEG, and NaCl, respectively, as shown in Figures 4(b), 4(e)–4(h).

We can verify this by the highest generation percentage of $NaHClO_3$ at 92%, generation energy at 23, germination energy in a week at 57.33%, the germination index at 897, and the germination rate index at 15.3, a significant difference from other treatments. Treatment with $NaHClO_3$ shows the mean germination time at 7.25 days; it isn't significantly longer than the control at 6.00 days or PEG at 5.72 days. (Table 2). The results of this experiment were consistent with those reported by Kumari [18]: $NaHClO_3$-treated seeds took on a green color, which had not been observed under laboratory conditions, and full germination was recorded in only 8 days in the nursery test. Thus, it confirms the role as a stimulatory agent.

The high emergence and uniform seedling size required for agricultural production of *A. paniculata* are not always achieved due to vigor differences among commercial seed lots. However, low vigor and a high mean germination time were caused by seed ageing, as indicated by mean germination time [41]. The mean germination time is a suitable index for the vigor evaluation of seeds [42]. In order of seed strength and seed age, it was found that NaCl had the highest seed strength and the lowest seed age compared to $NaHClO_3$. By the way, the seed sample from treatment $NaHClO_3$ was vigour and seed ageing show same level with control and PEG because mean germination time is not significant. Anyway, mean germination time from other treatments wasn't significantly different from control except for NaCl but NaCl wasn't significantly different from other treatments except for control and $NaHClO_3$. So, it meant that the selected seeds were similar in age and vigor in all treatments.

For growth, development can be observed based on the number of seedlings that develop from secondary roots and cotyledons. The secondary roots of the $NaHClO_3$ treatment showed the highest number, followed by $H_2SO_4$ and the control, with no significant difference. The cotyledons of the $NaHClO_3$ treatment showed the highest number and were no significantly different except PEG and NaCl (Table 2). According to the findings of Yadav et al. [43], scarification increases germination rate, greater hypocotyl elongation, and radicle growth, and produced in a substantial reduction in the time for germination.

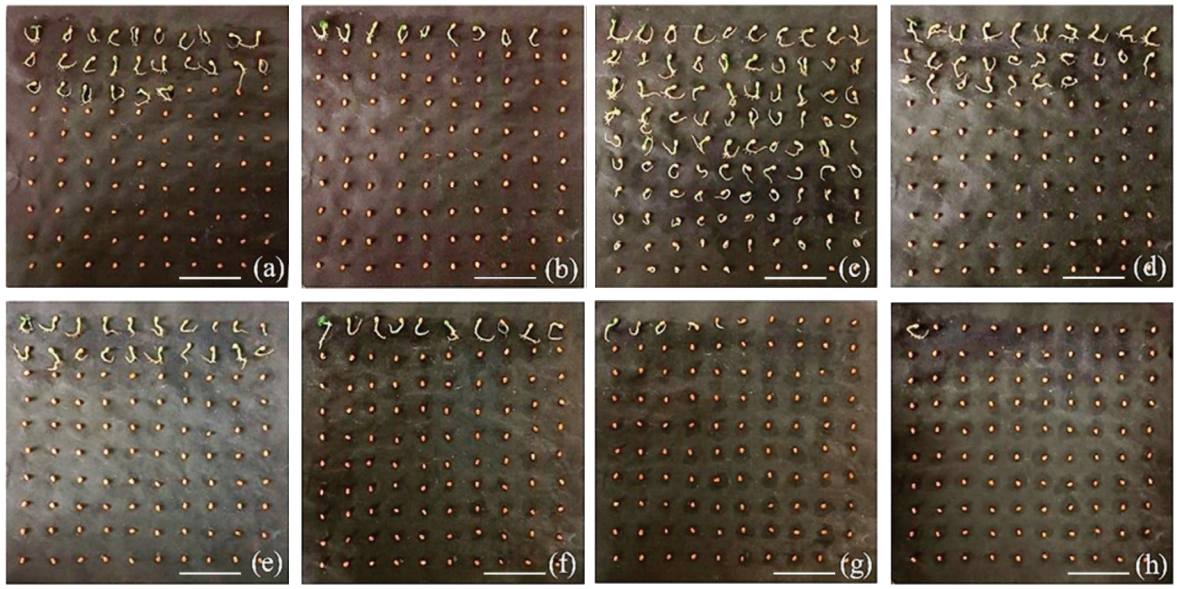


Figure 4: Number of seed germination on different chemical treats in microbubble aeration with optimum dissolved oxygen. One hundred *A. paniculata* seeds were shown for representative. (a) Nonchemical treat as control, (b) chemical treat with 200 ppm gibberellic acid ($GA_3$) for 30 min, (c) sodium hypochlorite ($NaHClO_3$) for 30 min conc., (d) 2% (v/v) sulfuric acid ($H_2SO_4$) for 10 min, (e) 150 mM potassium nitrate ($KNO_3$) for 30 min, (f) 2% (w/v) potassium chlorite (KCl) for 10 min, (g) 10% polyethylene glycol (PEG) for 24 h, and (h) 25 mM sodium chloride (NaCl) for 24 h (bar = 1 cm).

Table 2: Comparison parameter of means and variation for germination of different chemical scarification treatment.

| Chemical Treat* | Generation percentage in a week (GPW ± SD) | Generation percentage (GP ± SD) | Generation energy (GE ± SD) | Mean germination time (MGT ± SD) | Germination index (GI ± SD) | Germination rate index (GRI ± SD) | No. of seedling secondary roots (SSR ± SD) | No. of seedling cotyledon (SC ± SD) |
|---|---|---|---|---|---|---|---|---|
| Control (NC) | $22.67^{b} \pm 4.73$ | $25.33^{b} \pm 4.04$ | $6.33^{b} \pm 1.01$ | $6.00^{ab} \pm 0.93$ | $277.67^{b} \pm 43.14$ | $4.41^{bc} \pm 0.69$ | $23.33^{ab} \pm 2.52$ | $1.00^{ab} \pm 0.00$ |
| $GA_3$ | $8.67^{cde} \pm 2.08$ | $9.00^{d}e \pm 2.00$ | $2.25^{de} \pm 0.50$ | $4.46^{bc} \pm 0.39$ | $112.67^{cd} \pm 24.71$ | $2.12^{de} \pm 0.45$ | $8.33^{c} \pm 1.53$ | $1.00^{ab} \pm 0.00$ |
| $NaHClO_3$ | $57.33^{a} \pm 7.51$ | $92.00^{a} \pm 3.00$ | $23.00^{a} \pm 0.75$ | $7.25^{a} \pm 0.48$ | $897.00^{a} \pm 63.15$ | $15.30^{a} \pm 1.53$ | $34.33^{a} \pm 12.70$ | $1.50^{a} \pm 0.71$ |
| $H_2SO_4$ | $22.67^{b} \pm 2.89$ | $26.33^{b} \pm 2.08$ | $6.58^{b} \pm 0.52$ | $5.39^{bc} \pm 0.52$ | $305.33^{b} \pm 22.72$ | $5.45^{b} \pm 0.47$ | $23.33^{ab} \pm 2.52$ | $0.50^{ab} \pm 0.71$ |
| $KNO_3$ | $17.00^{bc} \pm 2.00$ | $19.67^{c} \pm 0.58$ | $4.92^{c} \pm 0.14$ | $5.35^{bc} \pm 0.89$ | $229.33^{b} \pm 22.59$ | $4.18^{bc} \pm 0.69$ | $13.33^{bc} \pm 1.53$ | $1.00^{ab} \pm 0.00$ |
| KCl | $11.67^{cd} \pm 2.52$ | $12.00^{d} \pm 2.00$ | $3.00^{d} \pm 0.50$ | $4.89^{bc} \pm 0.53$ | $146.00^{c} \pm 30.61$ | $2.72^{cd} \pm 0.71$ | $9.67^{c} \pm 3.06$ | $0.50^{ab} \pm 0.71$ |
| PEG | $4.00^{de} \pm 1.00$ | $4.33^{ef} \pm 1.53$ | $1.08^{ef} \pm 0.38$ | $5.72^{abc} \pm 0.86$ | $48.00^{de} \pm 13.12$ | $0.78^{e} \pm 0.17$ | $3.33^{c} \pm 0.58$ | $0.00^{b} \pm 0.00$ |
| NaCl | $1.33^{f} \pm 0.58$ | $1.33^{f} \pm 0.57$ | $0.33^{f} \pm 0.14$ | $4.17^{c} \pm 0.29$ | $17.00^{e} \pm 6.93$ | $0.32^{e} \pm 0.12$ | $1.33^{c} \pm 0.58$ | $0.00^{b} \pm 0.00$ |

*Control (NC) is a nonchemical treatment: 200 ppm gibberellic acid ($GA_3$) for 30 min, sodium hypochlorite ($NaHClO_3$) for 30 min, 2% (v/v) sulfuric acid ($H_2SO_4$) for 10 min, 150 mM potassium nitrate ($KNO_3$) for 30 min, 2% (w/v) potassium chlorite (KCl) for 10 min, and 10% polyethylene glycol (PEG) for 24 h and 25 mM sodium chloride (NaCl) for 24 h.

The best indicator of the depth of dormancy is thought to be the germination index [44]. Moreover, high germination index values indicate great seed quality and homogeneity [45]. The $NaHClO_3$ treatment showed the highest germination index and germination rate index, meaning that the seeds had a shorter dormancy and better quality than other treatments. The germination index and germination rate index of sulfuric acid were comparable to those of the control but other treatments show lower than control (Figures 5(e) and 5(f)). So, treatment with $H_2SO_4$ had no effect on seed dormancy destruction or seed quality improvement. On the other hand, other treatments tend to increase seed dormancy and decrease seed quality.

Most seeds remain dormancy related to the hard seed coat. It results in impermeable to water and gases. Dormancy in *A. paniculata* is influenced by both physical properties and physiological mechanisms (unknown protein). Chemical treatments in *A. paniculata* seeds can reduce seed coat layer or dissolve some inhibitor proteins that might be beneficial in breaking seed dormancy and improving germination [9]. Thus, this experiment concluded that $NaHClO_3$ was able to destroy proteins on the hard seed coat, allowing water and oxygen to penetrate into the seed, thereby stimulating seed germination in macrobubble aeration systems that are full of enough dissolved oxygen and water for seed germination. According to the research of Coelho et al. [46], physical

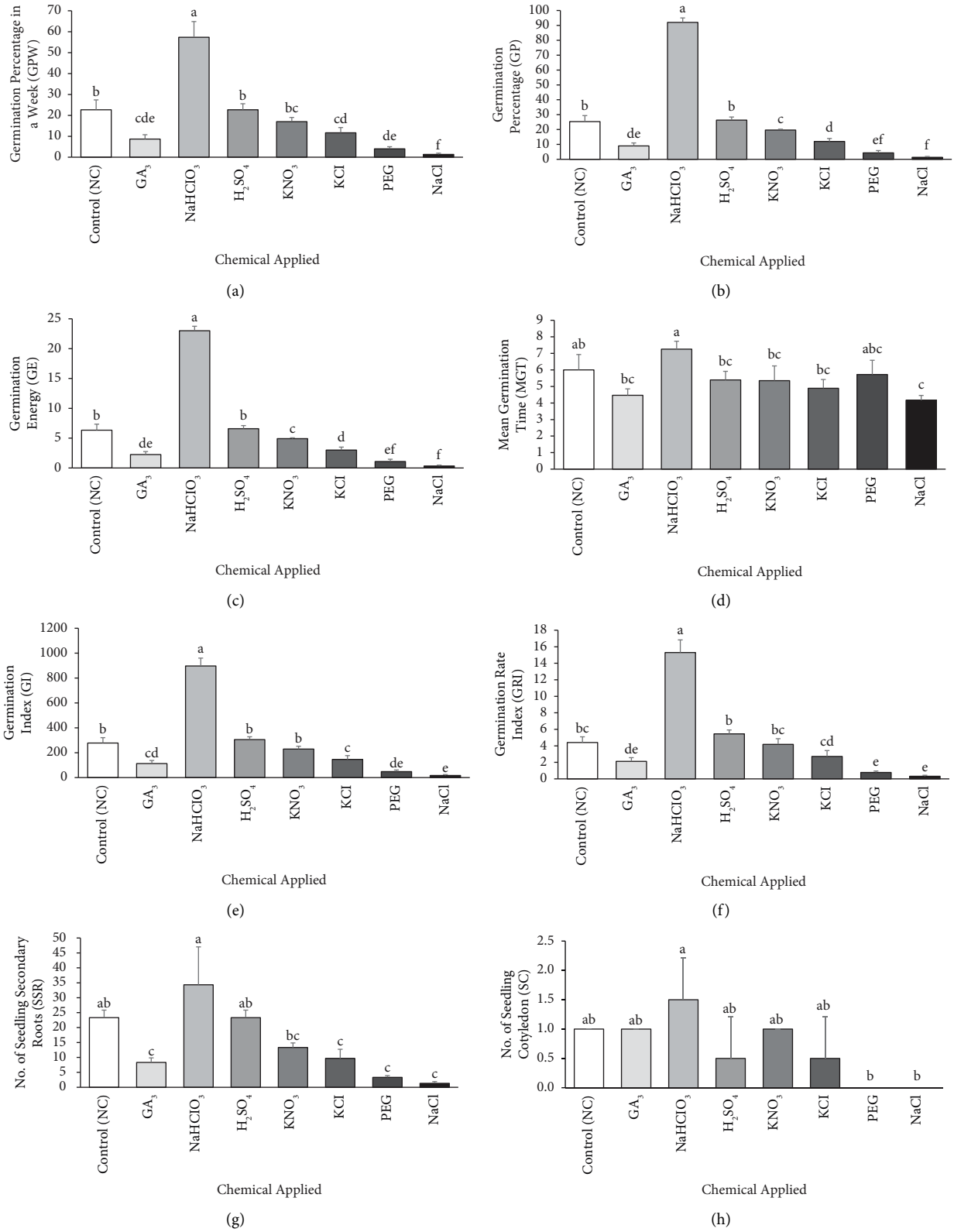


Figure 5: Effect of seed germination parameter on different chemical scarification treatment in macrobubble condition that control (NC) is nonchemical treat, 200 ppm gibberellic acid ($GA_3$) for 30 min, sodium hypochlorite ($NaHClO_3$) for 30 min conc., 2% (v/v) sulfuric acid ($H_2SO_4$) for 10 min, 150 mM potassium nitrate ($KNO_3$) for 30 min, 2% (w/v) potassium chlorite (KCl) for 10 min, 10% polyethylene glycol (PEG) for 24 h, and 25 mM sodium chloride (NaCl) for 24 h. (a) Germination percentage in a week (GPW), (b) germination percentage (GP), (c) germination energy (GE), (d) mean germination time (MGT), (e) germination index (GI), (f) germination rate index (GRI), (g) no. of seeding secondary roots(SSR), and (h) no. of seeding cotyledon (SC).

scarification can speed up the germination index of seeds. It is reported that using sodium hypochlorite as a disinfectant slowed the rate of decomposition of tamarind seeds during germination [47].

## 4. Conclusions

The destruction of *A. paniculata* seed dormancy by chemical scarification of the seed coat by immersing the seeds in a combination of chemicals and physical methods in swirling macrobubble water is a more effective method than using only macrobubble soaking. This is effective in stopping dormancy, stimulating seed germination, and also improving the quality of the seeds. This method also affects the physiological development of secondary root formation. The conduct of this study may serve as useful information in the production and improvement of germination because it allowed us to better understand the seed germination process of this plant.

## Data Availability

All data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

AP managed the research fund, conceptualized the experiment, conducted the work, analysed the data, and prepared manuscript. SS reviewed the manuscript and checked for writing. TK checked statistical analysis, reviewed the manuscript, and cooperated with the researchers.

## Acknowledgments

We would like to thank School of Agricultural Technology and Food Industry, Walailak University, for providing research area and facility. The research was supported by the Hydroponics Project, School of Agricultural Technology and Food Industry, Walailak University.

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