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Effects of nano-bubbles on seed germination of muskmelon

Quy Tung Le*, Chiti Sritontip

Rajamangala University of Technology Lanna, Thailand

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Abstract:

Nano-bubbles (NBs) demonstrate their full potential in fields like agriculture, aquaculture, food processing, and wastewater treating. This study investigated the effects of air NBs on the progress of seed germination of muskmelon. Distilled water was treated with NBs under time schedules of 10, 15, and 20 min and untreated distilled water was used as the control. Statistically significant differences in seed germination rate occurred between treatments. Growth indicators such as stem height, root length, and leaf area were measured after 14 d after treatment with water containing NBs. Statistical analysis showed that there were significant differences in the dry weight of samples between treatments although these differences were quite small.

Keywords: **muskmelon, nano-bubbles, seed germination.**

Classification number: **3.1**

Introduction

Applications of nano-bubbles (NBs) have been investigated across fields like agriculture, industry, and medicine. The unique properties recently discovered of these micro- and nano-scale bubbles in solutions present many intriguing characteristics such as long residence time, high efficiency of gas mass transfer, or the ability to increase the internal pressure and contact area between a liquid and a gas [1-3]. These properties lead to efficient chemical reactions and the dissolution and transfer of oxygen, nitrogen, ozone, or carbon dioxide in liquid environments, all of which are necessary for agriculture, aquaculture, food production, and environmental protection [4-6].

In agriculture, characteristics of NBs have been shown to outperform previous methods. One application that benefits most from studying NBs is seed germination [7-9]. Both in soil and soilless cultures, the germination stage is the first and most fundamental stage, which plays an important role in the growth, development, and vitality of plants. Many techniques applied to seed treatment research aims to improve the efficiency of a seed's emergence ability and also the quality of crops initiated from a seedling. Methods such as using bio-agents, chemical treatments, and heat treatments have been applied. Each technique includes advantages and disadvantages in particular aspects. While the NB technique was developed as a physical treatment, it can also affect the chemical characteristics of objects. Therefore, NBs have a rich potential to promote the sustainability of agriculture and the environment.

This experiment was conducted to evaluate the efficiency of NBs technique in seed treatments, and, in particular, the germination rate of hybrid muskmelon compared with traditional methods to identify the most effective formula. The disadvantages of the technique are also discussed.

Materials and methods

The seeds of the "Madam orange" hybrid muskmelon (Known-You Seed Co., LTD) were selected to test the germination rate under different nano-bubble treatments. There are four treatments with four replications of 20 seeds each. The seeds were placed on absorbent paper in Petri dishes and filled with different formulae of water. The four treatments are shown in Table 1.

*Corresponding author: Email: lequytung.mofa@gmail.com

Table 1. List of treatments used in this study.

Treatment 1	Control, distilled water.
Treatment 2	Distilled water was generated by nano-bubbles in 10 minutes.
Treatment 3	Distilled water was generated by nano-bubbles in 15 minutes.
Treatment 4	Distilled water was generated by nano-bubbles in 20 minutes.

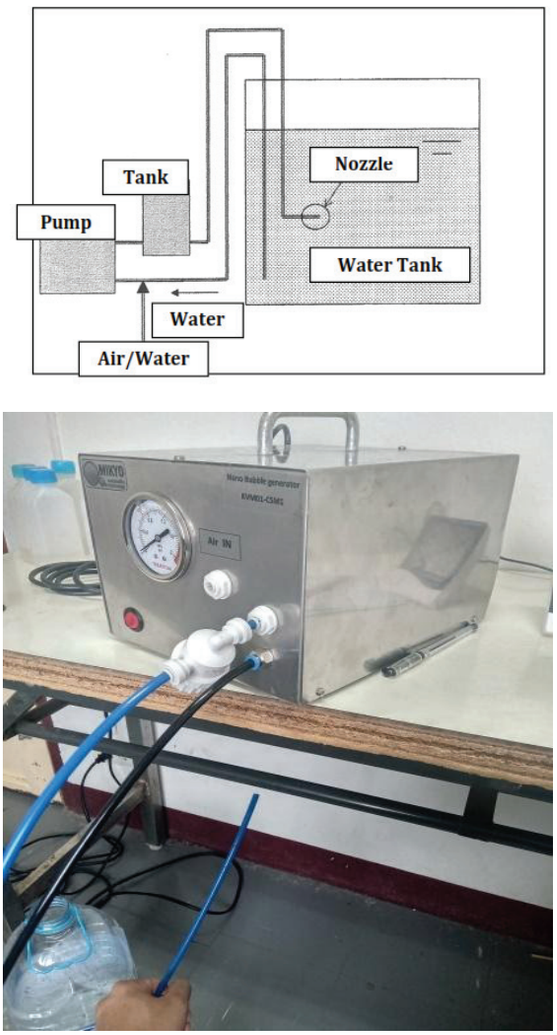

Fig. 1. Schematic diagram and image of the nano-bubble generator. The blue pipe is an inlet pipe in which water from the water tank and air from outside are absorbed. The black pipe is an outlet pipe, in which water carrying nano-bubble particles are released.

The water in Treatments 2, 3, and 4 was treated by a nano-bubble generator (modified and manufactured by Faculty of Engineering, Rajamangala University of Technology Lanna, Chiang Mai, Thailand) following the time as indicated in the formula of each treatment (Fig. 1).

Every 24 h, the water and absorbent papers were changed. After 7 d, the seeds were collected to observe the germination rate while the growth indicators were measured after 14 d (Fig. 2). The treatments were conducted following CRD (completely randomized design). Statistical analysis of variance was computed followed by an LSD multiple range test at $p \leq 0.05$.

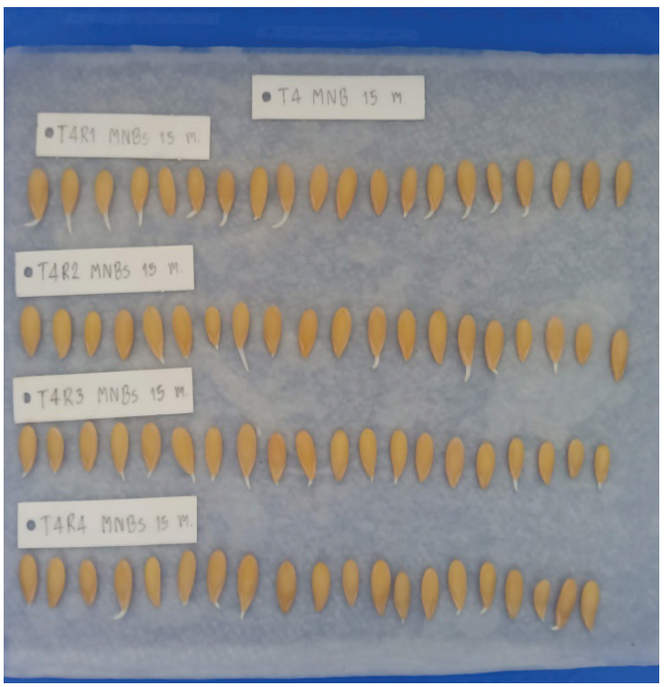

Fig. 2. Seeds treated with NBs.

Results and discussion

Before and after treatment with NBs-generated water, factors such as electrical conductivity (EC), water temperature, dissolved oxygen (DO), and pH did not significantly change. In general, EC, water temperature, and DO tended to increase, however, the differences were not significant (Fig. 3).

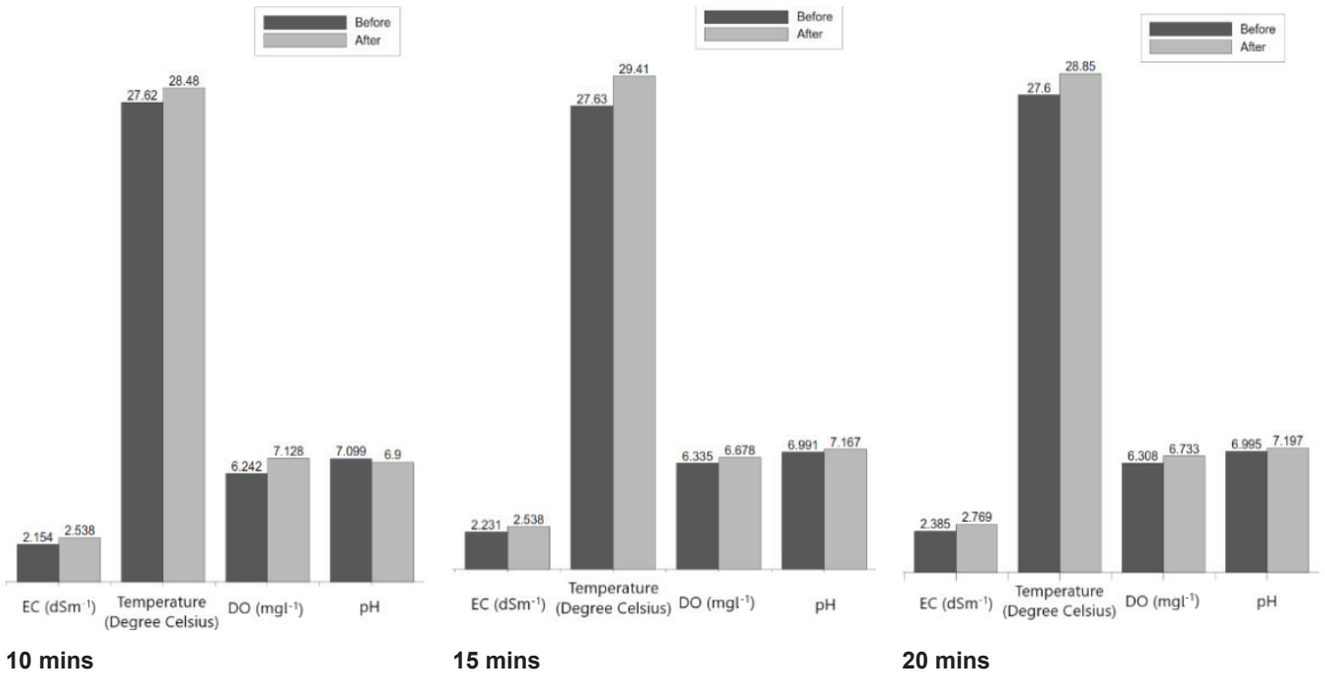

Fig. 3. Water factors before and after treatment with water generated with NBs for different generation times.

Dissolved oxygen plays an important role in agricultural applications such as in hydroponics and aquaculture. In crop science, seed germination is strongly influenced by oxygen concentration in soil and substrate. For seedling emergence, almost all seeds require a high and stable concentration of oxygen for respiration, except for some special cases in which seeds germinate anaerobically. Muhamad, et al. [10] indicated that various types of vegetable seed were sensitive to decreasing oxygen concentrations, which leads to reduced speed and percentage of germination. Moreover, the existence of nano-bubbles has proven that the residual time in solutions could last up to 2 weeks [11] and it can be considered as an advanced benefit in seedling treatment.

Figure 4 shows that the four treatments with water generated by NBs promoted the germination rate of muskmelon. After 7 d of submersion, the muskmelon germination rate reached more than 90% ($p \leq 0.05$) for all treatments. As a whole, the dormancy rates among the treatments were not significantly different, which was approximately 10% for all of treatments. The progress of the germination of the muskmelon seed is listed in the following stages: dormancy > emerge > root appearance > cotyledon appearance > stem appearance.

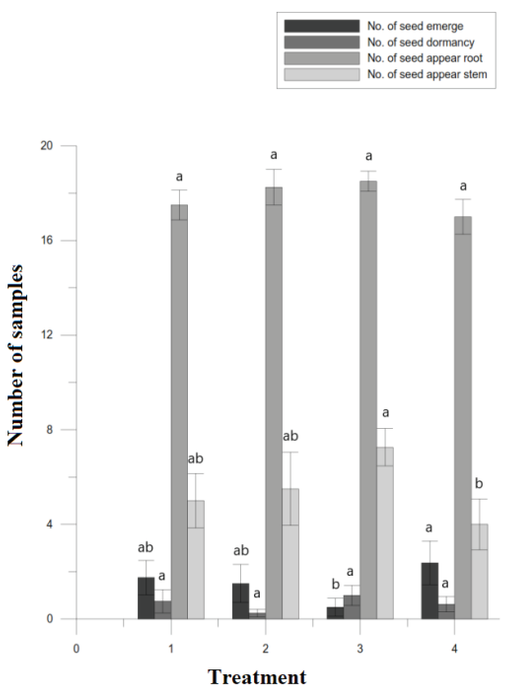

Fig. 4. Characteristics of samples observed after 7 d. A cluster bar chart shows the total number of seeds on the 7th day that existed in each of the four stages of germination. For example, all seeds that appeared to already possess a stem-initiated root were recorded in the column for "No. of seed appear root" and so on. The mean ± standard deviation is shown along with letters that label a significant difference according to the LSD multiple range test at $p \leq 0.05$.

The final state, stem appearance, obtained the highest observation rate with Treatment 3, i.e., 15 min water generation with NBs. The same result was observed in the emerge rate for all treatments. Conversely, with the number of observed stems, the emerge rate for Treatment 3 was the lowest and the highest belonged to Treatment 4. There was no significant difference in the observed emerge rate between Treatments 1 and 2. The emerge rate of the seeds depend on the quality of the seeds and the germination treatment. Also, if the state of emergence takes too long, the seeds will not have good development.

Fig. 5. Observed samples after 14 d.

Figures 5, 6 presents the growth indicators of muskmelon after 14 d under different treats of NBs. In comparison to the distilled water treatment, most growth indicators from NBs treatments show that there were significant differences ($p \leq 0.05$). The most significant difference belongs to Treatment 3 in terms of stem height, root length, and cotyledon area. In these cases, all the growth indicators showed significantly higher values. However, the stem diameter measurements did not show any significant differences between treatments. In general, treatment with NBs gave indicators that were equal or higher than the treatment with distilled water.

In term of cotyledon area, previous studies indicated that cotyledons could be used as a bioassay to determine the response of plant growth regulators (PGRs) such as kinetin, GA_3, and IAA under both light and dark germination conditions [12]. When homogenous seed material was used in experiment without the supplement of any type of PGRs, the specificity of the responses of plant hormones only provide the efficiency of internal PGRs among different treatments. Fig. 6 shows the highest cotyledon area belongs to Treatment 3. By linking this with other results from this experiment, the highest development indices appear in samples with Treatment 3 as well. This confirms the hypothesis that NBs not only impact physical factors, but also affect the biochemical activities of plants.

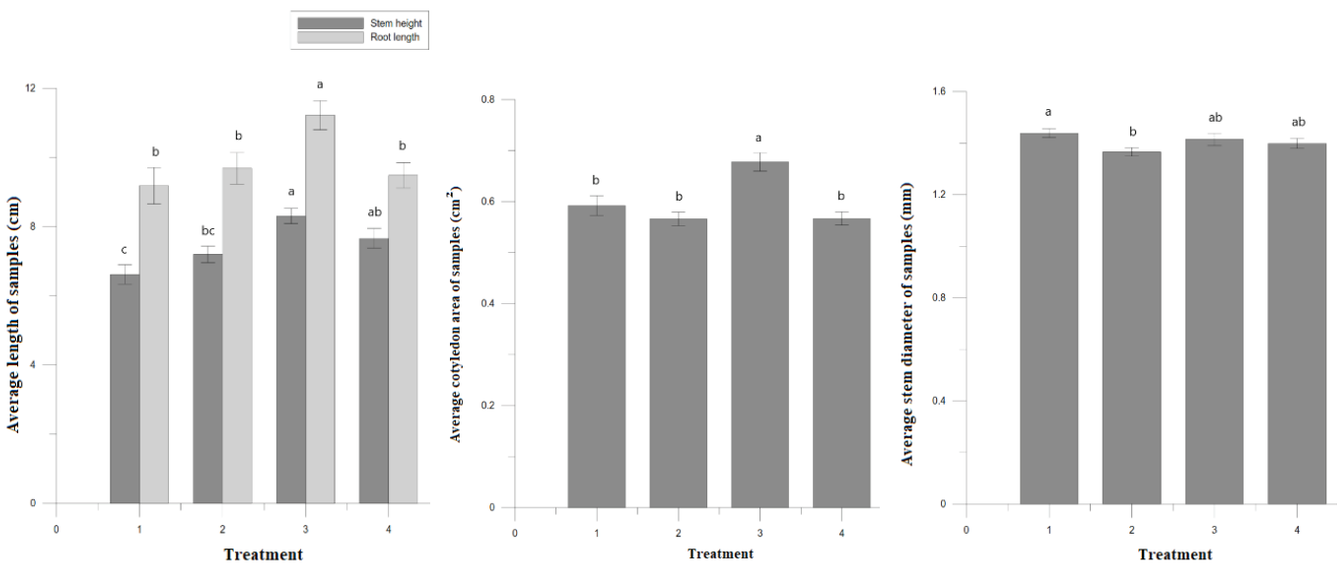

Fig. 6. Growth indicators of samples after 14 d. The mean ± standard deviation within treatments are followed by different letters that label a significant difference according to the LSD multiple range test at $p \leq 0.05$.

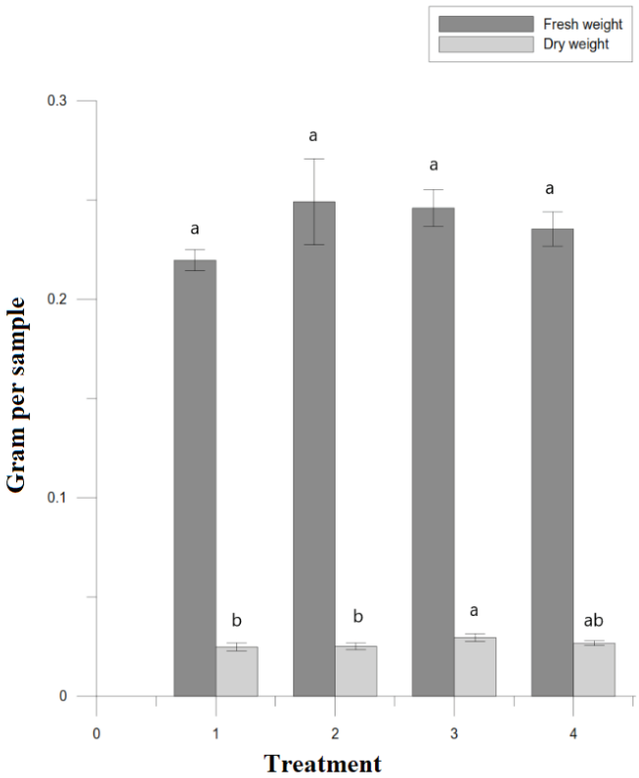

Fig. 7. Average weight of samples after 14 d. The mean ± standard deviation within treatments are followed by different letters that label a significant difference according to the LSD multiple range test at p≤0.05.

After 14 d, the weights of the samples were measured and the results are shown in Fig. 7. Even though there were significant differences in the growth indicators among treatments, the weights of the samples between treatments were quite similar. Statistical analysis results showed that there were differences in the dry weight of after each treatment where the largest difference was seen in Treatment 3, which was much higher than Treatments 1 and 2. However, the average amounts from real measurements were quite similar as significant differences are difficult to recognize without statistical analysis.

Conclusions

The influences of NBs on the seed germination of lettuce, carrot, fava bean, and tomato were investigated in other studies. Seeds in water containing NBs were found to exhibit 6-25% higher germination rates. Those treated with nitrogen NBs exhibited considerable effects in seed germination, whereas air and carbon dioxide NBs did not significantly promote germination. The growth of stem length and diameter, leaf number, and leaf width were promoted by NBs (except air) [13]. However, NBs were shown to enhance the growth of plants by improving the oxygen supply as nutrient elements [14]. Moreover, according to a previous study [15], rice growth did not differ between those irrigated with NBs water (water saturated by oxygen NBs) and those irrigated with control water (without NBs). Experiments have shown that there were differences in DO concentration of water before and after treatment of distilled water with NBs. However, DO is not the sole factor deciding the success of germination. Results from the research of germination rate of barley seeds showed that seeds submerged in water treated with nitrogen NBs and air NBs had higher germination rates (15-25%) in comparison to seeds treated in distilled water under the same concentration of DO [9]. In this case, an explanation of NBs carrying oxygen in the solution can provide the most convincing answer [11]. Even though the NBs in this experiment were ANBs (air nano-bubbles), treatments using ANBs showed significant differences in some growth indicators and development stages in the germination of muskmelon seeds. This suggests that the effects of NBs on the physiological progress of plants needs more investigation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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