Effect of ultrafine bubbles water on seed germination

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Abstract. Ultrafine Bubbles (UFBs) technology is a new and emerging science, capable of infusing UFB in liquids for long periods of time. UFBs is extremely small gas bubbles in liquid with diameter less than 10⁻⁶ m. This paper discussed the application of ultrafine bubbles water for seed germination. Seed germination is the process from seed to a sprout in which the metabolic process of a plant gets activated after a period of dormancy. A high seed germination percentage is important for vegetable production. Comparison experiments of seed germination rate were performed using distilled water and water containing ultrafine bubbles. This experiment utilized the dissolved oxygen concentrations of distilled water with approximately 10 and 20 ppm. The result indicates that the application of ultrafine bubbles water had a positive effect on the germination rate of seeds.

1. Introduction

Nowadays, application of ultrafine bubbles (UFBs) technology in agriculture, due to its tiny bubbles of diameter less than 100 µm including nano sizes, have been a subject of intensive research. UFBs have attracted considerable attention from various fields in recent years. It has been reported that UFBs can increase the physiological activity of living organisms. The characteristics of UFBs include the increased solubility of gases in liquids, reduced friction, zeta potentials and the generation of free radicals [1,2], leading to numerous promising applications. Fine bubbles (FBs) refer to all bubbles with a diameter d below 100 µm [3] and are subdivided into those above 1 µm (microbubbles) and the UFBs below 1 µm.

There have been many reports on the effects and applications of UFBs, such as purification of wastewater, water quality improvement, sterilization, cleaning of contaminated water and promotion of the physiological activities of living organisms. Shrinkage of microbubbles in water can be observed through an optical microscope. However, it is not possible to visually confirm whether the bubbles finally disappear by dissolving in water or they remain in the water as nanobubbles whose diameters are smaller than the spatial resolution of the microscope. Therefore, whether nanobubbles can remain for extended periods of time has still been a point to be discussed [4].

In recent years, the research on UFBs has been expanding rapidly in various fields due to their unique properties. One of the most astonishing effects of UFBs water is its promotion effect on the physiological activity of living organisms [5]. UFBs water and H₂O₂ solution both stimulated seed germination speed and had a similar pattern on the effect of gene expression profiles on barley seed sprouts [6]. The objective of this study was to demonstrate the application of ultrafine bubbles water for seed germination.
2. Methodology

2.1. Sample preparation
Soybean seeds cv. Argomulyo (6 months after being harvested) and cv. Grobogan (18 months after being harvested) were used as a sample. UFBs water was generated from distilled water using UFB generator ((FZ1N-10, IDEC Japan). Dissolved oxygen (DO) concentration was measured in both distilled water and distilled water containing UFBs at 20°C using a Winkler titration \[7\]. Distilled water and UFBs water have dissolved oxygen (DO) level of approximately 8 and 10 ppm. For those UFBs water injected with pure oxygen gas, the DO level was 20 ppm.

2.2. Germination tests
After being dipped in distilled water and distilled water containing and UFBs, seeds were put on moist filter paper in a plastic box (10 x 20 cm) and placed in the seed germinator with temperatures set at 30°C and dark condition. The number of soybean seeds sample was 100 for each replication. Dipping period was set for 1 and 2 h. Seeds were considered to have germinated if they exhibited a root or coleoptile length ≥2 mm. The germinated seeds were counted daily for 5 d. All experiments were carried out in three replications.

3. Results and Discussions
Seed germination is a crucial process in the seed-plant life cycle and is also important for plant establishment in natural and agricultural ecosystems \[8\]. During germination, the seeds rapidly recover physically from maturation drying, resume a sustained intensity of metabolism, complete essential cellular events to allow the embryo to emerge and prepare for subsequent seedling growth \[9\]. Therefore, a germination test is an appropriate method to verify the ability of water containing UFBs water to promote physiological processes. Comparison experiments were performed using distilled water and water containing UFBs produced from each batch of distilled water \[10\]. Figure 1 shows the germination rate of soybean seed dipped in distilled water and distilled water containing UFBs for 1 h and 2 h.

As shown in figure 1, three repetitive germination experiments showed that the germination rates of soybean seeds dipped in distilled water containing UFBs for 1 and 2 h were 10-20 percentage points greater than that of those dipped in distilled water; these results clearly verify the physiological effect of UFBs. The earlier germination might be explained by greater activities of germination-related enzymes, the early hydration of the membrane and greater molecular mobility of the bulk and hydration water fractions \[11\].

The difference reaches up to an average of 20-25% on the first day of germination. The dipped seed in distilled water containing UFBs was faster and more even germination. ROS (Reactive Oxygen
Species) caused by UFBs might play an important role in growth by facilitating the cell walls needed for cell extension [12]. As well as hydroxyl radicals produced in the cell wall during the extension of the radicle and the weakening of the endosperm in seeds [13]. On the other hand, ROS is capable of causing oxidative damage, against macromolecules so that it leads to lipid peroxidation, DNA damage and DNA breaking [14]. A sufficient amount of ROS is needed for practical applications. The appropriate amount of ROS will increase the physiological activity of living organisms. The increase in germination continued until the fifth day for the dipped seeds using UFB water. At day 3 to 5, the percentage of seed germination for those seeds dipped in distilled water containing UFBs reached about 10%.

For those seeds dipped in distilled water containing UFBs with DO level of 20 ppm shows more uniform results. The growth of soybean seed are significant differences among the seed dipped in distilled water and dipped in distilled ware containing UFBs with DO level of 10 and 20 ppm. This is caused by the amount of ROS that has a positive correlation with the amount of oxygen UFBs in the water. Hydroxyl radicals formed by the collapse of microbubbles in dilute solutions and oxygen nanobubbles can also produce ROS in water [15].

Dipping treatments for 1 and 2h resulted in different results. Dipping seeds in both distilled water and distilled water containing UFBs for 2h resulted in higher results than 1 h. Soybean seeds are very susceptible to imbibition damage, which is influenced by a variety of seed characteristics. Soybean seeds with water content lower than 11% [16] or even 13% [17,18] are usually more susceptible to imbibition damage. Whereas the soybean seed cv. Argomulyo contained 8% of moisture content. The imbibition process is the process of absorption of water by seeds to completely hydrated. Seeds that experience dipping for more than 1 h might experience better hydration to reach the water requirements for germination than the lesser ones.

Figure 2 shows the germination rate of soybean seed cv. Grobogan dipped in distilled water and distilled water containing UFBs for 1 h and 2 h. The germination rate in both treatments is less than 60%. Soybean seed cv. Grobogan is a soybean seed that has a long storage period. The longer storage period may result in a lower germination rate. Soybean seed germination decreases faster during storage than other grain crops [19]. Based on data from the Research Institute for Agricultural Biotechnology and Genetic Resources, seeds containing 12% moisture content have a shelf life of two years, the growth rate is 30%, while seeds containing 10% moisture content have 66% growth ability. The water content of the soybean seeds sample was 11%. It is considered that the water content and length of the storage period may relate to the growth ability. This result shows that on the first day the growth of the seed after being dipped in distilled water and distilled water containing UFBs are 0 and 1.3%. The higher germination rate was found for that seed dipped in distilled water containing UFBs 20 ppm at day 3 for 1h dipping time. There is no increase in germination rate after day 3. The similar result was found for those soybean seeds dipped in distilled water and distilled water containing UFBs for 2 h.

![Figure 2](image-url)  
**Figure 2.** The germination rate of soybean seed cv. Grobogan dipped in distilled water and distilled water containing UFBs for 1 h (a) and 2 h (b).
4. Conclusion
This study demonstrated the application of UFBs water for germination tests of seed. The germination rate of sample seeds dipped in the water containing UFBs were obviously higher than that in the distilled water. The use of UFBs water has a positive effect on enhancing germination rate for both different storage period of seeds.

5. References
[1] Serizawa A, Inui T, Yahiro T and Kawara Z 2003 Lamination of micro-bubble containing milky bubbly follows in a pipe 3rd European-Japanese Two-Phase Flow Group Meeting p 21-27
[2] Chu L B, Xing X H, Yu A F, Sun X L and Jurck B 2008 Enhanced treatment of practical textile wastewater by microbubble ozonation Process Safety and Environmental Protection. 86 (5) 389-393
[3] ISO 20480-1 2017 Fine Bubble Technology—General Principles for Usage and Measurement of Fine Bubbles Part 1: Terminology. Available online: https://www.iso.org/obp/ui/#iso:std:iso:20480:-:1:ed:1:v1:en (accessed on 26 May 2019).
[4] Ljunggren S, Eriksson J C 1997 The lifetime of a colloid-sized gas bubble in water and the cause of the hydrophobic attraction Colloids and Surfaces A: Physicochemical and Engineering Aspects. 129-130 151-155
[5] Park J, Kurata K 2009 Application of microbubbles to hydroponics solution promotes lettuce growth Hort Technology. 19 212-215
[6] Liu S, Oshita S, Kawabata S and Thuyet D Q 2017 Nanobubble Water’s Promotion Effect of Barley (Hordeum vulgare L.) Sprouts Supported by RNA-Seq Analysis Langmuir. 33 (43) 12478-12486
[7] Anonim 2019 The Winkler Titration. https://www.flinnsci.com/api/library/.../e3a5604c6d5 649fb6aa900bb28cac2e (accessed on 26 May 2019)
[8] Weitbrecht K, Müller K and Leubner-Metzger G 2011 First off the mark: early seed germination. J. of Experimental Botany. 62 (10) 3289-3309
[9] Nonogaki H, Bassel G W and Bewley J D 2010 Germination-still a mystery Plant Science. 179 574-581
[10] Liu S, Otsuka Y, Kawagoe Y, MakinoY and Oshita S 2012 Effects of the water containing nanobubbles on metabolic activities of barley seeds. Proc. of the 6th International Symposium on Machinery and Mechatronics for Agriculture and Biosystems Engineering (ISMAB) (Jeonju Korea) p 446-451
[11] Vashisth A, Nagarajan S 2010 Characterization of water distribution and activities of enzymes during germination in magnetically-exposed maize (Zea mays L.) seeds Indian J. of Biochemistry and Biophysics 47 311-318
[12] Passardi F, Longet D, Penel C and Dunand C 2004 The class III peroxidase multigenic family in rice and its evolution in land plants Phytochemistry. 65 1879–1893
[13] Müller K, Linkies A, Vreeburg R A M, Fry S C, Liszkay A K and Metzger G L 2009 In Vivo Cell Wall Loosening by Hydroxyl Radicals during Cress Seed Germination and Elongation Growth Plant Physiol. 150 (4) 1855–1865
[14] Tomizama S, Imai H, Tsukada S and Simizu T 2005 The detection and quantification of highly reactive oxygen species using the novel HPF fluorescence probe in a rat model of local cerebral ischemia Neuroscience Research. 52 304-313
[15] Li P, Takahashi M and Chiba K 2009 Enhanced free-radical generation by shrinking microbubbles using a copper catalyst Chemosphere. 77 1157-1160
[16] Simon E W, Raja H R M 1972 Leakage during imbibition J. of Experimental Botany 2 1076-1085
[17] Hobbs P R and Obendorf R I 1972 Interaction of initial seed moisture and imbibitional temperature on germination and productivity of soybean Crop Science. 13 664-667
[18] Obendorf R L and Hobbs P R 1970 Effect of seed moisture on temperature sensitivity during imbibition of soybean Crop Science. 10 563-566
[19] Tekrony D M and Egli D B 1991 Relationship of seed vigor to crop yield *Seed Science and Technology*. 21 127-137

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