Effect of storage temperature and ultrafine bubbles water treatment on the breaking dormancy of garlic bulb

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Abstract. Seed dormancy is a physiological phenomenon in plants, it allows us to overcome periods that are unfavorable for seedling established. Several processes are known to be involved in the induction of dormancy and the switch from dormant to germinating state. Garlic bulb has a dormancy period of more than 3 months. Understanding seed dormancy and breaking dormancy is important in the cultivation process of garlic. The objective of this research was to investigate the effect of storage temperature and ultrafine bubbles (UFBs) water on the breaking dormancy of garlic. Storage temperature was set at 5°C, 15°C and room temperature (29±2°C). Soaking treatments were carried using distilled water, UFBs water with dissolved oxygen 8 ppm and 20 ppm for 24 h and the control was carried without soaking treatment. After being soaked, the sample of garlic was placed at room temperature (29±2°C). The result shows that the highest percentage of sprouting 55.85% was observed for that garlic stored at 15°C using UFBs 8 ppm soaking treatment after placed at room temperature (29±2°C) during 2 weeks. This result indicates that UFBs water has an effect on enhancing the breaking dormancy of garlic bulbs.

1. Introduction

Garlic is one of Indonesia's important horticultural commodities. In Indonesia, garlic is known for its seasoning and medicine. In 2017, the consumption for household and industrial needs reached 556,000 tons, but only 21,000 tons of it was planted domestically. The remaining 535,000 tons or 99.53% were imported from China and India [1]. Indonesia's garlic productivity has been decreasing due to the loss of agricultural land and the seed dormancy period. Seed dormancy is a period where the activity of plant such as growth and development are stopped temporarily and garlic dormancy period is about more than 3 months [2] until 6 months before replanting (depend on the varieties). Planting and growing periods are approximately 3-4 months; therefore, Indonesia's garlic only replanting once a year.

One of the main causes of productivity problems of garlic is the garlic dormancy period. Understanding seed dormancy and breaking dormancy is important in the cultivation process of garlic. Low-temperature storage was observed by some researchers and it can be applied to shorten the dormancy period and increase the growth rate. Garlic (Tawangmangu varieties) was stored at 0°C for 1 month reach 83.33% of sprouting [3]. Garlic breaking dormancy occurred most rapidly at 5-10°C [4] or an intermediate storage temperature of 5-18°C signaled by the internal development of the sprout [5]. However, longer low-temperature exposure can make growing defects caused by the vernalization response becomes saturated [6].

Generally, a local farmer in Indonesia used the soaking method with water or chemical solutions to increase the growth rate. But this method can be hazardous and less effective. This research tried to used technological water named ultrafine bubbles (UFBs) water. Ultrafine bubbles (UFBs) water is technological water containing high oxygen with the diameter of the bubble about 100 µm-submicrometer [7]. This water is produced by the UFB generator, which works based on Henry's law,
the more pressure is given, the more gas will be dissolved [8]. Water under gas pressure will split the molecules of water into nano-sized bubbles (NBs) containing Reactive Oxygen Species (ROS) compounds [9]. ROS is a compound known as free radical compounds derived from oxygen [10]. ROS works by weakening the cell wall of a plant so that the seed embryos can grow faster. The more high accumulation of ROS, the more fast germination rate [11]. ROS is a compound that plays a role in the formation of sprouts and shortens seed dormancy [12].

Based on the previous research, UFB water can shorten the dormancy period of rice seeds and can accelerate soybeans seed germination [13,14]. There is no information about the application of UFB water on garlic yet especially for breaking dormancy combination with storage temperature. The objective of this research was to investigate the effect of storage temperature and ultrafine bubbles (UFBs) water on the breaking dormancy of garlic. The length of the sprout or plumule will be measured after treatments.

2. Material and Method

2.1. Materials and tools

100 kg sample of garlic was used in this research. Garlic was harvested from Ciwidey's farmer orchard (Lumbu Hijau), West Java, and already dried for 2 weeks until 50-61% of moisture content (figure 1.a), distilled water, UFBs water with dissolved oxygen 8 ppm and 20 ppm, oxygen refill, plastic, materials for measure dissolved oxygen, UFB generator manual FNIN-10 IDEC and oxygen tube (figure 2.c), thermometer, digital caliper, tools for measure dissolved oxygen, hygrometer, plastic jar, net, analytical balance, oven and refrigerator.

2.2. Soaking treatment and sprouting/plumule measurement

Garlic storage was set in three temperatures consists of 5°C, 15°C and room temperature (29±2°C) (RH 70-80%). The storage was set under dark conditions (figure 1.b, 1.c). After 3 weeks of storage, garlic was submerged in a plastic jar (figure 2.a). Soaking treatments were carried using distilled water, UFBs water with dissolved oxygen 8 ppm and 20 ppm for 24 hours and control without soaking treatment. After being submerged, the sample of garlic was placed at room temperature (figure 2.b). The soaking treatments were carried out in three replications using a completely randomized design factorial intime. The change in length, the percentage of plumule or sprout length were measured in 2 weeks after soaking treatment. Plumule is a prospective stem and leaves that grow during germination period. The length of plumule and clove counted using calipers by compare the ratio of plumule length and clove length (figure 3) and the percentage of plumule were calculated by equation 1. The plumule measurement method was destructive observation thus the samples that used in every experiments was different clove.

\[ \text{Plumule} \% = \frac{\text{The plumule length}}{\text{The clove length}} \times 100 \]  

(1)

![Figure 1. Drying and storage condition of garlic](image-url)
Figure 2. Soaking and placed in room temp treatment and UFBs generator

Figure 3. Clove and plumula of garlic

As an addition, this research also observed the quality of the garlic during storage temperature consists of moisture content, weight loss and garlic damage every 2 weeks, 300 g, 500 g, and 500 g of garlic sample were weighted, respectively. The moisture content of samples were calculated by equation 2, weight loss of samples were calculated by equation 3, and garlic damage of samples were calculated by accumulate the damage single garlic clove (on gram) every 2 weeks.

\[
\text{Moisture content} \, (\%) = \frac{(M2-M3)}{(M2-M1)} \times 100
\]  

Where:  
M1 = Empty cup weight  
M2 = Cup weight + weight of sample before drying  
M3 = Cup weight + weight of sample after drying

\[
\text{Weight loss} \, (\%) = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100
\]  

3. Results and Discussion

This research consisted of three experiments where each experiments was carried out in different week used different clove. The observation start with measured the length of plumule clove before did any soaking treatment (pre-treatment) (figure 4) and 2 weeks after soaking treatment (2nd weeks) (figure 5) as a further observation. The result showed that the data experiment for all temperature fluctuated greatly.
Figure 4. Plumule length before soaking treatment
Figure 5. Plumule length 2 weeks after soaking treatment
The highest percentage of plumule was observed for that garlic stored at 15°C using UFB 8 ppm soaking treatment. The average percentage was 50.44%, 55.85%, and 52.69%, respectively for each experiments and the highest percentage was 55.85% observed in the second experiment (figure 6). This result indicated that the intermediate temperature storage has an effect on enhancing the breaking dormancy of garlic bulbs. The optimum temperature for garlic germination is in range 10-20 °C [15]. This was more rapidly than stored at 5°C and room temperature. The average percentage of plumule length for garlic stored at 5°C were 29.22%, 36.46%, 35.67% respectively for each experiments and the highest percentage was 36.46% in the second experiment soaking used distilled water (figure 7). The average percentage of plumule length for garlic stored at room temperature was 26.05%, 25.30%, 30.12% respectively for each experiments and the highest percentage was 30.12% in third experiment soaking used distilled water (figure 8).

**Figure 6.** The percentage of plumula length of 15°C

**Figure 7.** The percentage of plumula length of 5°C
The data was run with Analysis of Variance (ANOVA) and to measure specific differences using Duncan’s Multiple Range Test (DMRT) p<0.05. The results showed that storage temperature and soaking water had a significant effect on breaking dormancy when sample stored at 15°C using UFB 8 ppm soaking water (table 1).

**Table 1. Main effect of storage temperature and soaking water on breaking dormancy**

| Storage Temp (°C) | UFB 8 ppm | Distilled Water | UFB 20 ppm | Control | Mean | Duncan Grouping |
|-------------------|-----------|-----------------|------------|---------|------|-----------------|
| 5                 | 31.5±4.1ab| 30.7±3.6b       | 29.3±3.2b  | 28.3±2.3b | 29.9 | B               |
| 15                | 45.3±11a  | 34.9±10.5ab     | 33.8±11.3ab| 34.2±8.8ab| 37.1 | A               |
| Room Temp         | 27.6±5.5ac| 27.3±4.4bc      | 23.8±2.9bc | 24.6±3.8bc| 25.8 | C               |
| Mean              | 34.8      | 31.0            | 29.0       | 29.0     |      |                 |
| Duncan Grouping   | A         | B               | B          | B        |      |                 |

Dissolved oxygen is related to the amount of micro and nano bubbles (MNBs) in water. MNBs are able to increase the mobility of water and affect the plant bioelectrics for plant growth [16]. MNBs can generate ROS and the amount of ROS from MNBs have a positive correlation to the density of NBs in water [17]. ROS accumulation could plays a role in the development of germination by weakening the cell wall which is need to cell elongation [18]. The excess ROS concentration have an oxidation effect and moderate ROS concentration show physiological promotion effect [17]. We measured distilled water contain 6-9 ppm dissolved oxygen, UFB 8 ppm contain 7-14 ppm dissolved oxygen and UFB 20 ppm contain 20-38 ppm approximately (data not shown) but in some condition high dissolved oxygen is unstable especially in the high temperature condition because basically oxygen easy to evaporate in high temperature therefore, to keep high dissolved oxygen in water, low temperature exposure is very necessary. In this research, the highest percentage of plumule length was garlic clove soaked in UFB 8 ppm and distilled water. According to this result, we assume that moderate dissolved oxygen contain nano bubbles have an effect on the garlic germination process.
Distilled water contain 4-8 ppm dissolved oxygen, it also allows there are nano bubbles and ROS in small amount thus, it has the ability to increase germination. This is related to the statement of Liu S et al (2013), they conclude that control water may also contain a small number of NBs although substantially fewer than water containing NBs [19]. However, UFB 20 ppm probably has the same effect as the others due to the higher dissolved oxygen the more stable NBs and ROS in water because of the high dissolved oxygen concentration could suppress gas dissolution [16]. Furthermore, further experiments are needed to confirm result above. Moreover, according to Wu (2015) statement [20], storage temperature has the same case, there are many contradiction on the effect of pre planting low temperature treatment on garlic, some researchers have reported increases, others observed no significant differences while the others reported depressive effect.

As an addition, this research also observed the quality of the garlic during storage temperature consists of moisture content, weight loss and garlic damage every 2 weeks. The results showed that the moisture content had fluctuate data approximately 63%-67% on average (figure 9), weight loss increase significantly during storage in all the storage temperature. The highest percentage of weight loss for garlic stored at 5°C approximately 16.08%, then for 15°C and room temperatures were 11.29% and 7.22% respectively (figure 10). The highest percentage of damage for garlic storage at room temperature of approximately 3.87 g then for 5°C and 15°C approximately 0.919 and 0.917 g, respectively (table 2). Initial garlic condition such as moisture content, weight and damage on clove are the important factors related to seed quality especially for consumption. Garlic must be conditionated in approximately 60% of moisture content so as not attacked by microorganism and reduce damage cloves. Garlic clove that have decreased weight indicate not suitable as a sample for UFBs treatment because there is a possibility that there has been a maximum germination on it or activity of microorganism. During germination process, garlic cloves lose some of water content therefore the weight decreases.

![Figure 9. The percentage of garlic moisture content](image-url)
The amount of garlics damage (gram)

| Temp      | 0     | 2     | 4     | 6     | 8     | 10    |
|-----------|-------|-------|-------|-------|-------|-------|
| 5°C       | 0     | 0     | 0.868 | 0.919 | 0.919 | 0.919 |
| 15°C      | 0     | 0.007 | 0.679 | 0.917 | 0.917 | 0.917 |
| Room Temp | 0     | 1.601 | 2.881 | 3.234 | 3.473 | 3.871 |

4. Conclusion

The highest percentage of sprouting signaled with the highest percentage of plumule 55.85%, was observed in the garlic stored at 15°C using UFB 8 ppm after 2 weeks of soaking treatment. This result indicates that UFBs water has an effect on enhancing the breaking dormancy of garlic bulbs.

5. References

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