Effects of ultrasound irradiation on the growth of Japanese radish sprouts

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Abstract: The growth characteristics of the sprouts were evaluated using the number of germinated seeds, amount of water absorbed, and the length and mass of plants. In this study, although the experiment was performed in an air-conditioned room, environmental conditions such as temperature and humidity were not precisely controlled. To remove the differences between environmental conditions, results of experiments without ultrasound irradiation were also investigated as reference values. Seeds were chosen so that differences in the number of germinated seeds was measured one day after seeding.

1. Introduction

The safe and stable production of crops is desired in the agricultural industry. Recently, plant factories have become popular, where crop production is controlled by light [1,2], and temperature [3]. In addition, the effects of ultrasound [2,4,5] and electric field on the growth of plants have been reported [6]. Ultrasound has other potential applications in the agricultural industry, because its irradiation conditions such as power and frequency show good controllability. For example, several methods of food dehydration using airborne ultrasound have been developed [7,8]. However, the quantitative relationship between condition of ultrasound irradiation and growth characteristics has not been investigated in detail. In this letter, the relationship between the frequency and pressure of ultrasound and the growth characteristics of a vegetable was experimentally investigated.

2. Experimentation

Multiple replicated experiments were required for the investigation. Therefore, the Japanese radish sprout was chosen as the specimen, which grows in a short period. The number of germinated seeds, amount of water absorbed, and the length and mass of plants were evaluated.

2.1. Experimental system

The experimental equipment used is shown in Fig. 1(a). Two bolt-clamped Langevin ultrasonic transducers (BLTs) were set under a water tank (width, 160 mm; depth, 80 mm; and height, 100 mm). The design features of the two BLTs were the same, where two lead zirconate titanate (PZT) transducers of 35 mm diameter and 5 mm thickness were used. The BLTs were electrically connected in parallel. The seeds of Japanese radish sprouts were put on a sponge located on the water surface. The distance between seeds was in the range of 30–130 mm. When the BLTs were vibrated, the bottom of the water surface. The distance between seeds was in the range of 30–130 mm. When the BLTs were vibrated, the bottom of the water surface oscillated and ultrasound was irradiated on to the seeds 30–130 mm. When the BLTs were vibrated, the bottom of the water surface. The distance between seeds was in the range of 30–130 mm. When the BLTs were vibrated, the bottom of the water surface. The distance between seeds was in the range of 30–130 mm. When the BLTs were vibrated, the bottom of the water surface. The distance between seeds was in the range of 30–130 mm. When the BLTs were vibrated, the bottom of the water surface.

In this study, two sets of equipments were used. The resonance frequencies of the BLTs attached to the equipment were 91 and 185 kHz. The Q values and motional admittances for these resonances were 180 and 4 mS at 91 kHz and 84 and 7 mS at 185 kHz, respectively. The frequencies of 90 and 180 kHz were used in experiments, where the water heights were set at 44 and 48 mm for the frequencies of 90 and 180 kHz, respectively.

The distribution of sound pressures was measured using a hydrophone (B&K, Type 8103). When the applied peak-to-peak voltages were 50 V, the average pressures and standard deviations were 1.50 and 1.07 kPa at a frequency of 90 kHz, and 3.44 and 1.61 kPa at a frequency of 180 kHz, respectively. In Sect. 2.3, growth characteristics are evaluated in relation to the average pressures of 10 Pa, 100 Pa, 1 kPa, and 10 kPa at 90 and 180 kHz. The experiment was performed in an air-conditioned room controlled at 18°C.

2.2. Number of seeds

Generally, the reliability of statistical data increases as the number of seeds increases. In this study, a sample size of 100 was chosen. The reliability of the change in mass was estimated by the T-test (see Sect. 4.1), assuming that the mass has a normal distribution and the variance is constant. The masses of 100 plants were measured one week after seeding. The distribution of the masses is shown in Fig. 2. The average and standard deviation of the masses were 0.125 and 0.0352 g, respectively. The results of the T-test with a reliability of 95% show that the mass can be used for evaluation with high reliability when the mass changes by less than 8%.

2.3. Evaluation method

The growth characteristics of the sprouts were evaluated using the number of germinated seeds, amount of water absorbed, mass, and length of plants. In this study, although the experiment was performed in an air-conditioned room, environmental conditions such as temperature and humidity were not precisely controlled. To remove the differences between environmental conditions, results of experiments with ultrasound irradiation were normalized by those of experiments without ultrasound irradiation. Four set of experiments were conducted at four different acoustic pressures (10 Pa, 100 Pa, 1 kPa, and 10 kPa). For each set of experiments, the characteristics at the frequencies of 90 and 180 kHz were investigated. The characteristics in the case without ultrasound irradiation were also investigated as reference values. Seeds were chosen so that differences between the average masses of seeds were less than 0.0001 g for each set of experiments. In total, 12 experimental data sets were obtained.

2.3.1. Germination

To evaluate the growth rate until germination, the number of germinated seeds was measured one day after seeding. The...
increasing rate of germination was defined by $(G_u - G_n)/G_n$, where $G_u$ and $G_n$ are the numbers of seeds that germinated with and without ultrasound irradiation, respectively.

2.3.2. Water absorption

To evaluate the effect of ultrasound irradiation, amount of water absorbed was investigated. The amount of water absorbed $K$ was defined as

$$K = M_2 - M_1,$$

where $M_1$ and $M_2$ were the initial average mass and the average mass one day after seeding, respectively. The increase rate of the amount of water absorbed was defined as $(K_u - K_n)/K_n$, where $K_u$ and $K_n$ are the amounts of water absorbed with and without ultrasound irradiation, respectively.

2.3.3. Mass and length of plants

Next, the average mass and length of plants were evaluated. First, the average $A$ was calculated for the masses $a_i$ (total number: $N$) for the seeds without ultrasound irradiation.

$$A = \frac{\sum a_i}{N}$$

Second, the normalized masses $B_i$ were calculated by dividing each mass $b_i$ for the seeds with ultrasound irradiation by $A$.

$$B_i = \frac{b_i}{A}$$

Third, average $C$ was calculated for the masses $B_i$ for the seeds with ultrasound irradiation.

$$C = \frac{\sum B_i}{N}$$

Then, the increasing rate of the mass of plants was calculated as $C - 1$. The increasing rate of the length of plants was similarly calculated.

2.4. Timeline of experiment

For the evaluation, 100 seeds of sprouts were grown in a water tank for a week. The timeline of the experiment is shown in Fig. 3.

**Day 0:** The measurement started at 14:00. Initial masses were measured. Ultrasound was irradiated for 24 h.

**Day 1:** The masses of seeds and the numbers of germinated seeds were measured at 14:00.

**Days 2–5:** Ultrasound was irradiated for 3 h.

**Day 6:** The masses and lengths of plants were measured at 14:00. The measurements were complete on this day.

3. Experimental results

3.1. Increasing rate of germination

Figure 4(a) shows a photograph of seeds without ultrasound irradiation. Figure 4(b) shows a photograph of seeds with ultrasound irradiation at a frequency of 90 kHz and a pressure of 100 Pa. The numbers of germinated seeds were 27 and 49 for the cases of without and with ultrasound irradiation, respectively. Figure 5 shows the increasing rate of germination. The maximum increasing rate of 81% was obtained at a frequency of 90 kHz and a pressure of 100 Pa.

3.2. Increasing rate of amount of water absorbed

Figures 6(a) and 6(b) show distributions of the mass of seeds with and without ultrasound irradiation, respectively. The average normalized mass increased by 6% in the case of ultrasound irradiation at 90 kHz and 100 Pa. Figure 7 shows the increasing rate of the amount of water absorbed. As shown in the figure, for all of the specimens irradiated with
ultrasound, the amount of water absorbed increased. The increasing rate became the maximum, 14%, when the sound pressure was 100 Pa.

3.3. Increasing rate of mass and length of plants

Figures 8(a) and 8(b) show the increasing rate of the average mass and length of plants, respectively. The increasing rate decreased in all plants when ultrasound was irradiated at a frequency of 180 kHz. The highest increasing rate was obtained at a frequency of 90 kHz and a pressure of 100 Pa.

4. Discussion

The reliability of the measured data and acoustic power under optimal conditions was investigated.

4.1. Statistical test

First, the reliability of measured data was investigated. Using the data under the optimal conditions (90 kHz, 100 Pa), the T-test was performed. The mass and length are assumed to be normally distributed. \( T \) was calculated as follows:

\[
T = \frac{\bar{X} - \bar{Y}}{\sqrt{\frac{\sigma_X^2}{m} + \frac{\sigma_Y^2}{n}}}
\]

Here, \( \bar{X} \) and \( \bar{Y} \) are average values for the cases with and without ultrasound irradiation, respectively. \( \sigma_X^2 \) and \( \sigma_Y^2 \) are the variances for the cases with and without ultrasound irradiation, respectively. \( m \) and \( n \) are the numbers of samples (= 100).

**Mass of seeds and amount of water absorbed**

Increases by 6% (mass) and 14% (absorp.)

\[
\begin{align*}
\bar{X} &= 0.02549 \text{ [g]}, \quad \bar{Y} = 0.02409 \text{ [g]} \\
\sigma_X^2 &= 2.326 \times 10^{-5} \text{ [g]^2}, \quad \sigma_Y^2 = 2.325 \times 10^{-5} \text{ [g]^2} \\
T &= 2.056, \text{ Reliability: 96%}
\end{align*}
\]

**Mass of plants (Increase by 8%)**

\[
\begin{align*}
\bar{X} &= 0.19992 \text{ [g]}, \quad \bar{Y} = 0.18524 \text{ [g]} \\
\sigma_X^2 &= 0.003092 \text{ [g]^2}, \quad \sigma_Y^2 = 0.0033675 \text{ [g]^2} \\
T &= 1.830, \text{ Reliability: 94%}
\end{align*}
\]
Length of plants (Increase by 5%)

\[ X = 91.402 \text{ [mm]}, Y = 87.586 \text{ [mm]} \]
\[ \sigma_x^2 = 886.2 \text{ [mm}^2], \sigma_y^2 = 1046.2 \text{ [mm}^2] \]

\[ T = 0.868, \text{ Reliability: 60\%} \]

The results show that the masses are reliable when their reliabilities are higher than 90\%. To increase the reliability for the length, a larger number of samples should be used for evaluation.

4.2. Acoustic power

Next, the acoustic power of ultrasound irradiating sprouts was investigated. Here, the acoustic power indicates energy, that propagates in the direction perpendicular to the water surface per unit time and area [9]. The acoustic intensity \( I \) in the propagation direction is

\[ I = \frac{P^2}{\rho c}, \quad (6) \]

where \( P \) is the effective amplitude of the sound pressure, \( \rho \) is the density of water, and \( c \) is the sound speed of water. When \( P = 100 \text{ Pa} \), \( \rho = 1,000 \text{ kg/m}^3 \), and \( c = 1,500 \text{ m/s} \), the acoustic intensity was 6.7 mW/m\(^2\). The irradiated area was on the order of \( 10^{-2} \text{ m}^2 \). Thus, irradiated energy was on the order of \( 10^{-5} \text{ W} \).

5. Conclusion

In these experiments, a frequency of 90 kHz and a pressure of 100 Pa provided optimal conditions for all the germination parameters: the number of germinated seeds, amount of water absorbed, mass, and length. The increasing rates of germination, amount of water absorbed, mass, and lengths were 81\%, 14\%, 8\%, and 5\%, respectively. The acoustic power was 6.7 mW/m\(^2\) at 100 Pa. We found that such a small power affects the growth of the radish sprouts. In addition, amount of water absorbed increased with the ultrasound irradiation regardless of the frequency and pressure. The results show that ultrasound could promote water absorption. On the other hand, a frequency of 180 kHz and pressures of 10 Pa, 1 kPa, and 10 kPa prevented the growth of the radish sprouts but not amount of water absorbed. As a result, we conclude that the growth of Japanese radish sprouts can be controlled by ultrasound irradiation.

In this study, environmental conditions such as temperature and humidity were not precisely controlled. Therefore, the evaluation results could vary. As a further study, the evaluation method including the determination of the number of seeds should be improved. Reproducibility should be also verified. The mechanism of the growth control of plants and the effect of direct ultrasound irradiation of plants should also be investigated. In this work, we used only Japanese radish sprouts; however, there are a variety of plants. The optimal conditions should be investigated for specific plants.

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References
