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Effect of sound stimulation on *Dendranthema morifolium* callus growth[1]

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Abstract

We designed a set of devices for generating sound fields and studied the effect of sound stimulation on *Dendranthema morifolium* callus growth. We came to a conclusion that the promotion effect was most obvious in a frequency of 1000 Hz and a strength of 100 dB sound stimulation. However, when we increase the frequency and strength out of a certain range, this effect was reduced, or even represented a restraint effect when reached another higher range. It was found in our experiment that sound stimulation of a frequency of 1000 Hz and a strength of 100 dB obvious promoted the soluble protein and sugar in cytoplast, which is a sigh of high metabolism level and vigorous divide state in strong sound stimulation administrated *D. morifolium* callus.

Keywords: Sound stimulation; *Dendranthema morifolium*; Growth

1. Introduction

Acting as a special form of alternative stress, strong sound stimulation has an obvious effect on plant growth. It was found in many studies that in some low strength and frequency sound stimulation administrated plant; sound stimulation did not damage its round structure, but promoted the metabolism, permeability and selectivity of cell membrane, as well as the combination of DNA and synchronization of cell cycle [1–3]. In addition, sound stimulation of low frequency and strength would also increase the activity of enzymatic biocatalyst. However, the material used in these former experiments is complex in variety and genetic background, and thus is not suitable for further understandings. *Dendranthema morifolium* which is a conventionally used material in experi-

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ment, has fewer chromosomes, shorter growing period, more seeds and smaller genome. Thus, using *D. morifolium* which is simple in genetic background will benefit the further research, and it was used in our experiment to study the effect of sound stimulation on *D. morifolium* growth.

2. Material and method

2.1. Material

Well growing *D. morifolium* seedling was transplanted into flowerpot, in which the soil was suffocated to kill the entire ovum, pest, and weed seeds. Carbendazol wettable powder (0.5%) was insufflated to keep the soil asepsis, sterile, and weed free. The *D. morifolium* transplanted was then moved into green house for growing, ready for inoculation.

We used the lamina, stem, and stem apex as explants for inoculation, and found the callus of stem was best growing. Therefore, it was used in our latter experiment as explant. The callus of *D. morifolium* was cultured in 50 ml triangular flask, with 20 ml MS solid medium in each (adding 1.0 mg/l NAA, 1.5 mg/l BA), and cultured in incubator at 26 °C, for 14 h, 4000 lx.

2.2. Sound stimulation

The sound stimulation was generated by sound generating device we designed (Fig. 1). Vigorous growing *D. morifolium* callus was cut into small pieces for generation. Steadily generated *D. morifolium* callus was stimulated by sine-sound-wave as a treatment two times a day, lasting 30 min each time. In the 2 weeks treatment, the control samples were put in the same environment.

2.3. Measurement of callus growth rate

Electronic balance was used to measure the fresh weight of sound stimulated *D. morifolium* callus.

$$\text{callus growth rate} = \frac{(\text{fresh weight after stimulation} - \text{fresh weight before stimulation})}{\text{time}}$$

2.4. Measurement of soluble protein content

The stimulated callus and control were separately added into 20 mmol/l phosphoric acid buffer

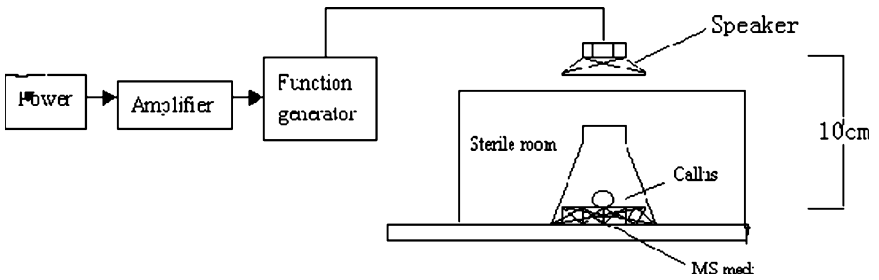

Fig. 1. Scheme sound stimulation generating setup.

solution (pH 6.8) according to a ratio or 1:5 (w/v), rubbed in ice bath, centrifuged for 10 min at $1000 \times g$. The deposition was double solved by 0.85% NaCl into a constant volume. The content of protein was measured according to the method of Bradford [4], using BSA (product by Sigma) as an internal standard.

2.5. Measurement of soluble sugar content

Callus (1 g) was selected and put into condensing taper bottle, adding 2% HCl 150 ml, and hydrolyzed in boiled water for about 3 h. It was then cooled to room temperature, and phenolphthalein indicator was added. NaOH (30%) was then used to counteract the solution just into rosiness, then immediately adding 2% HCl until the rosiness color just disappears. After that, 10% lead acetate was added to eliminate the other proteins and still into a constant volume. 3 ml dinitrosalicylic acid (DNS) was then added, for colorimetric analysis in 540 nm, using dextrose as an internal standard.

3. Results

3.1. Effect of sound stimulation at different frequency on the growth of D. morifolium callus

As shown in Fig. 2, at a certain strength (100 dB), low frequency sound stimulation can promote the growth of *D. morifolium* callus, which becomes even more obvious when the frequency increases, and reaches a peak at around 1000 Hz. Whereas, when exceeding a certain range (around 2000 Hz), the promotion was obviously reduced, or even becomes restraint.

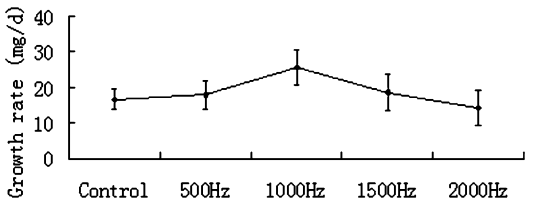

Fig. 2. Effect of 100 dB sound with different frequency on the growth of *D. morifolium* callus.

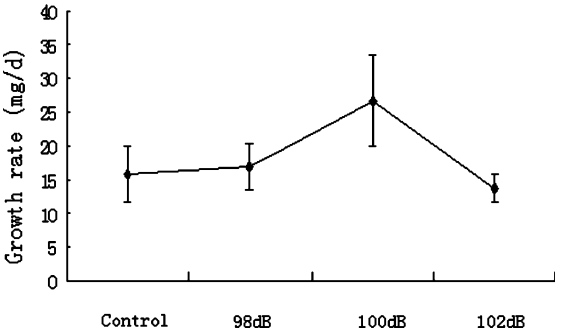

Fig. 3. Effect of 1000 Hz sound with different strength on the growth of *D. morifolium* callus.

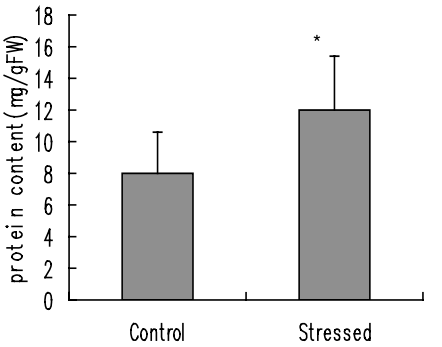

Fig. 4. Effect of sound stimulation on the protein content of *D. morifolium* callus.

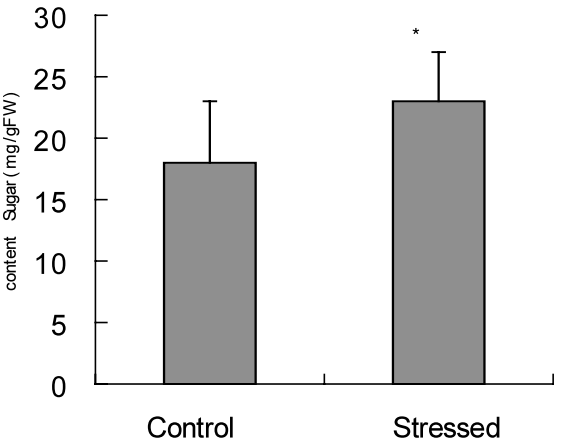

Fig. 5. Effect of sound stimulation on the sugar content of *D. morifolium* callus.

3.2. Effect of sound stimulation at different strength on the growth of D. morifolium callus

As shown in Fig. 3, at a certain frequency (1000 Hz), the growth of callus increases with the increase of strength, and reaches a peak at 100 dB. However, when it goes higher, the promotion decreased, and even becomes restraint too.

It is concluded that the effect of 1000 Hz and 100 dB sound stimulation to the *D. morifolium* callus was most obvious, and this frequency and strength was used in all the following experiments.

3.3. Sound stimulation at a certain strength and frequency on the content of soluble protein and sugar in D. morifolium callus

The analysis of content of soluble protein and sugar in *D. morifolium* callus after sound stimulation (Figs. 4 and 5) indicates that, after 100 dB 1000 Hz sound stimulation, the content of soluble protein and sugar is obviously higher than the control. Variance analysis also shows an obvious difference between. Former researches indicated that the content of soluble protein changes with the growth, divide, and differentiation, and is related to the cell differentiation in the exponential phase of growth [5]. The increase of soluble protein under the sound stimulation at certain frequency and strength provides a fine substance basis of cell growth and differentiation. In addition, soluble sugar, acts as a substrate of carbon metabolism, the increase of which content would also benefit the cell metabolism.

4. Discussion

It is unavoidable for plants to be affected by environment and human activities. The former studies consider that the growth and development of plant is a relatively passive process, where the environmental stress mainly acts either as an inhibiting or damaging effect [6,7]. The study of Lichtenthalar indicates that, slight stress could promote the assimilation of cell, but does not damage it even if lasting a long time. This kind of moderate stimulation benefits the plant [8]. It was also found by Wang that ultrasonic wave at a certain frequency and strength can also promote the growth and divide of carrot cell. So they developed a *bidirectional* theory between stress and cell growth, i.e. sound stimulation cannot only damage cell, but also can promote its growth when at a proper frequency and strength [9]. This theory is identical to our conclusion from our *D. morifolium* experiment and thus is well validated. The study of Xi [1,2] also shows that sound stimulation at a certain frequency and strength promoted the fluidity of membrane wall and membrane lipid in tobacco cells, which would benefit the absorb of nutriment and combination of DNA in S period, as well as synchronizing the cell cycle, which all benefit the growth of cell. Recent studies are more focused on the effect of electromagnetic stimulation, and resulted in a conclusion that the growth plant callus is obviously faster than that of control, as well as faster nutriment absorbability and protein combination [10,11]. Therefore, moderate electromagnetic treated plant callus is higher in metabolism level and divide and growth rate. It is also found in our experiment that sound stimulation can obviously increase the content of soluble protein and sugar in plant callus. It is also believed that the division and growth of plant tissue is fairly related to the content of protein in tissue, where the content of soluble protein reflect the accumulation of materials needed for cell division, as well as content of enzyme and relevant metabolism level. Sound stimulation at a certain frequency and strength induced increase of soluble protein and sugar content could provide a fine material basis for cell divide and growth. In addition, it is also found by other studies that mechanical vibration at a certain frequency can promote the growth of plant callus [12]. It is thus obvious that effect of various stresses by either nature environment or human activities is determined by the strength and time of stimulation on the plant. One possible explanation is that mechanical stimulation transfers energy into cell. This process not only drives the flow of nutriment but also affects on the membrane, whose structure and function is changed and thus induced various physiological reaction and response in cell.

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