ELSEVIER

Colloids and Surfaces B: Biointerfaces 24 (2002) 321–326

COLLOIDS AND SURFACES B

www.elsevier.com/locate/colsurfb

Effects of sound field on the growth of *Chrysanthemum* callus

Liu Yiyao [a], Wang Bochu [a,*], Long Xuefeng [a], Duan Chuanren [a], A. Sakanishi [b]

[a] *Key Lab for Biomechanics and Tissue Engineering under the State Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, PR China*

[b] *Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Japan*

Received 18 June 2001; accepted 17 August 2001

Abstract

The effects of sound field on *Chrysanthemum* callus were studied. The field of alternative stress was generated through a strong sound field system set up in our lab, and the activity of SOD, the content of soluble proteins, the activity of IAA oxidase and the absorption rate of calcium were measured. We found that the sound field stimulation has dual effects, which can enhance or inhibit the growth of *Chrysanthemum* callus, the growth effects of sound field on *Chrysanthemum* callus depended greatly on the intensity and frequency of sound field. The activity of SOD, the content of soluble proteins and the absorption rate of calcium in callus increased with the intensity and frequency increasing. However, those indexes began to decrease when the intensity and frequency went beyond the limit of 100 dB and 800 Hz, respectively. However, the changing tendency of IAA oxidase activity was reversed to the above three indexes. We draw a conclusion that the optimal stimulation conditions are 100 dB and 800 Hz. Under the conditions, the sound field can distinctly enhance the growth of *Chrysanthemum* callus. We think that moderate stress stimulation can enhance the assimilation of tissues or cells, improve their physiological activity and accelerate the growth of plants, this moderate stress stimulation is helpful to the growth of plant tissues.

Keywords: Sound field; *Chrysanthemum* callus; Biological effects

1. Introduction

Studies of physical stimulation on the growth of plant cells and tissues are a cardinal research field in Cell Biology [1]. In the last 10 years, the effects of stress stimulation on the growth and reconstruction of cells and tissues have been the focus in this research field [2,3]. Studies on the morphology, structure, function of plant cells and the growth, development, differentiation in different growth phases of plant cells are special significance in propagation good strains at large scale, researching plants adapting to outer environment and realizing the new technology of high efficient factory agriculture [4,5].

* Corresponding author. Tel.: +86-23-65-10257.
E-mail address: wangbc2000@yahoo.com.cn (B. Wang).

PII: S0927-7765(01)00275-2

As we know, a series of physiological and biochemical changes will take place during the growth of plants, the growth speed of plants depends on the change degree of all these indexes. Generally, outer Stress have obvious influence on many physiological and biochemical indexes in plant tissues, the stress intensity and its duration are very important for tissue growth, which determine the outer stress inhibits or promotes the tissue growth [6]. However, the intensity endured by tissues is different for different biological tissues, the most optimum intensity can be found through many trials and experiments. In this paper, we adopted sound field to stimulate the *Chrysanthemum* callus, the sound wave is a special alternative stress. On the other hand, we selected four physiological and biochemical indexes and measured their changes after the *Chrysanthemum* callus stimulated by sound field, according to the research, we can preliminarily explore the change tendency that sound field effects on the growth of *Chrysanthemum* callus. Base on those, in this article, we selected different intensities and frequencies of sound wave to stimulate the *Chrysanthemum* callus, and the activity of SOD, the content of soluble proteins, the activity of IAA oxidase and the absorption of calcium are measured.

2. Materials and methods

2.1. *Chrysanthemum callus culture*

The callus was cultured using the stem tip of *Chrysanthemum*. The MS culture media contains 3% sucrose, 0.9% agarose, 10 ml 0.1 mg/l NAA, and 15 ml 0.1 mg/l BA. After soaking, washing and disinfecting the stem tip of *Chrysanthemum*, we inoculated them in triangular bottles (25 ml) in which there were MS culture media. Ten days culture in the illumination culture apparatus at 25 °C, the *Chrysanthemum* callus come into being, then we subcultured these callus every 10 days for two times, so the callus were even. All these samples were divided into the control and eight treatment groups.

2.2. *Treatment of sound field stimulation*

The field of alternative stress was generated through a strong sound field system set up in our lab (see Fig. 1). The system mainly includes the power, amplifier, function generator and speaker. We can adjust the intensity and frequency by amplifier and function generator, respectively, so the magnitude of alternative stress can be controlled. The callus were put into a sterile room, the distance between the speaker and the callus is approximately 20 cm. We firstly used a given frequency (800 Hz) to continuously stimulate the callus for 10 days under different intensities (90, 95, 100, 105, 110 dB), twice every day and 30 min every time, then measured the SOD activity, the soluble proteins content, the IAA oxidase activity and the calcium absorption. On the other hand, we used a given intensity (100 dB) to stimulate the callus for 10 days under different frequencies (80, 800, 8000 Hz), twice every day and 30 min every time, and the above four indexes are also measured by the same methods. The cultur-

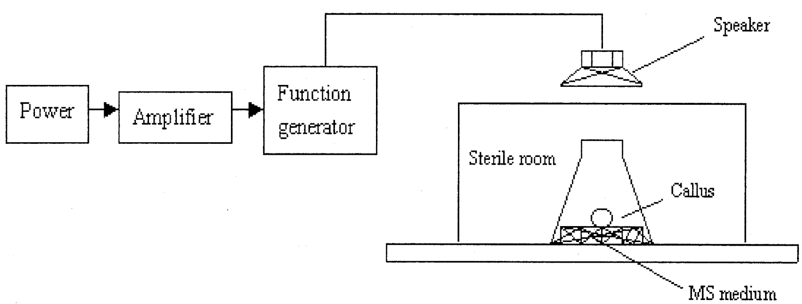

Fig. 1. The strong sound field apparatus.

ing condition of the control and treatment group were the same, namely, the illumination time was 12 h per day, illumination intensity was 1200 Lux, the culture temperature was 25 °C.

2.3. The measurement of superoxidise (SOD) activity

The measurement method is referred the Giannopolitia and Rie, which was mentioned in the [7].

2.4. The measurement of soluble proteins content

We adopt the method of Folin–phenol reagent, which referred the [8].

2.5. The measurement of indolizomycin (IAA) oxidase activity

The measurement is referred the method of Cai et al. [9]. The IAA can react with F_eCl_3 and then bred substance will come into being, we can measure the IAA oxidase activity by colorimetry.

2.6. The measurement of calcium absorption

The measurement method of Calcium Absorption is referred Zhao et al. [4]. We sliced a piece of culture medium under the *Chrysanthemum* callus, which thickness is approximate 1 mm, then we melted it diluted into double-distilled water for four times. The PLASMA–SPEC(I)ICP luminous spectrum apparatus is used to measured the content of calcium, which can indirectly reflect the calcium absorption of *Chrysanthemum* callus.

3. Results

According to our experiments, we found that the growth effects of sound field on *Chrysanthemum* callus depended greatly on the intensity and frequency of sound field. Our experiments also indicate that the sound field stimulation has dual effects, namely, which can enhance or inhibit the growth of *Chrysanthemum* callus. The activity of SOD, the content of soluble proteins and the absorption rate of calcium in callus increased with

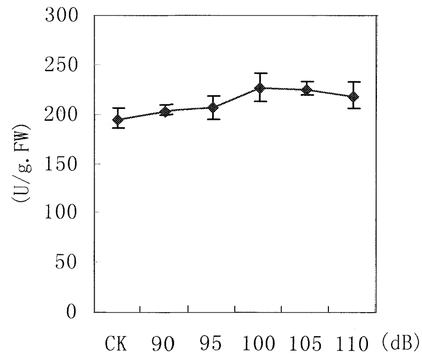

Fig. 2. The effect of intensity on the activity of SCD under given frequency.

the intensity and frequency increasing. However, those indexes began to decrease when the intensity and frequency went beyond the limit of 100 dB and 800 Hz, respectively (See Figs. 2–7 and 9). As for the activity of IAA oxidase, its changing tendency was reverse to the above three indexes (see Figs. 4, 8 and 9). As a result, we drown a conclusion that there were an optimum intensity and frequency for sound field stimulation. Under the conditions, sound field stimulation can remarkably enhance the growth of *Chrysanthemum* callus.

4. Analysis and discussions

Many studies about the effects of environment stress on the development, growth and morphogonesis of plant tissues have been reported [10,11]. Generally, outer stresses have greatly ef-

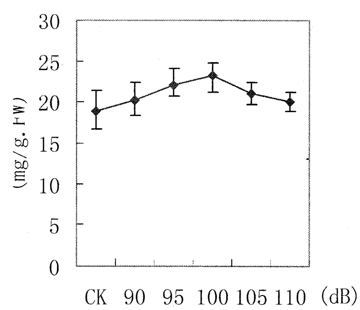

Fig. 3. The effect of intensity on the content of soluble protiens under given frequency.

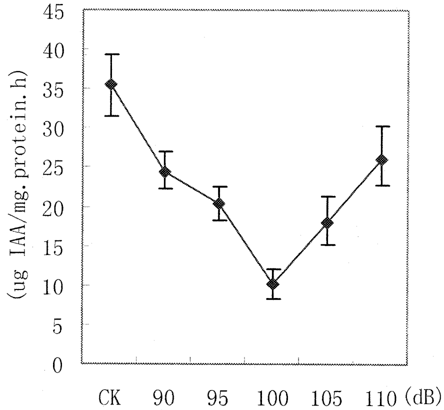

Fig. 4. The effect of intensity on the activity of IAA oxidase under given frequency.

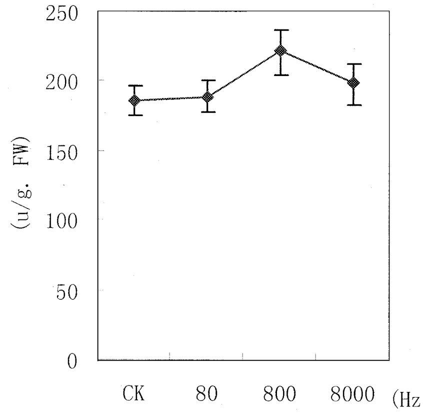

Fig. 6. The effect of frequencies on the activity of SCD under given intensity.

fects on the growth, existence, propagation and caducity of plants, moderate stress stimulation can enhance the assimilation of tissues or cells, improve their physiological activity and accelerate the growth of plants, this moderate stress stimulation is helpful to the growth of plant tissues [12]. From our experimental results, we also draw the similar conclusions. In a certain range of intensity and frequency, the sound field can obviously enhance the growth of plant tissues, however, its growth will be inhibited when the sound field is beyond the range. From our experiments, we found that the growth effects of sound field on *Chrysanthemum* callus is closely related to the intensity and frequency of sound field. As a result, we draw a conclusion that the optimal stimulation conditions are 100 dB and 800 Hz. Under the conditions, the sound field can distinctly enhance the growth of *Chrysanthemum* callus.

As we know, the mechanisms of biological effects caused by stresses are very complex, it is difficult to research about how the plant cells receive the mechanic signal and how to transfer the mechanic signals into the cells. We think that it is a good method to unveil the mechanisms of biological effects caused by stresses from the level of cell and molecule. In our experiments, we have measured the four physiological and biochemical indexes which is closely related to the growth of *Chrysanthemum* callus, we found that sound field greatly effects the growth, development and cell division of *Chrysanthemum* callus. SOD is an effective clear reagent of reactive oxygen that is harm to plant growth; the activity of SOD is related to antivirus, anti-stress and anti-caducity

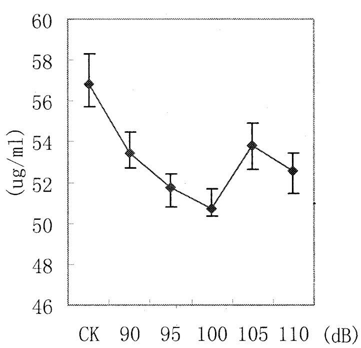

Fig. 5. The effect of intensity on the absorption of calcium under given frequency.

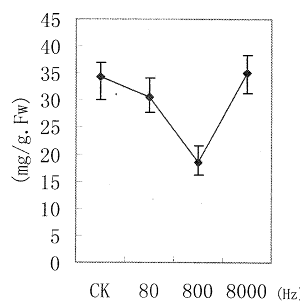

Fig. 7. The effect of frequencies on the content of soluble proteins under given intensity.

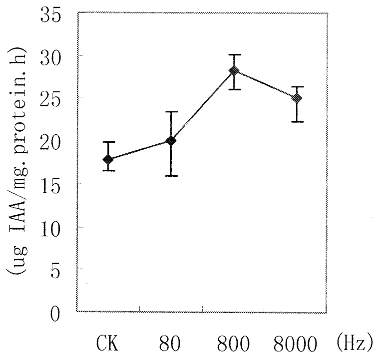

Fig. 8. The effect of frequencies on the activity of IAA oxidase under given intensity.

of plants. On the other hand, its activity has relation with differentiation and division of cells. Many experiments have demonstrated that the activity of SOD has a positive correlation with the growth of callus [13]. Studies have shown, one mechanism of reactive oxygen harming plant tissues is SOD initiates the membrane lipid peroxidation, and the structure of cell membrane destroyed, which will cause cells death.

The division and growth of plant tissues depend greatly on the content of soluble proteins in tissues. The accumulation level soluble proteins content not only reflects the indispensable substances accumulation for cell division, but also reflects the content of enzymes and the metablization level. Under 100 dB and 800 Hz of sound field, the content of soluble proteins is the highest, which provides a good condition for tissue growth.

IAA is a kink of auximone, which is an indispensable for cell division and growth. The content of IAA is related to the growth of callus, the

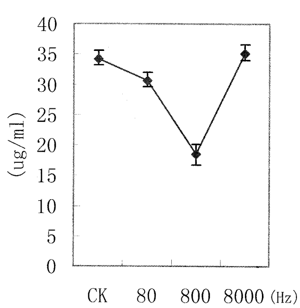

Fig. 9. The effect of frequencies on the absorption of calcium under given intensity.

decomposition of IAA is regulated by IAA oxidase in plant. If the activity of IAA oxidase is higher, then the content of IAA will decrease, so the growth of callus will be inhibited [14]. Our experiments demonstrated that the activity of IAA oxidase decreases in *Chrysanthemum* callus after sound field stimulation in different intensity and frequency (especially in 100 dB and 800 Hz). The lower activity of IAA oxidase is helpful to the growth of *Chrysanthemum* callus.

It is well known that calcium is an important nutrition element for plant growth. From our experimental results, it is showed that sound field has an important influence on the calcium absorption, and also in 100 dB and 800 Hz of sound field, the rate of calcium absorption is the highest. As we know, calcium plays an important role as a second messager in plant growth and development, and also in the response and adaptation of plants to the environmental stress [15]. C_a^{2+} may transfer the mechanical stress stimulation into other signal molecules, which is so-called cascade amplification, by this way, a series of physiological and biochemical changes will be caused.

Upto now, there are many explanations about the mechanisms that outer stresses can effect the growth of plant tissues [16]. It is reported that, in a certain range of intensity and frequency, the sound field decreased the phase transition temperature significantly. The decrease of thermodynamic phase transition denotes the enhancement of the fluidity of cell wall and membrane, which make the cell to growth and division faster and easily. The enhancement of cell wall fluidity might be one of the mechanisms of the promotion of plant growth by sound field. On the other hand, the electric potential of cell membrane will be changed after the sound field stimulation. The open or close of some ion channels (for example, C_a^{2-} channel and K^+ channel) can cause their concentrations between the two sides of cell membrane change, and accordingly the electric potential of cell membrane will change, which will cause the inner auximone and other growth substances transport. As a result, the absorption and transferring substances ability of cells enhance, so the plant growth is promoted.

Acknowledgements

The authors wish to thank the support of State Fund of Nature Science (No. 39770206) and the State Ministry of Education.

References

[1] J. Braam, R.W. Davis, Rain, wind and touched expression of camodulin-related genes in Arabidopsis, Cell 60 (1960) 359–364.
[2] Daniel J. Cosgrove, Wall extensibility: its nature, measurement and relationship to plant cell growth, New Phytol. 124 (1993) 1–23.
[3] Philip M. Lintilhac, Thompson B. Vesecky, Stress-induced alignment of division plane in plant tissues grown in vitro, Nature 307 (1984) 363–364.
[4] J. Zhao, W.J. Yong, F.R. Ma, et al., Effects of high voltage electostatic field (HVEF) on proliferation of callus from alfalfa leaf, Acta Biophys. Sinica Chin. 13 (2) (1997) 255–260.
[5] G.Y. Shi, Q.J. Zhou, Z.Q. Zhang, The effect of high voltage electrostatic field (HVEF) on growth of ginkgo biloba callus, Acta Biophys. Sinica Chin. 15 (3) (1999) 547–550.
[6] A. Haley, A.J. Russell, N. Wood, et al., Effect of mechanical signaling on plant cell cytosolic calcium, Proc. Natl. Acad. Sci. USA 92 (1995) 4127–4128.
[7] C.N. Giannopolitia, S.K. Ries, Superoxide Dismutasts I. occurrence in higher plant physiol. 59 (1977) 315.
[8] W.C. Cai, H.J. Yuan, Usual Analysis Methods of Biological Materials, Science press, Beijin, 1982, pp. 93–95.
[9] W.C. Cai, Experimental Guidance of Plant Physiology, High Education Press, Beijin, 1980, pp. 189–191.
[10] B.C. Wang, Effect on carrot cell growth response in suspension culture under ultrasonic condition, 10th ICB, Pecs, Hungary, July 1999.
[11] S.J. Roux, Calcium as a mediation of plants directional growth response to gravity, in: A.M. Asashima, G.M. Alacinski (Eds.), Fundamentals of Space Biology, Japan Sci. Soc. Press, Tokyo, Springer-Verlag, Berlin, 1990, pp. 57–67.
[12] R.G. Alscher, W. Cumming (Eds.), Stress Responses in Plants: Adaption and Acclimation as Mechanism, J. Wiley-Liss, NewYork, 1990.
[13] C. Bowler, Montagu Van I, D. Inze, Superoxidise dismutase and stress tolerance, Ann. Rev. Plant Physiol. Plant Mol Biol. 43 (1992) 83.
[14] C.X. Yuan, J. Ding, The effects of water stress on the content of IAA, IAA oxidase activity and peroxidase activity in cotton leaf, Plant Phys. Lett. Chin. 16 (2) (1990) 179–184.
[15] D.S. Bush, Calcium regulation in plant cell and its role in signaling, Ann. Rev. Plant Physiol. Mol. Biol. 46 (1995) 95–122.
[16] K.L. Sun, B.X. Xi, The effects of alternative stress on the thermodynamical properties of cultured tobacco cells, Acta Biophys. Sinica Chin. 15 (3) (1999) 579–583.