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Effect of sound stimulation on cell cycle of chrysanthemum (*Gerbera jamesonii*)

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Abstract

Plant growth can be considered as the sum of cell proliferation in the meristems and the subsequent elongation of cells. The continuous proliferative capacity of plant cells is crucial for the production of new organs and thus has a significant impact on plant architecture. Now it had been found that the relationship between environmental factors and growth of plant was very close. And in this paper, the effect of sound stimulation on the cell cycle of chrysanthemum was studied to further explore the mechanism of biological effect of sound stimulation.
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Keywords: Sound stimulation; Cell cycle; Environmental factors; Chrysanthemum

1. Introduction

Since their immobility, plants are inevitably affected by environmental stresses. The relationship between environmental stress and plant development is one of the important research fields focused by biologists and physicists. Now it was found that plants could respond to the environmental factors of wind, rain, touch, electric field and ultraviolet radiation and alter its physiological condition to adapt to the change of environment [1–3].

Plant growth can be considered as the sum of cell proliferation in the meristems and the subsequent elongation of cells. The continuous proliferative capacity of plant cells is crucial for the production of new organs and thus has a significant impact on plant architecture. The alteration of cell cycle is very close to the growth and development of plant. Now the regulation of cell cycle about mammal and yeast has been widely studied, but the research on the cell cycle about plant is relatively lacking. It had been found that the cell cycle in higher plant was regulated by many inner and outer factors. Tang [4] reported that the number of cell and the proportion of cell in S phase increased under the stimulation of low-frequency electromagnetic field. It had been found that the sound wave with some strength and

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frequency could accelerate the growth of plant [5], but the mechanism of promotion was still not clear. In this paper, we cultured the seedling of chrysanthemum under sound wave. The change of cell cycle under the stimulation of sound was measured to explore the influence of sound wave on the growth and cell cycle of plant. We have chosen chrysanthemum as the experimental system because of the single hereditary character.

2. Materials and methods

2.1. Materials

Stems from chrysanthemum seedlings were inoculated in conical flasks with 20 ml MS solid medium each (supplemented with 0.001 mg/l IAA), and were cultured in illumination incubator at 26 °C.

2.2. Sound stimulation

Alternating stimulation field was achieved by sound generator, which was designed in our lab. Exuberantly growing chrysanthemum seedlings were cut into even stems and inoculated in mediums. Inoculated stems were stimulated by sound wave with a certain intensity (100 db) and frequency (1000 Hz) for 9 days, and each day for 60 min. Control group were placed in the same environment with stressed ones.

2.3. Preparation of protoplast

Referring to Galbraith [6].

2.4. Measurement of cell cycle using flow cytometry (FCM) [9]

Referring to Laurent [7].

3. Results

Figs. 1 and 2 were the photographs of cell cycle for the control and stressed chrysanthemum. The proportion of DNA in different phase was shown in Table 1. In the method of flow cytometry (FCM), the proportion of DNA in S phase was usually looked on as the mark of cell proliferation. In this paper, the results of FCM showed that the cell cycle of chrysanthemum changed greatly and the proportion of cell in S phase increased and that of cell in G_0/G_1 decreased (Table 1).

4. Discussions

Natural plants inevitable suffer from the environmental stress but the research found that plant could usually adapt to the change of environment by means of changing their outer form, inner structure and physiological character. Peng [14] studied the effect of gradient magnetic field on the physiologic and biochemical functions of rice seedling, and he found after the treatment of gradient magnetic field, the content of chlorophyll, proline, soluble protein increased remarkably, and SOD activity raised greatly, while membrane permeability and POD activity decreased. In addition to this, the root and seedling were higher than the control. Our research suggested that sound stimulation could accelerate the growth of chrysanthemum callus. So far, the researcher had studied the effect of environmental factors on the growth of plants from physiological and biochemical direction. And the research from cell cycle direction about the effect of environmental factors, especially sound stimulation, is relatively rare.

Cell proliferation is one of the most important character of living activity and the basis of growth and development of plant, which is usually realized by the process of cell cycle. The mitotic cell cycle consists of alternating rounds of DNA replication (which occurs during the S phase) and chromosome segregation (mitosis or M phase) interrupted by gaps known as G_1 (the interval before S phase) and G_2 (the interval after S phase) [7]. Progression through the major transitions of the eukaryotic cell cycle is driven by a family of serine/threonine kinases known as cyclin-dependent kinases (CDKs). The catalytic activity of these protein kinases is regulated by the association with their regulatory subunits, cyclins. The activity of the

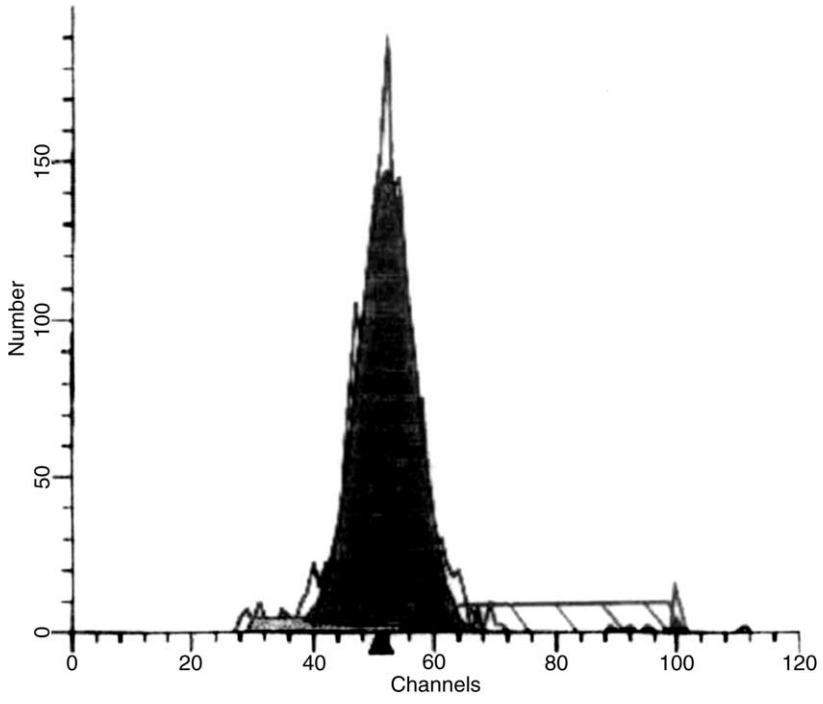

Fig. 1. Cell cycles chart of non-stimulated *Gerbera jamesonii*.

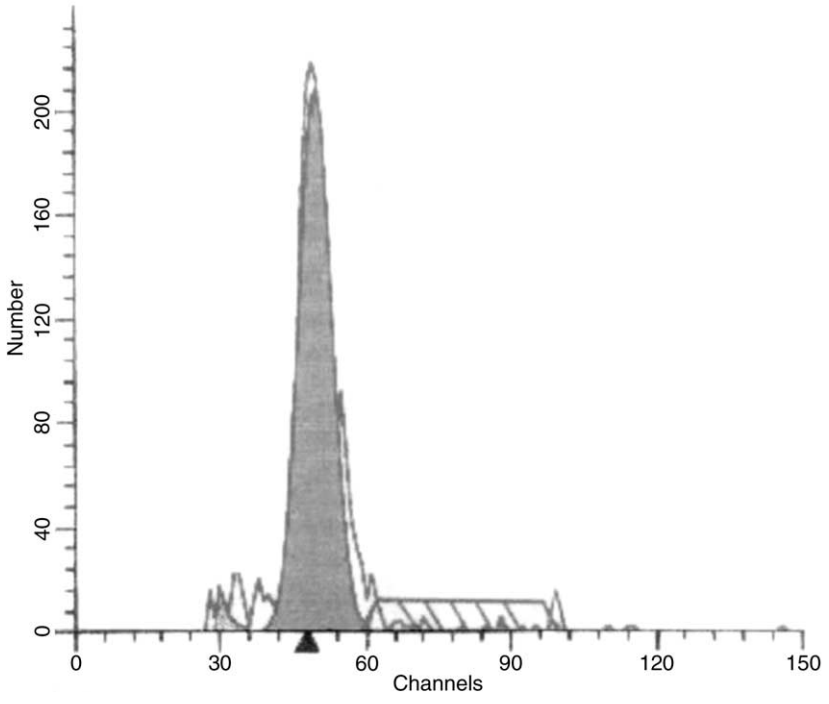

Fig. 2. Cell cycles chart of stimulated *G. jamesonii*.

Table 1
Change of cell cycles of chrysanthemum under sound stimulation

	G_0–G_1 (%)	S (%)	G_2 (%)
CK	78.18	21.76	0.06
T9	58.63	41.33	0.04

complexes is further controlled by a number of mechanisms including phosphorylation/dephosphorylation, interaction with inhibitory proteins, proteolysis, and intracellular trafficking [10]. CDKs are key regulators of the cell cycle and their activities are consequently tightly regulated. Recent developments in the field of CDK regulation have included the discovery and characterization of CDK inhibitors. These developments have had an impact on our understanding of how other signaling pathways may be linked to the cell cycle machinery [11]. Plants, like animals, possess also an array of CDK-like kinases, but their functions are poorly defined [12].

FCM allows the simultaneous measurement of multiple fluorescences and light scatter induced by illumination of single cells or microscopic particles in suspension, as they flow rapidly through a sensing area. FCM is increasingly used for basic, clinical, biotechnological, and environmental studies of biochemical relevance [8]. The cell cycles for different cells are significantly diverse, and even for the cells of the same kind, the cell cycle may change with the variation of physiological behavior, nutrition condition and environment. Tang [4] studied the effect of electric magnetic field (EMF) with low frequency on the cell cycle and apoptosis of osteoblast using FCM, he found that EMF could enhance the proportion of cells in S phase and reduce the ratio of apoptosis cells, which indicated that the effect of EMF on the cell proliferation might work by the means of the change of cell cycle and apoptosis. So the environmental factors could highly influence the cell cycle of life. In this paper, we studied the effect of sound wave on the cell cycle of chrysanthemum using FCM. The results showed sound wave could greatly change the cell cycle of chrysanthemum and the number of cell in G_0/G_1 decreased while that in S phase increased, which indicated sound wave accelerated the growth of chrysanthemum. Li [13] reported that sound wave could diversely work on the cell cycle and apoptosis of different kinds of cells and accelerate the growth of tobacco cells. But what does CDKs function as in the effect of sound stimulation? How are the signaling pathways linked to the cell cycle machinery? So the mechanism of effect of sound wave on the cell cycle is very complex and the relative research is further needed.

Acknowledgements

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