Bot. Bull. Academia Sinica 24: 169-173 (1983)

FUSICOCCIN DOES NOT INHIBIT THE EMBRYOGENESIS OF CULTURED WILD CARROT CELLS[1,2]

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(Received May 13, 1983; Accepted June 10, 1983)

Abstract

The effects of fusicoccin (FC) on H^+ transport and membrane potential in cultured cells of *Daucus carota* L. (wild carrot), and its effects on somatic embryogenesis were studied. H^+ transport and membrane potential were measured separately with a pH electrode and glass microelectrodes. FC stimulated H^+ extrusion and made the membrane potential more negative in these cells, but no inhibitory effect on all stages of embryogensis was observed at FC concentrations up to 10 μM.

Introduction

Cultured cells of *Daucus carota* L. (wild carrot) can be manipulated to regenerate whole plants through somatic embryogenesis. The switch from callus proliferation to embryogenesis can be obtained by removing 2,4-dichlorophenoxyacetic acid (2,4-D) from the medium (Halperlin and Wetherell, 1964; Sung *et al.*, 1979).

Auxins can induce cell enlargement in plant stems and coleoptiles (Cleland, 1971), and stimulate H^+ extrusion (Cleland, 1973; Marré *et al.*, 1973) and membrane hyperpolarization (Etherton, 1970; Marré *et al.*, 1974). Fusicoccin (FC) has similar effect on these stated tissues and the response to FC is usually more rapid and drastic than to auxins (Marré, 1977).

Since FC is also reported to stimulate H^+ extrusion in cultured cell suspensions and protoplasts (Rollo *et al.*, 1977; Rubinstein and Tattar, 1980) and to hyperpolarize the membrane potential of oat mesophyll protoplasts (Rubinsten and Tattar, 1980), the effect of FC on embryogenesis of wild carrot was studied.

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[1] Paper No. 264 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

[2] This work was supported by the National Science Council and Academia Sinica, Republic of China.

Materials and Methods

The strain of wild carrot callus culture (W001C) was donated by Dr. Z. R. Sung at the University of California, Berkeley, CA, USA. Fusicoccin was provided by Dr. G. Michieli at the Farmoplant, Milan, Italy.

To study the efficiency of embryo formation, the procedure developed by Sung *et al.* (1979) was followed with minor modification. Embryogenesis was initiated by transferring suspensions of carrot cells maintained in Murashige and Skoog's (MS) medium (Linsmeir and Skoog, 1965) supplemented with 0.1 mg/l of 2,4-D (R1 medium) to the medium devoid of 2,4-D (A1 medium). After the cells were incubated one day in A1 medium, they were filtered through a 205-μm nylon filter and washed twice with the same medium. The cells were then resuspended with the same medium but supplied with various concentrations of FC and buffered with 10 mM of 2-[N-morpholino]ethanesulfonic acid (MES). By means of optical density (Klett unit; Sung, 1976), cell densities were estimated and adjusted to 3×10^4 cells/ml. The cells were incubated in sterilized plastic petri dishes and later scored as callus, globular-stage, heart-stage, torpedo-stage embryos and plantlets.

Membrane potential of cultured cells was measured with glass microelectrodes of tip resistance between 10 and 15 MΩ, and connected to a WPI model 750 dual microprobe amplifier (W-P Instruments, Inc., Conn., USA). Cells in A1 medium filtered through a 205-μm nylon filter were added to a narrow chamber of 1 mm inner gap. When cells settled at the bottom of the chamber, a cell was impaled with a glass microelectrode coming downward vertically. After a steady resting membrane potential was observed, FC or 2,4-D was administered to the chamber with a micro-syringe. The membrane potential was recorded continuously.

Changes in external pH were measured with a combination pH electrode (Radiometer model GK 2320C) connected to a Radiometer model PHM 26 pH meter and recorded with a recorder. Cells in R1 medium were filtered through a 205-μm nylon filter and washed three times with medium containing 163.5 mM glucose, 3 mM $CaCl_2$, 10 mM KCl and 0.1 mM MES, pH 6.0. After the cells were equilibrated for 40 min at 26°C on a rotary shaker (150 rpm), pH change was traced. When pH was stabilized, FC or 2,4-D at a final concentration of 10 μM was added. pH changes were converted to amounts of H^+ extrusion by back titration with 0.01 N HCl. For routine study, 8 ml of cells at 3×10^6 cells/ml was used.

Results and Discussion

The frequency of embryo formation was scored according to the development stages, namely callus, globular, heart, torpedo and plantlet, until most of the cells regenerated into plantlets. In the presence of FC up to 10 μM, the formation frequencies were virtually indistinguishable from those observed in A1 medium at

any of those stages (Fig. 1). No inhibition of embryogenesis by FC was then observed. The results were similar in the presence or absence of MES in A1 medium (data not shown).

The effects of FC on H^+ extrusion and membrane potential are shown in Table 1. FC at 10 μM induced acidification of medium and cause hyperpolarization of the membrane potential in less than 5 seconds. The result was similar to that of FC on cultured oat protoplasts (Rubinstein and Tattar, 1980). FC was active in hyperpolarizing membrane potential and stimulating H^+ extrusion, while it did not inhibit embryogenesis. On the other hand, 2,4-D was inactive in stimulating membrane hyperpolarization and H^+ extrusion, but was highly active in blocking any stages of the full embryogenesis process (Sung *et al.*, 1979). We may conclude that the enhanced H^+ extrusion or membrane hyperpolarization does not involve in blocking wild carrot embryogenesis. Therefore, the mechanism of 2,4-D inhibition of embryogenesis might differ from that of 2,4-D induced cell enlargement. Although an overall membrane hyperpolarization did not seem to have significant effect on wild carrot embryogenesis, the result did not rule out the possibility that the localized gradient of electric field or ion(s) could occur and may contribute a driving force to polarizing the formation of plumule-radical axis.

Table 1. *Effect of fusicoccin on membrane potential (E_m) and H^+ extrusion in cultured wild carrot cells*

Experimental conditions were described in "materials and methods". The basic medium used for potential measurement was A1 medium, and for pH measurement was 163.5 mM glucose+3 mM $CaCl_2$+10 mM KCl+0.1 mM MES, pH 6.0. In the assays of pH, the initial pH was 6.0.

Treatment	E_m	Increase in H^+ excretion
	mV	neq/10^7 cells/min
None	−56	
10 μM 2,4-D	−55	not detectable
10 μM FC	−68	7.9±2.4

Acknowledgement

Fusicoccin and wild carrot culture were kindly provided by Dr. G. Michieli and Dr. Z. R. Sung, respectively. This work was supported by a grant from the National Science Council and in part from Academia Sinica, Republic of China.

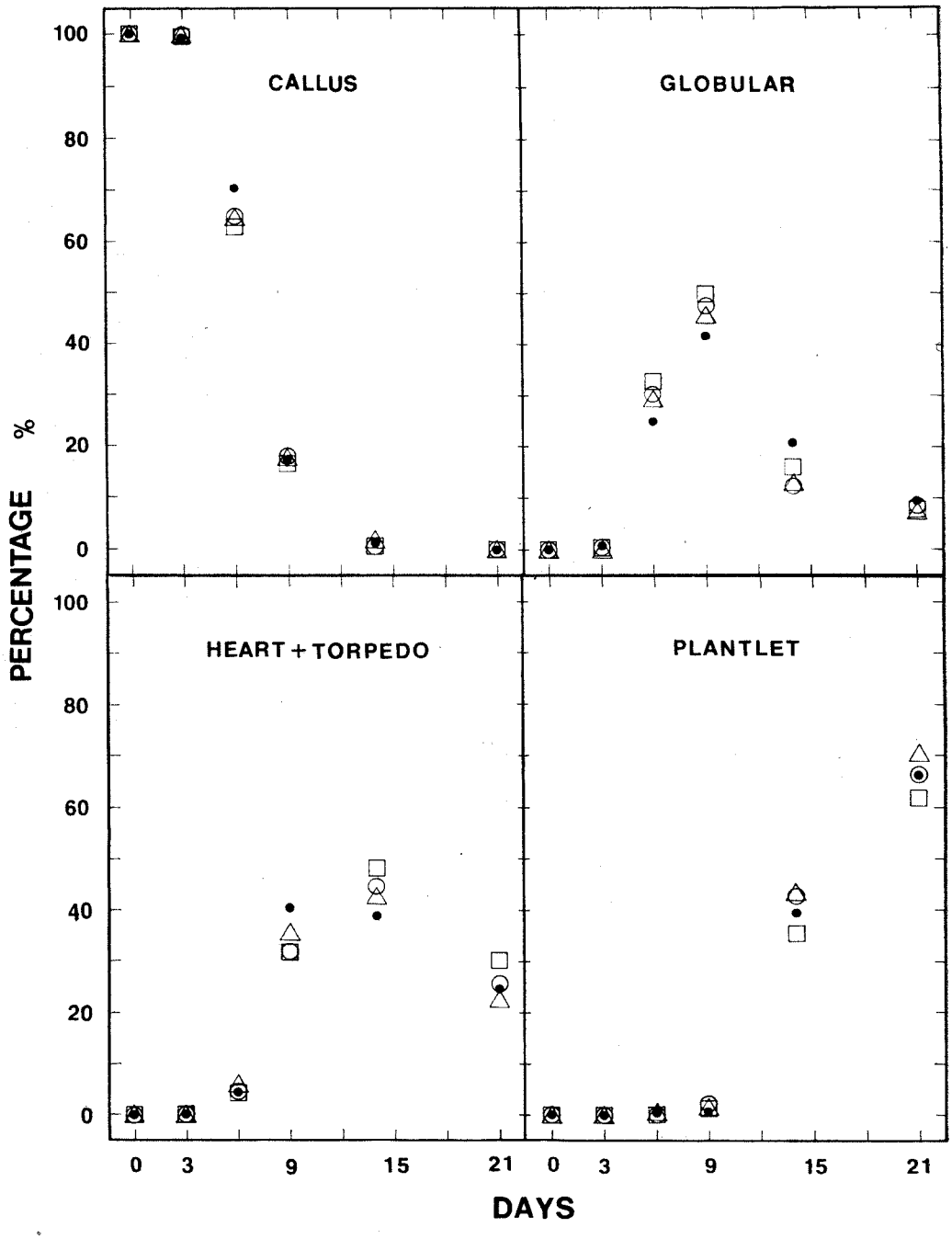

Fig. 1. Effect of fusicoccin concentration on frequencies of embryo formation in cultured wild carrot cells. The frequency was presented as percentage of the total cell clumps (callus) or embryos at the particular stage(s). Experiments began on day 0 when cells were washed and incubated in petri dishes containing A1 medium or A1+FC.
● A1; □ A1+1 μM FC; ○ A1+2 μM FC; △ A1+10 μM FC

Literature Cited

Cleland, R.E. 1971. Cell wall extension. Ann. Rev. Plant Physiol. **22**: 197-222.

Cleland, R.E. 1973. Auxin-induced hydrogen ions excretion from *Avena* coleoptiles. Proc. Natl. Acad. Sci. USA **70**: 3092-3093.

Etherton, B. 1970. Effect of indole-3-acetic acid on membrane potentials of oat coleoptile cells. Plant Physiol. **45**: 527-528.

Halperin, W. and D.F. Wetherell. 1964. Adventive embryony in tissue cultures of the wild carrot, *Daucus carota*. Amer. J. Bot. **51**: 274-283.

Linsmeir, E.M. and F.C. Skoog. 1965. Organic growth factor requirements of tobacco tissue culture. Physiol. Plant. **18**: 100-127.

Marré, E. 1977. Physiological implications of the hormonal control of ion transport in plants. *In* E. Marré (ed.), Plant Growth Regulation. Springer-Verlag, Berlin, pp. 54-66.

Marré, E., P. Lado, E.R. Caldogne, and R. Colombo. 1973. Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation. Plant Sci. Lett. **1**: 179-184.

Marré, E., P. Lado, A. Ferroni, and A.B. Denti. 1974. Transmembrane potential increase induced by auxin, benzyladenine and fusicoccin. Correlation with proton extrusion and cell enlargement. Plant Sci. Lett. **2**: 257-265.

Rollo, E., E. Nielsen, F. Sala, and R. Cella. 1977. Effect of fusicoccin on plant cell cultures and protoplasts. Planta **135**: 199-201.

Rubinstein, B. and T.A. Tattar. 1980. Regulation of amino acid uptake into oat mesophyll cells: a comparison between protoplasts and leaf segments. J. Exp. Bot. **31**: 269-279.

Sung, Z.R. 1976. Turbidimetric measurement of plant cell culture growth. Plant Physiol. **57**: 460-462.

Sung, Z.R., R. Smith, and J. Horowitz. 1979. Quantitative studies of embryogenesis in normal and 5-methyltryptophan-resistant cell lines of wild carrot. Planta **147**: 236-240.

杏枝枯病毒素對野生胡蘿蔔組織之體胚形成無抑制作用

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杏枝枯病毒素（fusicoccin）雖可促使野生胡蘿蔔（*Daucus carota* L.）之細胞排出氫離子，及增加細胞膜之負值電位，但是該毒素之濃度高達 10 μM 時，仍然不能抑制組織形成體胚。