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Tissue culture response to fusicoccin

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Fisiologia vegetale. — *Tissue culture response to fusicoccin* (*). Nota (**) di MILENA BANDIERA e GIORGIO MORPURGO, presentata dal Socio G. MONTALENTI.

RIASSUNTO. — Sono stati condotti esperimenti al fine di stabilire se la fusicoccina, un glicoside fitotossico, che determina una vasta gamma di modificazioni nei tessuti vegetali, possa sostituire le auxine come fattore di crescita di colture di tessuti di *Daucus carota* e *Nicotiana* sp. È risultato che la fusicoccina stimola nettamente alcuni cloni ed inibisce altri della stessa specie. La crescita è dovuta a moltiplicazione cellulare.

INTRODUCTION

Fusicoccin A, the phytotoxic glycoside produced by *Fusicoccum amygdali* [1, 2] strongly stimulates water uptake in various plant tissues [3]. The stimulation of cell enlargement, accompanied by increased cell wall plasticity, and the increase in fresh weight, both promoted by the toxin [4, 5, 6, 7, 8, 9], are typical effects of auxins. Fusicoccin however lacks some auxinic characteristics such as ability to inhibit cell elongation in root tissues [3].

The present research was aimed at investigating whether the action of fusicoccin in stimulating cell multiplication of plant tissue culture is similar to that of auxins. In fact it is well known that continuous growth and cellular multiplication of tissue cultures, in synthetic medium, are strictly dependent on adequate exogenous supply of auxins [10].

MATERIALS AND METHODS

Media. In all experiments the Murashige and Skoog [11] basal medium (MS) was used.

Isolation and, in some cases, maintenance of strains were carried out on White [12] medium (CM) as modified by Steward [13] or in Lindsmaier and Skoog [14] medium (LS).

Chemicals. Fusicoccin A was a gift from prof. S. Russi and prof. A. Ballio. Stock solution of FC was prepared as follows: 20 mg were dissolved in 1 ml of ethanol 95° α and water was added to a final concentration of 25 mg % *w/v*.

Kinetin (K), i.e. 6-furfuril-amino-purin, was purchased from Fluka, auxins from NBC.

(*) Lavoro eseguito presso il Centro di Genetica Evoluzionistica nell'Istituto di Genetica dell'Università di Roma.

(**) Pervenuta all'Accademia il 5 agosto 1975.

Strains. Tissue cultures of *Daucus carota* derived in this laboratory from discs of root, according to the technique of Gautheret [15], were subcultured on agarized CM supplemented with 2–4 dichloro-phenoxy-acetic acid (2–4 D) 0.1 mg/l.

Tissue cultures of *Nicotiana tabacum* (var. Barley) were obtained directly from the seeds on CM supplemented with 2–4 D (0.1 mg/l). On this medium, at germination time, roots do not develop: the presence of the hormone leads to the complete dedifferentiation of the tissue and consequently to the growth of undifferentiated callus.

Nicotiana langsdorffii callus, kindly supplied by prof. M. Buiatti, was grown on LS supplemented with 2–4 D (0.5 mg/l).

Growth measurement. From six to ten callus fragments of about 200 mg fresh weight, were transferred from the maintenance medium to MS in various experimental hormonal conditions.

Cultures were incubated in continuous light at 24 °C and aseptically weighed and transferred on fresh medium, every 10–15 days till the end of the experiment.

In two cases titration of cellular content in thymine was carried out. The rationale of the method is the assumption that DNA increased proportionally to cell number. For microbiological thymine titration a mutant strain of *E. coli* K 12 with a specific requirement for thymine has been used. Callus cells were disrupted with a Potter homogenizer, hydrolyzed 60 minutes with perchloric acid 70 % at 100 °C and neutralized with KOH.

After centrifugation (30 minutes–8500 rpm) the supernatant was dried at 70 °C, resuspended in distilled water and used for the " fish spin " method of titration [16].

RESULTS

Growth curves of *Daucus carota.* 1) Culture established on 1969 (DC 69). *Daucus carota* callus was used to test the effects of different doses of FC and indol-3-acetic acid (IAA) with and without the addition of K. Results are shown in figs. 1, 2, 3 and 4. FC stimulates the increase of fresh weight of the culture at a concentration between 0.001 and 0.1 mg/l and becomes inhibiting at a concentration higher than 0.1 mg/l.

At the end of the experiment samples from three cultural conditions were tested for thymine content (Table I).

The relative concentration of thymine supports the idea that, in this case, FC stimulates growth of the callus by cell division. Experiments on this strain were not repeated because the culture suddenly became " anergee " and therefore auxin-independent [17].

2) Culture established on 1967 (DC 67). The hormonal reponse test gave the results that are presented in fig. 5. FC at all concentrations is completely ineffective in stimulating growth. On the contrary it causes a remarkable inhibition at concentrations higher than 1 mg/l.

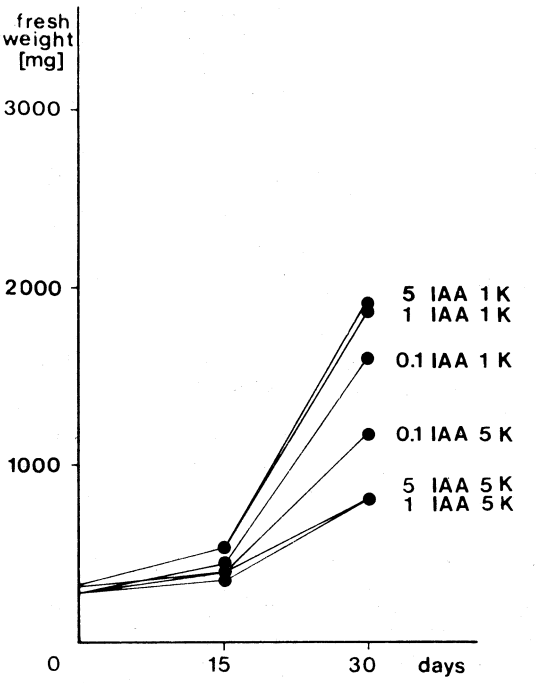

Fig. 1. – Growth curves of *Daucus carota* (DC 69) on MS medium supplemented with different concentrations (mg/l) of indol-3-acetic acid (IAA) and kinetin (K).

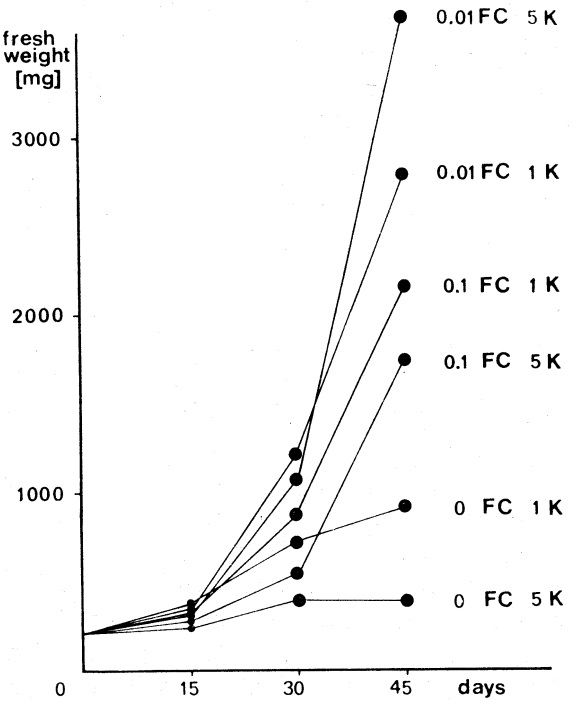

Fig. 2. – Growth curves of *Daucus carota* (DC 69) on MS medium supplemented with kinetin (K) and fusicoccin (FC) (concentrations in mg/l).

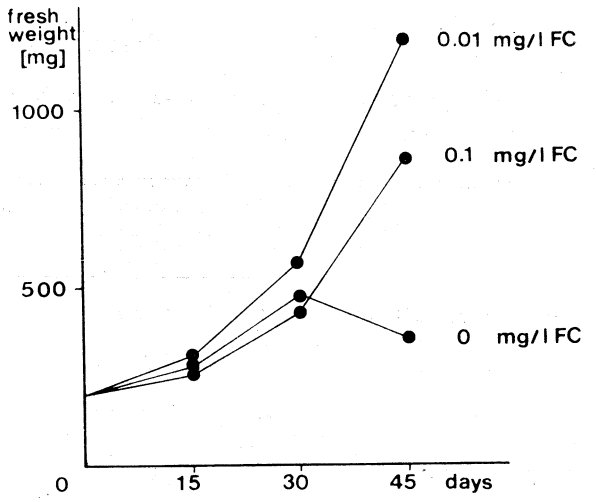

Fig. 3. – Growth curves of *Daucus carota* (DC 69) on MS medium with and without fusicoccin (FC).

Growth curves of *Nicotiana*. In our experiments two strains of independent origin were used: the original one (see Material and methods) and a second strain also originated by seed of the same variety of tobacco. The latter has been isolated as follows: 1500 seeds were aseptically plated on MS supplemented with various concentrations of FC and 2–4 D. 2–4 D alone, in the concentration range between 0.1 and 10 mg/l inhibits the growth of the root, stimulating callus formation.

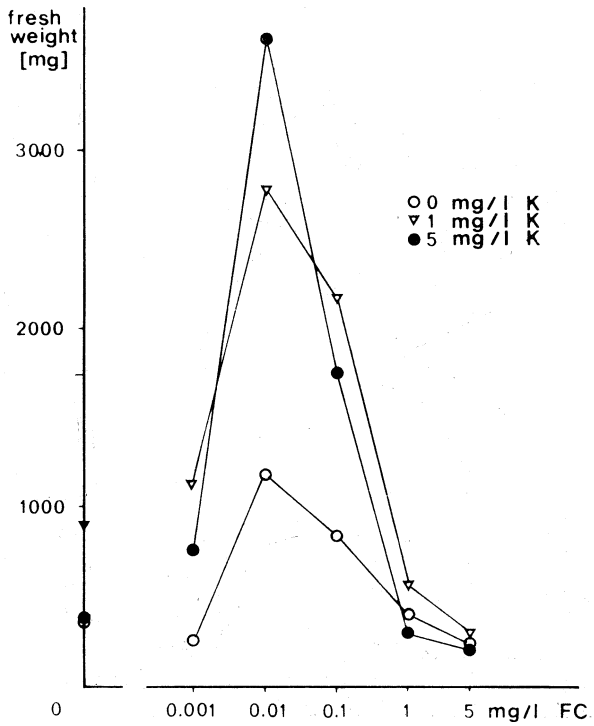

Fig. 4. – Growth response of *Daucus carota* (DC 69) on MS medium to various concentrations of kinetin (K) and fusicoccin (FC) (45 days old cultures).

TABLE I.

Thymine concentrations in cultured samples of Daucus carota (DC 69) *and fusicoccin stimulated* Nicotiana tabacum *on variously supplemented MS medium.*

	Hormonal supplement (mg/l)	Mean weight of samples (mg)	Thymine γ % g fresh weight
Daucus carota (DC 69) . . .	1 K 1 IAA	2.040	19,9
	1 K 0,1 2–4 D	2.750	25,0
	1 K 0,01 FC	1.160	38,7
Nicotiana (FC stim.)	10 IAA	7.620	60,0
	1 NAA	7.830	41,0
	1 2–4 D	4.410	90,0
	0,1 FC	2.430	42,0
	0,0001 FC	4.310	38,0

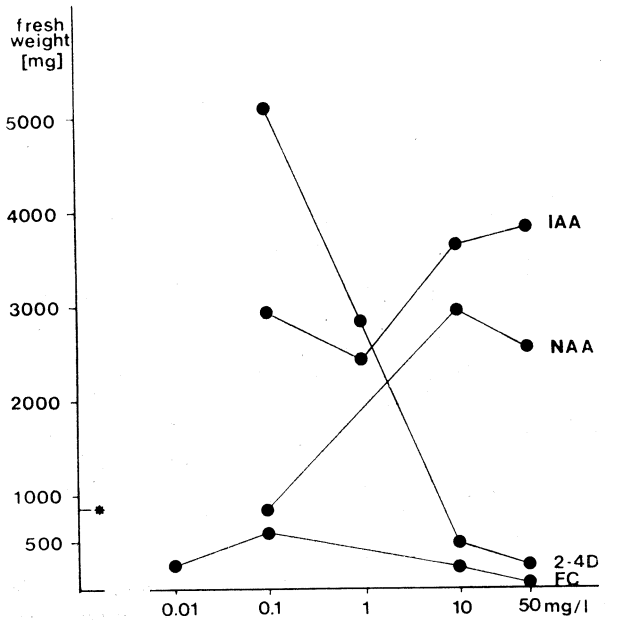

Fig. 5. – Growth response of *Daucus carota* (DC 67) on MS medium to fusicoccin (FC) and auxins (45 days old cultures) * mean weight of the inocula. (Abbreviations were settled in the text).

FC alone never induced callus formation at any concentration tested (0.001–10 mg/l), although at some concentrations it stimulates growth of seedlings (Bandiera in preparation). When seeds were plated on a medium where FC and 2–4 D were simultaneously present, only in one case was a vigorously growing callus obtained, in the presence of 0.1 mg/l FC and 1 mg/l 2–4 D. The callus turned out to be stimulated by FC.

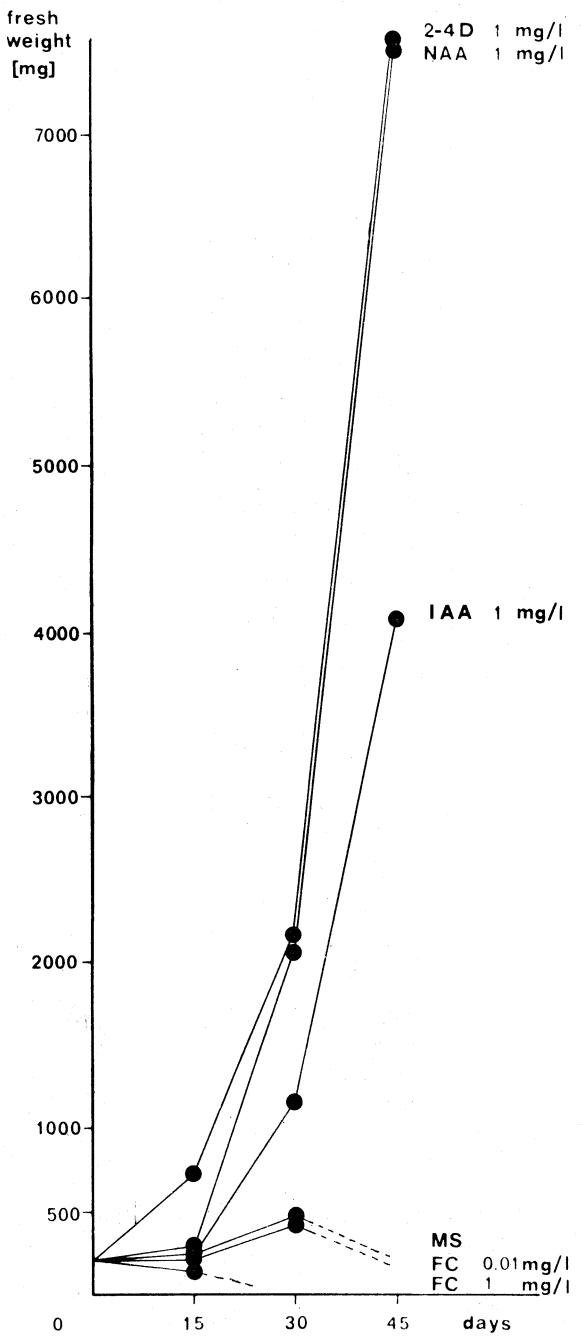

Fig. 6. – Growth curves of *Nicotiana tabacum* on MS medium supplemented with auxins and fusicoccin (FC).

Fig. 6 shows the growth curves in the presence of different concentrations of auxins (IAA, 2–4 D and naphtalene-acetic acid–NAA) and FC, of the original strain. It is evident that FC, in this strain, at any concentration cannot substitute auxins as a growth factor.

Fig. 7 shows the growth curves of the callus stimulated by FC. The doses of FC indicated in the figure are the most effective in the range tested

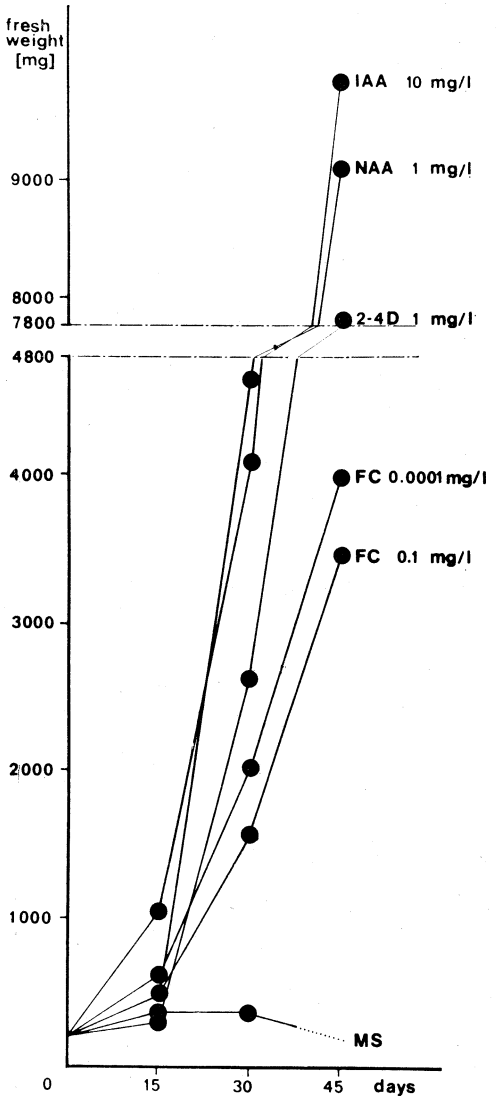

Fig. 7. – Growth curves of *Nicotiana tabacum* callus "stimulated by fusicoccin", on MS medium supplemented with auxins and fusicoccin.

(from 0,00001 to 50 mg/l). It is clear that FC, although less effective than auxins, can support continuous growth in this particular strain.

The analysis of the total content of thymine (Table I) confirms that growth is due mainly to cell multiplication, not only to cell enlargement.

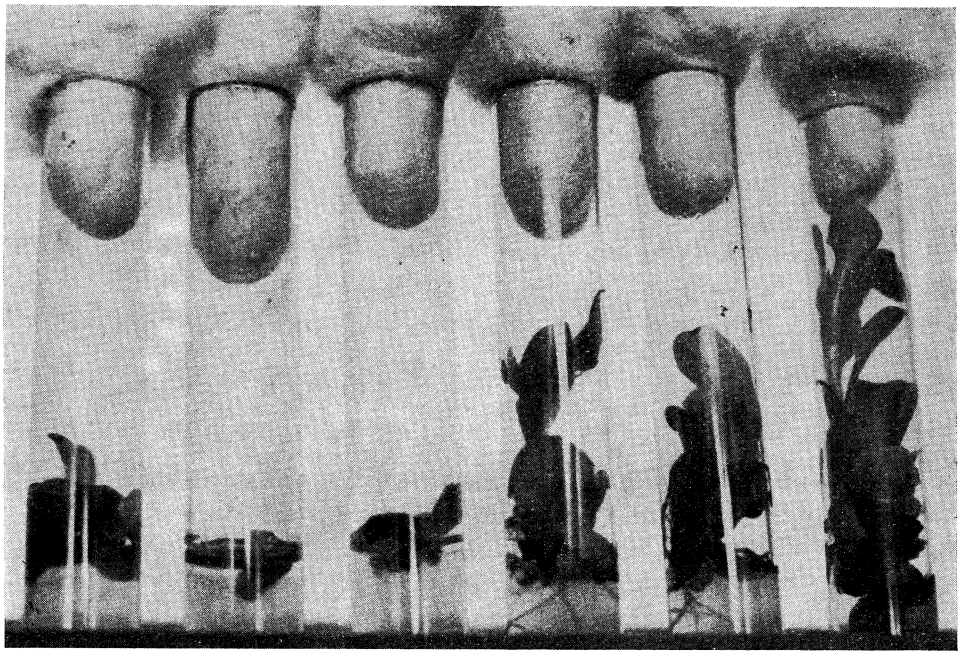

Fig. 8. – Plantlets from fusicoccin stimulated callus one month after transfer on MS medium supplemented with fusicoccin. FC concentrations are 0–0.001–0.01–0.1–1–10 mg/l (left to right).

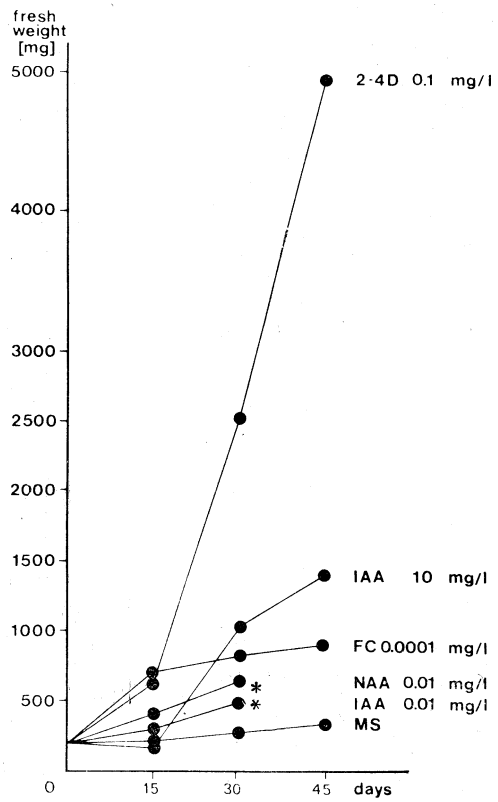

Fig. 9. – Growth curves of *Nicotiana langsdorffii* on MS medium supplemented with optimal doses of auxins and fusicoccin. * callus undergoes differentiation.

Cells of the *Nicotiana tabacum* strain stimulated by FC were also induced to differentiate into whole plants by transferring the callus onto MS supplemented with K (0.1 mg/l) and IAA (1 mg/l).

The possibility of an inhibitory effect of FC on these newly formed plantlets was tested. A strong stimulatory effect of FC on the growth rate of the plantlets was evident (fig. 8) at the highest concentrations. No wilting was observed even at the stage of well developed leaves.

Fig. 9 finally reports the growth curves of *Nicotiana langsdorffii* at the optimal doses of auxins and FC. It is evident that there is no stimulatory effect by FC with regard both to weight increase and to bud formation.

Discussion

The data here presented lead to the conclusion that on some particular strains FC may stimulate indefinite growth of plant cell cultures, substituting auxins as growth factor. In those cases weight increase does not apparently depend on cell enlargement, but on actual cell multiplication; this conclusion is based on the finding that the increase of fresh weight is accompanied by the increase of the total DNA content.

However the stimulatory effect of FC is not a constant feature, some clones being severely inhibited by FC, and it is not species specific because one of two clones of *Daucus carota* and one of two clones of different species of *Nicotiana*, were similarly stimulated, while the other clones were inhibited.

It is very difficult at present to speculate on the nature of the condition that permits cells to be stimulated by the toxin in some particular clones. Unfortunately the action either of FC or of auxins at biochemical level is unknown.

FC, when it does not stimulate growth, shows a pronounced toxic effect, proving that the drug still enters the cells. This seems to rule out the possibility that a mutation acts making the cells impermeable to FC.

In the case of stimulated strain of *Nicotiana tabacum*, the process that produces the resistance acts also on the whole plant. This fact again suggests that the response of all tissue to FC may depend on the genetic constitution of the strain.

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