Bol. Botânica, Univ. S.Paulo
6: *67-73, 1978*

EFFECT OF GAMMA-RADIATION ON GROWTH OF CYTOKININ-HABITUATED AND CYTOKININ-DEPENDENT TOBACCO TISSUE CULTURES

EFEITO DA RADIAÇÃO GAMA SOBRE O CRESCIMENTO DE TECIDOS DE TABACO, CITOCININA- HABITUADOS E CITOCININA-DEPENDENTES

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SUMMARY - The possibility to induce restorations in the growth of 2Krad gamma irradiated cells of cytokinin-dependent and cytokinin-habituated tobacco (*Nicotiana tabacum* cv W-38) tissue cultures was investigated. The fresh weight yields of the two strains proved to be different when submitted to the same radiation dose and grown under identical conditions. These fresh weight yields were dependent on the growth substance concentration in the media into which the irradiated tissues had been transferred. The addition of kinetin to irradiated cytokinin-habituated tissues did not result in growth restoration. Restorative effects were noticed after the addition of high concentrations (in the inhibitory range) of IAA to cytokinin-dependent tissues.After 2 Krad gamma radiation, habituated tissues grown in absence of exogenous cytokinins showed an increased reactivity to IAA additions; on the other hand, dependent tissues showed increased reactivities to kinetin additions (in the presence of 11.5 μM IAA) and to 10 or 100 μM IAA additions (in the presence of 0.1 μM kinetin).

RESUMO - A possibilidade de induzir a restauração do crescimento de tecidos irradiados com 2 Krad de radiação gama foi investigada em linhagens celulares de tabaco (*Nicotiana tabacum* cv W-38), citocinina-habituadas e citocinina-dependentes. O peso fresco das duas linhagens, após tratamentos idênticos, revelou-se diferente. O aumento do peso depende da concentração das substâncias de crescimento presentes no meio para o qual os tecidos foram transferidos. A adição de cinetina a tecidos citocinina-habituados não resultou em restauração no crescimento. Restauracões foram observadas após a adição de altas concentrações (na faixa inibitória) de AIA a tecidos citocinina-dependentes. Após o tratamento com2 Krad de raios gama, os tecidos habituados mostraram-se mais sensíveis que os controles à adição de AIA (em ausência de citocininas exógenas). Por outro lado, os tecidos dependentes mostraram-se mais sensíveis à adição de cinetina (em presença de 11,5 μM de AIA) e à adição de 10 ou 100 μM de AIA (na presença de 0,1 μM de cinetina).

INTRODUCTION

The reduction in the growth of irradiated sunflower crown-gall tumor cells was reversed, wholly or in part, by supplying the tumor cells with appropriate concentrations of indoleacetic acid (Klein & Vogel 1956). Different yields of irradiated cytokinin--habituated tissues of *Nicotiana tabacum* cv W-38 were obtained after the addition of appropriate kinetin concentrations to the growth media (Hell 1978). Decreases in growth inhibitions due to post radiation treatments with yeast extracts, fractional parts of ribonucleic acids, amino acid solutions and several cytokinins, on tissue cultures of *Helianthus tuberosus* and crown-gall tissues of *Scorzonera hispanica* were also reported (Jonard & Bayonove 1976a, b).

This paper presents further results of investigations on the possibility to induce restorations in the growth of irradiated cells, by changes in the auxin/cytokinin ratio in the culture media.

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MATERIAL AND METHODS

Two strains of tobacco callus (*Nicotiana tabacum* L cv Wisconsin-38) were used as test material: a cytokinin-dependent strain isolated from pith of young tobacco plants and a cytokinin-habituated strain isolated from callus of cytokinin-dependent tissues. Callus stock cultures were maintained on RM-65 medium with 2mg/liter IAA (Linsmaier & Skoog 1965). No exogenous cytokinins were added to the stock culture media of the cytokinin-habituated tissues and 0.2mg/liter kinetin was added to the culture media of the cytokinin-dependent tissues. Prior to irradiation, the cytokinin-dependent tissues were cultured on RM-65 with 0.03mg/liter kinetin and 2mg/liter IAA for 21 days in each of 2 transfers.

Irradiation was performed three weeks after the last transfer of stock cultures. Callus tissues to be irradiated were placed, under aseptic conditions, on moist filter paper in a 5 cm Petri dish. The tissues were exposed to 2.0 Krad gamma radiation at a dose rate of 64.2 rad/min from a ^{137}Cs source in a Caesatron model E (Atomic Energy of Canada Ltd.) After irradiation, the tissues were transferred to RM-65 media with several different combinations of IAA and kinetin (as described under "Results"). One piece of tissue (0.05g) was transferred into each 50 ml Erlenmeyer flask with 20 ml medium. The cultures were kept in the dark at about 28°C.

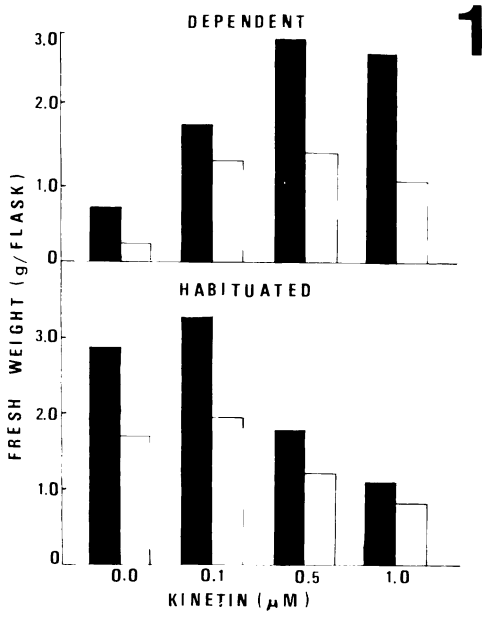
The tissues were harvested after 21 days in culture and their fresh weight was determined. Each treatment consisted of 10 - 13 replicates. From these replicates the mean fresh weight was calculated. Relative growth at each level of growth substance treatment was expressed in percent of the fresh weight yield in absence of added kinetin or in presence of 0.1 μM IAA.

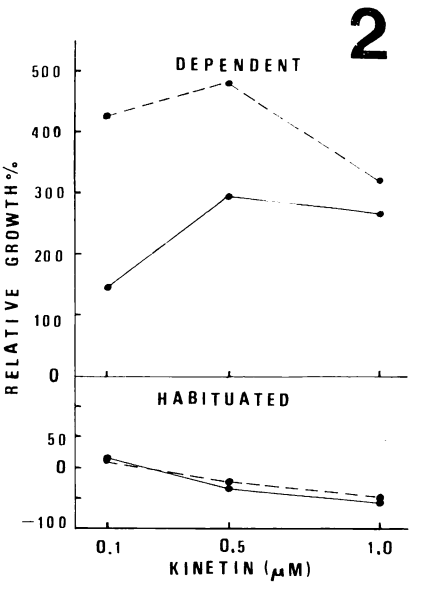
RESULTS

Fresh weight yields of control and irradiated tissues, grown in the presence of 11.5 μM IAA and several different kinetin concentrations are show in figure 1. Cytokinin-dependent tissues presented only a limited fresh weight yield in the absence of exogenously added kinetin. Additions of kinetin to the media resulted in marked fresh weight increases in these tissues, the highest yield being observed at 0.5 μM kinetin.

Fig. 1 – Effect of kinetin on growth of cytokinin-dependent and cytokinin-habituated tobacco tissue cultures: control (black) and 2Krad gamma-irradiated (white). Fig. 2 – Effect of kinetin on relative growth of cytokinin-dependent and cytokinin-habituated tobacco tissue cultures: control (full line) and 2Krad gamma-irradiated (dashed line). Fig. 3 – Effect of indoleacetic acid, in absence of exogenously added cytokinins, on the growth of cytokinin-dependent and cytokinin-habituated tobacco tissue cultures: control (black) and 2Krad gamma-irradiated (white). Fig. 4 – Effect of indoleacetic acid, in absence of exogenously added cytokinins, on relative growth of cytokinin-dependent and cytokinin-habituated tobacco tissue cultures: control (full line) and 2Krad gamma-irradiated (dashed line).

Fig. 1 – Efeito de cinetina sobre o crescimento de culturas de tecidos de tabaco citocinina-dependentes e citocinina-habituados: controles (preto) e irradiados com 2Krad (branco). Fig. 2 – Efeito de cinetina sobre o crescimento relativo de culturas de tecidos de tabaco citocinina-dependentes e citocinina-habituados: controles (linha contínua) e irradiados com 2Krad (linha interrompida). Fig.3 – Efeito do ácido indolilacético, na ausência de citocininas exogenas, sobre o crescimento de culturas de tecidos de tabaco citocinina-dependentes e citocinina-habituados: controles (preto) e irradiados com 2Krad (branco). Fig. 4 – Efeito de ácido indolilacético, na ausência de citocininas exogenas, sobre o crescimento relativo de culturas de tecidos de tabaco citocinina-dependentes e citocininas-habituados: controles (linha contínua) e irradiados com 2Krad (linha interrompida).


1
DEPENDENT
HABITUATED
FRESH WEIGHT (g/FLASK)
3.0
2.0
1.0
0
0.0
0.1
0.5
1.0
KINETIN (μM)


2
DEPENDENT
HABITUATED
RELATIVE GROWTH %
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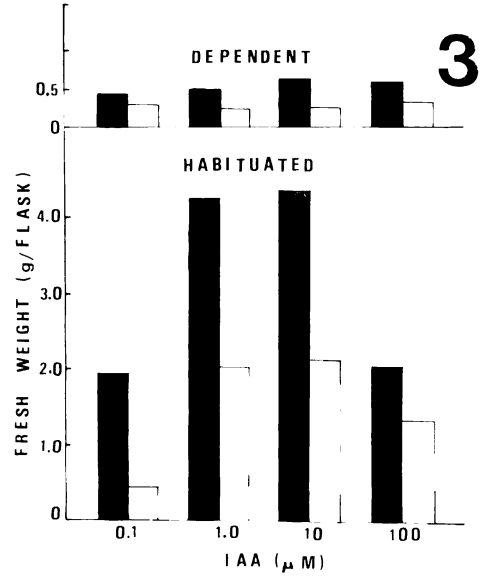
400
300
200
100
0
50
−100
0.1
0.5
1.0
KINETIN (μM)


3
DEPENDENT
HABITUATED
FRESH WEIGHT (g/FLASK)
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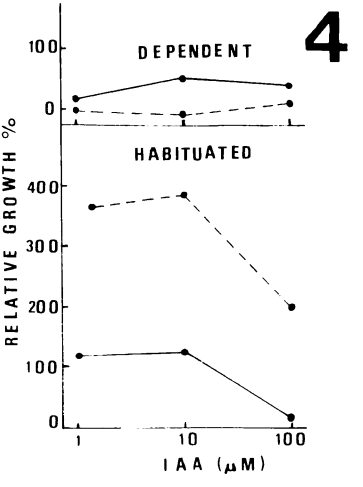
4.0
3.0
2.0
1.0
0
0.1
1.0
10
100
IAA (μM)


4
DEPENDENT
HABITUATED
RELATIVE GROWTH %
100
0
400
300
200
100
1
10
100
IAA (μM)

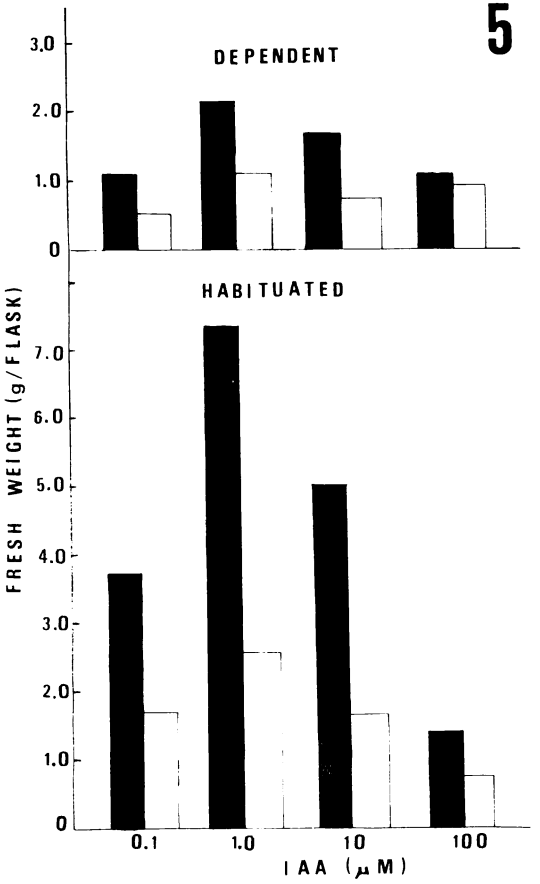
The fresh weight yields of irradiated tissues increased with increasing kinetin concentrations but were always smaller than those of the control tissues grown in the same media. Nevertheless, the relative growth values of irradiated cytokinin dependent tissues, in the presence of kinetin and IAA, were markedly larger than those of the respective control tissues (Figure 2). Cytokinin-habituated tissues produced a large amount of fresh weight in the absence of kinetin (Figure 1). Addition of 0.1 μM kinetin resulted in increased fresh weight yields, and addition of 0.5 and 1.0 μM kinetin proved to be inhibitory. Irradiated tissues presented similar responses, their fresh weight yields being always smaller than those of the corresponding control tissues (Figure 1). Furthermore, no apreciable changes in relative growth values of cytokinin-habituated tissues could be detected after 2 Krad gamma irradiation (Figure 2).

Fresh weight weight yields of control and irradiated tissues grown in the absence of exogenous cytokinins and under several different IAA concentrations are shown in figure 3. Cytokinin-dependent tissues, whether irradiated or not, when grown on media lacking cytokinins were practically unable to respond to the addition of auxins to the media. Nevertheless, irradiation descreased even more this low reactivity to auxins, as shown by the values of the relative fresh weight growth (Figure 4). Cytokinin-habituated tissues responded with large increases in fresh weight to the increase in IAA concentration in the media (Figure 3). In all instances fresh weight yields of irradiated tissues were smaller than those of the respective non-irradiated tissues (Figure 3). The irradiated cytokinin-habituated tissues showed larger relative growth increases than non-irradiated tissues grown in the same IAA concentrations (Figure 4).

Fresh weight yields of control and irradiated tissues grown in the presence of 0.1 μM kinetin and several different IAA concentrations are shown in figure 5. Addition of 0.1 μM kinetin to the media triggered responsiveness of the cytokinin-dependent tissues to the concomitant addition of IAA. Addition of 1.0 or 10 μM IAA enhanced the fresh weight yields, but the 100 μM IAA concentration proved to be inhibitory. The same was observed in irradiated tissues, but relative growth values obtained in response to 10 and 100 μM additions were larger than those noticed in non-irradiated tissues (Figure 6). Cytokinin-habituated tissues grown in presence of 0.1 μM kinetin responded with increased fresh weight yields to the presence of 1.0 or 10 μM IAA, but with decreased values to 100 μM IAA. The same tendency was demonstrated by the irradiated tissues (Figure 5). Relative growth values of cytokins-habituated tissues after gamma irradiation proved to be smaller than those of non-irradiated tissues, at the 1 μM concentration showing no differences at 10 or 100 μM IAA (Figure 6).

DISCUSSION

Tobacco callus tissues irradiated with the same dose of gamma radiation, produced quite different fresh weight yields in accordance with the growth-substance combination in the media. This effect may be the result of an increase in the mass of an essentially unchanged number of cells, possibly by early differentiation (Wangenheim & Howard 1978) or to an increase in the number of surviving cells. The two tobacco tissue strains, treated and grown in absolutely identical conditions, also presented different fresh weight yields, showing that radiation effects are also dependent on their ability to synthesize the cytokinins needed for continued growth (Einset & Skoog 1973). Since the growth of tobacco pith callus depends on the presence of auxins and cytokinins in the culture (Das *et al.* 1956), this means that the effect of the addition of one of these growth substances to an irradiated tissue will depend on the availability of the other



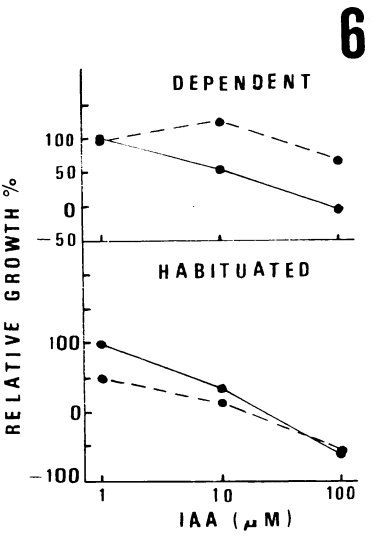


Fig. 5 – Effect of indoleacetic acid, in presence of 0.1 μM kinetin, on the growth of cytokinin-dependent and cytokinin-habituated tobacco tissue: control (black) and 2Krad gamma-irradiated (white). Fig. 6 – Effect of indolcacetic acid, in presence of 0.1 μM kinetin, on relative growth of cytokinin--dependent and cytokinin-habituated tobacco tissue cultures: control (full line) and 2Krad gamma--irradiated (dashed line).

Fig. 5 – Efeito do ácido indolilacético, na presença de 0.1 μ M de cinetina, sobre o crescimento de culturas de tecidos de tabaco citocinina-dependentes e citocinina-habituados: controles (preto) e irradiados com 2Krad (branco). Fig. 6 – Efeito do ácido indolilacético, na presença de 0.1 μM de cinetina, sobre o crescimento relativo de culturas de tecidos de tabaco citocinina-dependentes e citocinina-habituados: controles (linha contínua) e irradiados com 2Krad (linha interrompida).

regulator. This availability has to be considered in absolute terms, i. e. the presence or not of this particular substance (see dependent tissues, figures 3 and 5), and also in relative terms, i. e. its concentration in relation to the other growth-factor present in the system: callus plus media (see dependent and habituated tissues, figure 1).

Radiation-induced changes in relative growth values show that at least three different situations may be considered: a) Growth inhibition is not related to the particular growth substance under investigation. This seems to happen with habituated tissues grown in presence of several additions of kinetin (Figure 2) or in presence of 0.1 μM kinetin together with 10 or 100 μM IAA (Figure 6). b) There is an increased response of the irradiated tissues to the addititon of the growth substance (see habituated tissues, figure 4 and dependent tissues, figure 2 and also figure 6 at 10 or 100 μM IAA additions). c) The response to the additions of the growth substance showed by the irradiated tissues is less conspicuous than presented by non-irradiated tissues (see dependent tissues, figure 4). The above hypothesized situations could be related to radiation effects on the growth substance content (Skoog 1935), its biosynthesis (Gordon 1957) or on the reactivity of the irradiated tissues (Miura *et al.* 1974). Radiation-induced shifts from conditions favorable for callus growth to those favouring cell differentiation have also been reported and ascribed to changes in growth substance content or activity (Degani & Pickholz 1973, Hell *et al.* 1978).

Striking differences in radiation-induced growth-inhibitions (in % of non-irradiated tissues) of cytokinin-dependent tissues (Figure 5) were observed when the tissues were grown in the presence of 0.1 μM kinetin and 1 μM IAA (50% inhibition) as compared with the same tissues grown in presence of 0.1 μM kinetin and 100 μM IAA (11% inhibition). This kind of restorative effect of high concentrations of growth substance (in the range of inhibitory concentrations) was noticed before, with kinetin additions to cytokinin-habituated tissues treated with 3 Krad gamma radiation (Hell 1978).

Cytokinins play an important role when their supply is growth limiting, i. e. the growth of the tissue depends on an exogenous supply of kinetin. On the other hand, auxins seem to play an important role in all treatments since all tissues are dependent on their exogenous supply. Nevertheless, the IAA additions trigger the responsiveness of these tissues only in presence of an adequate amount of cytokinin, be it from exogenous or endogenous source.

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