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The effect of ionizing irradiation on the tissue culture of *Coronilla varia*

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Abstract

Long-term callus cultures of crownvetch (*Coronilla varia* L.) grown on the Murashige and Skoog's medium with 2,4-D (1 mg l^{-1}) and cultures of somatic embryos cultivated on the same basic medium but with IAA (1.0 mg l^{-1}) were exposed to ionizing irradiation. The irradiation caused a growth inhibition excepting the lowest dose of 2.5 Gy. The highest dose of 160 Gy induced browning of the culture but this colour change was not lethal. The amount of "giant cells" present in both cultures was dependent on the dose of irradiation.

Introduction

Cultures of plant explants represent a suitable material for testing of physiological and genetic effects of ionizing irradiation. The significant resistence of plant tissue cultures to ionizing irradiation in comparison with intact plants was found. With growing disintegration of the organism and with decreasing functional specialisation of cells being influenced, the total sensitivity to ionizing irradiation of the influenced cell population decreased (Venketeswaran *et al.* 1966, Bajaj *et al.* 1970, Opatrný 1971). Killing or damaging a certain percentage of cells of the tissue culture was manifested far less on its further growth than damaging or killing of the same proportion of cells in the apical meristem or cambium on the growth of the irradiated plant.

The aim of these experiments was to find out the influence of ionizing irradiation on the total biomass production, morphogenic and metabolic activity of crownvetch cultures. The dedifferentiated callus cultures and embryogenic cultures of the same kind were compared.

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Material and methods

Callus cultures (Dušková *et al.* 1987) and cultures of somatic embryos (Dušková *et al.* 1990) were exposed to γ-irradiation. The irradiation source was ^{60}Co, distance 1 m, dosing rate 9.93 mGy s^{-1}.

Callus cultures cultivated in darkness were exposed to irradiation: 2.5, 5, 10, 20, 40, 80, 120 and 160 Gy - part before and part after inoculation. In the first case the callus was grown on a non-irradiated medium, in the second case on an irradiated medium. In both cases the fresh mass increase was determined after 4 weeks of cultivation (inoculum = 100 %). In the cultures irradiated before inoculation, the growth curves were made for doses of 2.5, 80 and 160 Gy.

A characteristic manifestation of the effect of irradiation on a cell population is the occurrence of "giant cells" attesting the defects in karyokinesis and then cytokinesis (mitotic death of cells). Determination of the occurrence of "giant cells" in irradiated calluses after 4 weeks of cultivation was performed on spreading preparations with an eyepiece with a grid. Three calluses from each variant were evaluated and from each callus three preparations were made (from the base, surface and middle). In each preparation 10 fields (1000 × 1000 μm) were evaluated. Cells bigger than 100 × 100 μm were considered to be "giant".

Somatic embryos. The influence of irradiation on the formation and further development of somatic embryos (50th subculture) cultivated on the Murashige and Skoog's medium with added IAA (1.0 mg l^{-1}) in the light was studied as follows: Cultures of somatic embryos (inoculum = 5 embryos + 5 embryoids) were exposed to γ-irradiation of 2.5 and 5 Gy before inoculation and transferred to a fresh medium of the same composition. After 4 weeks of cultivation the developed somatic embryos (significantly bipolar formations) and embryoids (organized structures with a slightly differentiated root pole) were counted. Both formations were, moreover, differentiated into one-, two- and multi-cotyledonal. In another subculture, embryos and embryoids (inoculum = 5 embryonic formations) were transfered separately to a medium without growth substances and after 4 weeks, *i.e.* 8 weeks since the irradiation, the second evaluation was performed.

The second variant consisted of transferring the somatic embryos (inoculum = 5 embryos + 5 embryoids) which had been exposed to irradiation (doses of 2.5 and 5 Gy) before inoculation, directly to a non-irradiated medium without growth substances. The evaluation was also carried out after 4 weeks of cultivation.

The presence of the cardio-active glycosides hyrcanoside and deglucohyrcanoside was determined chromatographically. 3 g of dried and powdered callus mass were extracted for 20 min in 75 cm^3 of boiling water. The ballast substances were removed from the extract by lead acetate, redundant lead ions were rinsed through a column of an ion exchanger (*Ostion KSGL*) and the extract was evaporated untill dry. The evaporation residue was dissolved in purified water in such a way that 1 cm^3 corresponded to 0.5 g of the dried callus. For TLC, *Silufol* plates (*Kavalier*, Czechoslovakia) were used. They were developed in a chloroform-methanol-water

(10:4:0.15) system and detected with a Baljet's agent. The standards were saturated methanol solution of hyrcanoside and deglucohyrcanoside.

Results and discussion

The influence of γ-irradiation on growth of callus cultures: Irradiation both before and after inoculation inhibited growth of the undifferentiated callus. The exception was the lowest dose used of 2.5 Gy. The highest dose used of 160 Gy caused the callus to turn brown and inhibited the growth very strongly but it did not destroy the culture (Fig. 1).

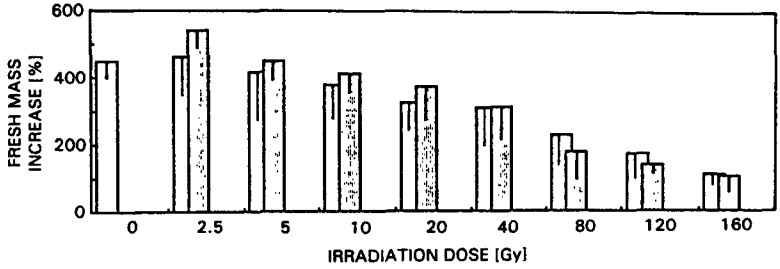

Fig. 1. Influence of various doses of γ-irradiation applicated before (*open columns*) or after (*hatched columns*) inoculation on the growth of callus culture of *Coronilla varia* L. evaluated after 4 weeks of cultivation (the fresh mass of the inoculum = 100 %). Means with 95 % confidence intervals.

On the other hand, Bajaj *et al.* (1970) demonstrated a growth stimulation of tissue cultures of *Phaseolus vulgaris* exposed to an irradiation of 5 Gy. Low irradiation doses of 2 - 10 Gy also stimulated the growth and differentiation of *Datura inoxia* callus (Jain *et al.* 1984). In comparison with reactions of tissue cultures of other plants the culture of the crownvetch seems to be relatively sensitive to irradiation. Callus cultures of *Nicotiana tabacum* were surviving even after a dose of 208 Gy (Hell *et al.* 1978) and calluses of *Phaseolus vulgaris* completely ceased growth only at a dose of 400 Gy (Bajaj *et al.* 1970). The lethal dose for callus cultures of *Citrus sinensis* was between 280-320 Gy (Spiegel-Roy 1973). The tissue culture of *Cajanus cajan* showed the largest growth when exposed to an irradiation dose of 50 Gy and only doses of 100 and 200 Gy inhibited the growth (Shama Rao *et al.* 1975). The same sensitivity to irradiation was found in undifferentiated calluses of two very different kinds - the crownvetch (*Coronilla varia, Fabaceae*) and the bearberry (*Arctostaphylos uva-ursi, Ericaceae*) (Dušková *et al.* 1988).

The growth curves (Fig. 2) done for cultures that have been exposed to irradiation of 2.5, 80 and 160 Gy before inoculation show a later begining and culmination of the exponential phase of the growth for doses of 2.5 and 80 Gy. This was probably caused by reparation of damages by irradiation. The growth of culture exposed to irradiation of 160 Gy was inhibited so much that individual growth phases could not be noticed.

An intervention into the mitotic apparatus of the cells was demonstrated by the growing number of "giant cells", greatly varying in shape. They occurred very rarely in the non-irradiated calluses. Their number in irradiated cultures grew in dependence on the irradiation dose (Fig. 3).

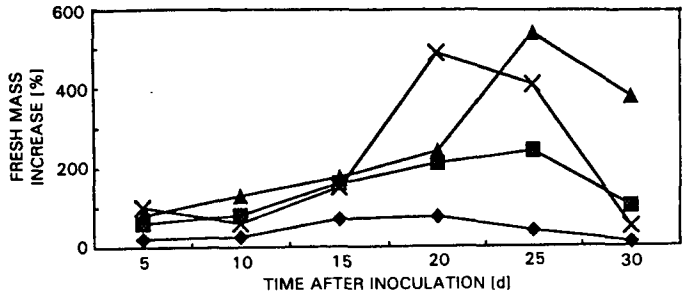

Fig. 2. Influence of various doses of γ-irradiation (*squares* - control, *triangles* - 2.5 Gy, *open circles* - 80 Gy, *closed circles* - 160 Gy) on the growth curve of the culture of *Coronilla varia L.* irradiated before inoculation .

The number of "giant cells" was higher in the variant where the irradiated callus was cultivated on a non-irradiated medium. For explanation we can, *e.g.*, assume that the processes of reparation coming after irradiation of the cultures were impaired by a "subcultural shock". The damage of the cells by irradiation was added to the unfavourable factors of subcultivation. Higher number of "giant cells" than in the control was found in the irradiated culture of *Arachis hypogaea.* A greater influence of irradiation was also demonstrated when the culture was grown on a non-irradiated medium rather than on an irradiated one (Verma *et al.* 1971).

The influence of irradiation on embryogenic cultures: In the cultures containing spontaneously developed somatic embryos exposed to ionizing irradiation in low doses (2.5 and 5 Gy), a significant stimulation of their further production occurred (Table 1). The number of newly developed embryoids and embryos rose to 43 (= 100 %) from the original number of inocula (10 embryonic formations) in the control by the process of secondary somatic embryogenesis during 4 weeks, due to irradiation of 2.5 Gy it rose to 222 (*i.e.* 516 %), and of 5 Gy to 93 (*i.e.* 216 %). After another inoculation of these cultures on the medium without growth substances (*i.e.* 8 weeks after irradiation and 4 weeks after separated cultivation of somatic embryos and embryoids), the stimulating effect was still apparent and it reached 156 % at a dose of 2.5 Gy and 169 % at a dose of 5 Gy.

The stimulating effect on embryogenesis was lower when irradiated embryos were transferred directly to the medium without growth substances. The total number of embryos reached 402 % after irradiation of 2.5 Gy and 198 % after irradiation of 5 Gy. Both fully developed embryos and embryoids originated with an apparent shift to the benefit of embryoids.

Chromatographic analysis did not demonstrate the influence of ionizing irradiation on the spectrum of substances reacting with Baljet's agent either in callus cultures or in embryogenic clutures. They are mainly hyrcanoside (R_F 0.6), deglucohyrcanoside (R_F 0.28).

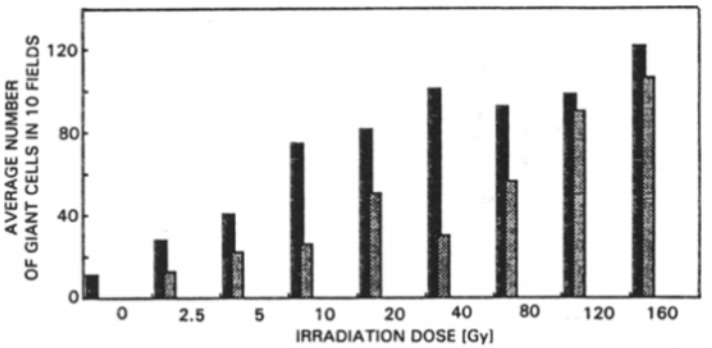

Fig. 3. Influence of γ-irradiation on the occurrence of "giant cells" in the callus culture of *Coronilla varia* L. irradiated before (*open columns*) and after (*hatched columns*) inoculation.

Table 1. The effect of single-dose of ionizing irradiation (2.5 or 5 Gy) on the morphogenesis of the embryogenic cultures of *Coronilla varia* L.

Irradiation dose [Gy]	Number of embryos		Number of embryoids	
	1-2 cotyledons	more cotyledons	1-2 cotyledons	more cotyledons
0	15	2	20	6
2.5	18	25	61	118
5	23	23	18	29

Fully regenerated plants were also obtained from irradiated cultures. No significant deviations in either their phenotype or in their growth abilities were found.

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