Theor. Appl. Genet. 59, 391-393 (1981)

Radiation Stimulated Increase of Plating Efficiency of Free Plant Cells

P.A.Th.J. Werry and K.M. Stoffelsen
Radiation Biophysics Group, Association Euratom, ITAL, Wageningen (the Netherlands)

Summary. Radiation induced stimulation of plating efficiency of free plant cells was observed following irradiation with X-rays (1.25 Gy, dose rate 3.1 Gy.min^{-1}) and fission neutrons (1.5 Gy, dose rate 0.05 Gy.min^{-1}). The dose range where the radiation stimulation effect is manifest is inversely correlated with the applied dose rate.

The results are discussed in view of the radiation induced stimulation as it is applied in agricultural practice.

Key words: Radiation induced stimulation – Plating efficiency – *Haplopappus gracilis* – Cell suspension culture

Introduction

When seeds of crop plants are irradiated with appropriate doses of X-rays or γ-rays (between 2 and 20 Gy) before being sown, an increased yield can often be obtained. This phenomenon, the 'radiation induced stimulation effect', is extensively documented and the principle is widely applied in agricultural and horticultural practice (Simon and Bhattachariya 1977).

Practical application, however, is often criticized because of the low degree of reproducibility of the stimulation effect. It is supposed (Bhattachariya anfd Joshi 1977) that this inconsistancy is due to an incomplete knowledge of environmental and other factors that may modify the response of seeds to the possibly stimulating doses. In addition, inadaequate understanding of the primary response of plant cells to radiation hampers the search for those factors influencing the stimulation effect. Thus, it is not yet understood why radiation stimulation treatment of seeds generally will result in a higher yield, but sometimes will not.

Recently a reproducible radiation stimulation effect was observed (Werry and Stoffelsen 1979) in a study on the cell killing effect of ionizing radiation of free plant cells. A more detailed study – especially with respect to the influence of radiation quality and dose rate – is presented in this report.

Materials and Methods

Details on initiation and maintenance of the cell suspension culture of *Haplopappus gracilis* (Nutt.) *Gray* have been published previously (Werry and Stoffelsen 1978).

Free cells were obtained by filtering a suspension culture through a series of nylon sieves having successive mesh widths of 297, 210 and 88 μm. The resulting suspension contains about 2.5×10^3 viable units per mililitre. Of these units, approximately 80 per cent are unicellular and 20 per cent bicellular; a negligible proportion are multicellular.

Free cells suspended in their own growth medium were irradiated with X-rays (160 kVp, filtration 0.25 mm Cu and 1.0 mm Al) and fission neutrons (mean energy 1.7 MeV, gamma contamination less than 5 per cent of the neutrons dose) (BARN reactor; Chadwick and Oosterheert 1969). Immediately after irradiation, the replating technique was employed: an aliquot of the irradiated cells was rapidly mixed with 2.3 volumes of liquid (40°C) B-5 agar medium and poured on top of feeder agar (preplating step). After a 2 weeks incubation at 28°C in darkness, the soft agar containing small colonies was carefully mixed with liquid (40°C) soft agar so as to give a maximum density of 0.5×10^3 small colonies per mililitre. Then 1 ml of this mixture was poured on top of solid feeder agar in Petri dishes (replating step). The Petri dishes were sealed with parafilm and incubated at 28°C in darkness.

After 2 weeks those cells that had developed into a colony large enough to be observed by eye – after about 12 cell divisions – were defined as survivors and counted. The plating efficiency (PE) is defined as

$$PE = \frac{\text{(number of visible colonies)}}{\text{(number of preplated units)}} \times 100 \text{ per cent.}$$

Survival S is defined as the PE at a given dose relative to the PE of the unirradiated control.

Corrections for the occurrence of bi- or tricellular aggregates in the filtered suspensions were made by applying the functions (Howland 1977)

0040/5752/81/0059/0391/$ 1.00

$S_{obs} = 1 - L$

where S_{obs} is the observed fractional colony survival and L the observed fractional colony inactivation,

$L = pf_1 + p^2 f_2 + p^3 f_3$

where f_1, f_2 and f_3 are the fractions of uni-, bi- and tricellular aggregates in the irradiated suspension (these proportions are determined microscopically in each experiment) and p is the fraction of inactivated free cells (it is assumed that p has the same value for free cells and cells in aggregates) and

$S = 1 - p$

where S is the free cell survival.

In general the PE in the untreated control is about 80% and never exceeds 90%; when in an experiment the PE of the untreated control is lower than 60%, this experiment is discarded. It was demonstrated previously (Werry and Stoffelsen 1978) that within a single set of experiments, where experimental conditions are fully identical, the PE is strictly correlated to the volume of the inoculum in the replating step. It is inferred from this correlation that the size of the aggregates – independent of the depth of the inoculum layer – determines the PE. In the procedure employed in this study the diameter of the aggregates resulting from unirradiated free cells, is such that approximately 20 per cent of the aggregates are covered with a layer of soft agar and therefore cannot develop into visible colonies because of inadequate gas exchange.

Results

The Effect of X-rays on PE

Irradiation of free cells results at first in an increase of the survival and, at increased dose, in a more or less substantial – depending on the applied dose rate – cell killing (Fig. 1). The effect of dose rate is such that maximum S occurs at a lower dose when the applied dose rate is higher.

It should be borne in mind here that the 'stimulated' PE can never be higher than 100% in a properly executed experiment. Extensive and repeated counting of the aggregates before and after the replating step showed that the increased S cannot be attributed to fragmentation of aggregates in the replating step.

The Effect of Irradiation with Fission Neutrons

No stimulation of survival could be detected when the cells were irradiated with fission neutrons at a dose rate of 1 $Gy.min^{-1}$ (Fig. 2). However, when a dose rate of 0.05 $Gy.min^{-1}$ was applied a pronounced stimulation effect could be noticed, with a maximal effect at a dose of 0.5 Gy.

When fission neutrons are applied at a dose rate of

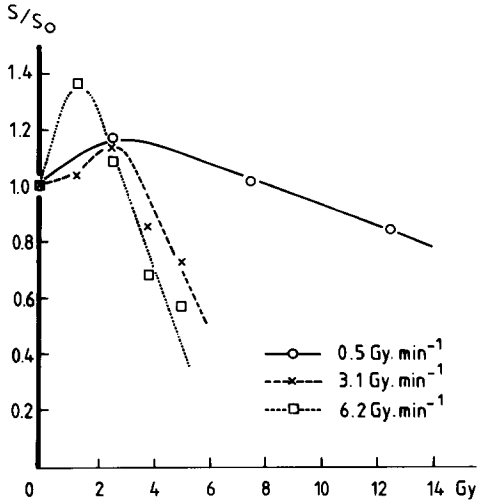

Fig. 1. Free cell survival of *H. gracilis* after X-irradiation

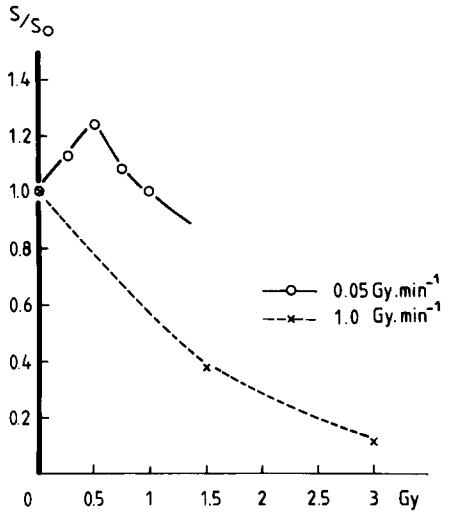

Fig. 2. Free cell survival of *H. gracilis* after irradiation with fission neutrons

1 $Gy.min^{-1}$, which was always used in previous experiments (Werry and Stoffelsen 1979) it is for practical reasons impossible to apply doses lower than 1.5 Gy. Based on the approximately exponential dose effect relationship for cell killing it was supposed that a radiation stimulation effect could not be effected by irradiation with fission neutrons. Following the observation with X-irradiation, however, that the dose range where the radiation stimulation effect is manifast, is inversely correlated with the applied dose rate it was hypothesized that a radiation stimulation effect probable could be effected by fission neutrons when applied at a sufficiently low dose rate. Figure 2 shows that at a dose rate of 0.05 $Gy.min^{-1}$ a radiation stimulation effect was indeed observed.

Discussion

The observed increase in S at appropriate doses of X-rays and fission neutrons is an unexpected and unique phenomenon in cellular radiation biology. The effect can be understood when the following arguments are considered:

1. Growth stimulation in callus cultures of *Haworthia mirabilis*, effected by radiation induced cytokinins, has been recently demonstrated by Pandey et al. (1978).

Preliminary experiments in our laboratory have shown that extracts of *Haplopappus* cells grown in suspension for 3 weeks after irradiation with X-rays to doses of 2.5 and 5 Gy stimulated growth when applied to growing suspension cultures. This stimulation was expressed as increased fresh weight. No increase in dry weight, however, was found, indicating that the size of the cells was increased but not the number of cells.

2. The main factor that influences the plating efficiency is the size of the aggregates at the replating step.

It can be considered from these arguments that following irradiation with X-rays and fission neutrons – at appropriate doses and dose rate – the diameter of the cells is increased due to the action of induced cytokinins, and, subsequently the size of aggregates at the replating step is also larger. The result is a higher plating efficiency which – when the cell killing effect of the radiation is not severe – exceeds that of the unirradiated controls.

The dose rate is apparently an important factor which influences the stimulation effect: the higher the dose rate the lower the dose where maximal stimulation occurs. Two separate aspects may be distinguished in this effect:

1. The cell killing effect of radiation is less severe when the radiation is applied at low dose rate. This is most probably due to the more ample possibility for the cells to repair the sublethal damage (Ben Hur et al. 1974; Werry and Stoffelsen, in preparation). Consequently the stimulation effect – which is only manifest when the cell inactivation effect is low – is apparent over a greater dose range at low dose rate than at high dose rate.

2. In analogy to the more efficient cell killing activity of radiation when applied at high dose rate it may be supposed that also the cytokinin induction is more efficient at high dose rates. This would mean that with a high dose rate already at a low dose sufficient cytokinin is induced to bring about the stimulation effect.

From these arguments it can be inferred that the observed dose rate effect is the result of two different effects: increased cell killing at high dose rate and enhanced cytokinin induction.

The cellular response to ionizing radiation – as it is observed in this study – at a dose that is well within the dose range that is normally used in practical radiation stimulation programs, may provide the basis for an explanation of the apparent low degree of reproducibility of this phenomenon: since cell elongation is a main factor in seed germination it seems most probable – in view of the present study – that radiation stimulates preeminently this cellular activity. The result is an increased seedling height and root length. Whether or not this increased size of the seedling results in bigger plants and higher yield may be depend on how such environmental factors as (micro)climate, soil condition etc. affect seedlings of abnormal size.

A correct assessment of whether a radiation stimulation treatment should be applied in a given situation or not must necessarily include an assessment of the factors that affect – positively or negatively – the stimulated seedling.

Acknowledgement

Stimulating discussions with Drs. Gilissen, Puite and Blaas are greatly acknowledged.

This research was financially supported by the Commission of the European Communities (Radiation Protection Programme of DG XII. Division Biology, Health Protection, Medical Research).

Literature

Ben Hur, E.; Elkind, M.M., Brouck, B.V. (1974): Thermally enhanced radio response of cultured Chinese hamster cells: Inhibition of repair of sublethal damage and enhancement of lethal damage. Radiation Res. 58, 38-51

Bhattachariya, S.; Joshi, R.K. (1977): Factors modifying radiation induced stimulation in plants: Preirradiation seed moisture content. Radiation Environm. Biophys. 14, 47-51

Chadwick, K.H.; Oosterheert, W.F. (1969): Neutron spectometry and dosimetry in the sub-score facility of a swimming pool reactor. Atompraxis 15, 1-4

Howland, G.P.; Hart, R.W. (1977): Radiation biology of cultured plant cells in: Plant Cell, Tissue and Organ Culture (eds.: Reinert, J.; Bajaj, Y.P.S.), pp. 731-754. Heidelberg, New York: Springer

Pandey, K.N.; Sabharwal, P.S.; Kemp, T.R. (1978): Cell division factors (cytokinins) from irradiated plant tissue. Nature 271, 449-450

Simon, J.; Bhattachariya, S. (1977): The present status and future prospect of radiation stimulation in crop plants. Budapest: Phylaxia

Werry, P.A.Th.J.; Stoffelsen, K.M. (1978): Conditions for a high plating efficiency of free cell suspensions of *Haplopappus gracilis* (Nutt.) *Gray*. Theor. Appl. Genet. 51, 161-167

Werry, P.A.Th.J.; Stoffelsen, K.M. (1979): The effect of ionizing radiation on the survival of free plant cells cultivated in suspension cultures. Int. J. Radiat. Biol. 35, 293-298

Received January 6, 1981
Communicated by H.F. Linskens

Dr. P.A.Th.J. Werry
Dr. K.M. Stoffelsen
Radiation Biophysics Group
Association Euratom, ITAL
P.O. Box 48
NL – 6700 AA Wageningen (the Netherlands)