Investigation of UVC-induced DNA damage and its repair by SCGE assay in barley

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Alkaline single cell gel electrophoresis (SCGE), or the "comet assay", is used to detect the DNA damage that occurs after treatment with a number of physical or chemical factors *in vivo* or *in vitro*. The purpose of this study was to determine whether UVC-inducible DNA damage in nuclei isolated from barley root meristems is the subject of repair. The kinetics of DNA repair was studied using an SCGE assay, measuring the number of nuclei with fragmented DNA as a function of recovery after UVC irradiation (3000 J/m^2 and 6000 J/m^2) time. After 2 h of recovery period, increased DNA fragmentation was observed. Reduction of DNA fragmentation in barley c. 'Auksiniai II' root meristem nuclei appeared after 8 h of incubation. This dependence could reflect the repair process of DNA strand breaks.

Key words: SCGE, UVC irradiation, DNA repair

INTRODUCTION

The comet assay, also called single cell gel electrophoresis (SCGE), was first introduced by Östling and Johanson in 1984 (Östling et al., 1984) as a microelectrophoretic technique for direct visualisation of DNA damage in individual cells. Originally applied to irradiated mammalian cells, SCGE has gone through a number of modifications, the most significant being the introduction of alkaline conditions enabling to disclose single strand breaks and alkali labile sites (Singh et al., 1988). Individual cell nuclei, suspended in a thin agarose gel layer on a microscope slide and exposed to a DNA-damaging agent are afterwards placed into alkali conditions to achieve DNA unwinding. Nuclei are then electrophoresed and stained with a fluorecent dye to reveal single strand breaks. The comet tail is in direct correlation with the level of DNA damage. SCGE can theoreticaly be applied to every type of eukaryotic cells, including plant cells. In 1996 SCGE has been applied for genotoxicity evaluation in *Vicia faba* (Koppen et al., 1996), *Allium cepa* (Navarrete et al., 1997) and *Nicotiana tabacum* (Gichner et al., 1998; Gichner et al., 2000) cells. As a continuation of the application of SCGE method to plant tissues we have applied this method to *Hordeum vulgare* root meristem cells. The cell nuclei were irradiated with a 3000 J/m^2 and 6000 J/m^2 UVC dose and left at room temperature for recovery period. The reduction of DNA fragmentation during post-irradiational incubation was measured in order to evaluate DNA repair. Thus DNA damage processing observed using SCGE in post-irradiation period could be important to determine DNA repair and its kinetics in barley nuclei.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L.) cultivars 'Auksiniai II' were obtained from Dr. L. Balčiūnienė (Botanical Garden of Vilnius University).

Plant growth conditions. Barley seeds were placed into Petri dishes on filter paper, watered with distilled water and kept in the dark at room temperature for 4–5 days.

Isolation of nuclei. All operations were performed under dim light. Root tips were cut off with a new blade about 2 mm from the apex in a Petri dish on ice and soaked in a modified Sörensen buffer (SB) drop (50 mM sodium phosphate pH 6.8, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% dimethyl sulfoxide (DMSO)) (Navarrete et al., 1997).

UVC irradiation. Isolated nuclei were exposed to a 3000 or 6000 J/m^2 UVC dose (БД-30, λ_{max} = 254 nm) in modified SB. Doses were selected according to the dimerisation curve of UV-irradiated DNA from *Bacillus subtilis* (Žukas et al., 1997). After 0, 2 and 8 h of a recovery period at room temperature in the dark the nuclei were exposed to a

temperatured 4 °C to stop the processes of repair in the nuclei, and agarose slides were made.

Single cell gel electrophoresis was performed according to Navarrete et al. and Gichner et al. (Gichner et al., 1998; Gichner et al., 2000; Navarrete et al., 1997). Slides previously coated with 1% normal melting agarose (NMA) were dried overnight. 50 µl of nuclear suspension and 50 µl of 37 °C 1% low melting agarose (LMA) prepared with SB was added onto each slide. Afterwards the slide was covered with 100 µl of 0.5% LMA. Two repared slides were immersed for 1 h into a lysing solution consisting of 2.5 M NaCl, 1% sodium sarcosinate, 100 mM Na_2EDTA, 10 mM Tris (pH 10) with 1% Triton X-100 and 10% DMSO and afterwards placed in electrobuffer for 20 min and electrophoresed (20 V, 300 mA) at 4 °C (electrophoresis buffer 1 mM Na_2EDTA and 300 mM NaOH, pH > 12). After electrophoresis the slides were washed with 400 mM Tris buffer pH 7.5 and stained with 70 µl ethidium bromide (20 µg/ml) for 5 min. and covered with a coverslip. For each slide 30–80 randomly chosen nuclei were analysed using a fluorescence microscope.

Statistical analysis. SCGE experiment was repeated three times. Data from experiments were analysed using Fisher's exact test (GraphPad InStat).

RESULTS AND DISCUSSION

The nuclei isolated from barley c. 'Auksiniai II' root meristems were irradiated with 3000 and 6000 J/m^2 UVC doses. SCGE showed the induction of DNA strand breaks, which caused the disappearance of the distinct outline of the nuclei (Fig. 1. B, D). The nuclei of that type were detected in samples that received both UVC doses and were attributed to damaged nuclei. The disappearance of the distinct outline of the nuclei was apparently determined by a slight fragmentation of the DNA. Alongside the nuclei without a distinct outline there were some nuclei which formed comets. The number of the comets correlated with UVC dose (Fig. 1. C, E). The nuclei without distinct outline or nuclei forming comets during SCGE were chosen as a criterion for UVC-inducible DNA damage. The quantity of the latter was conciderably lower in control samples.

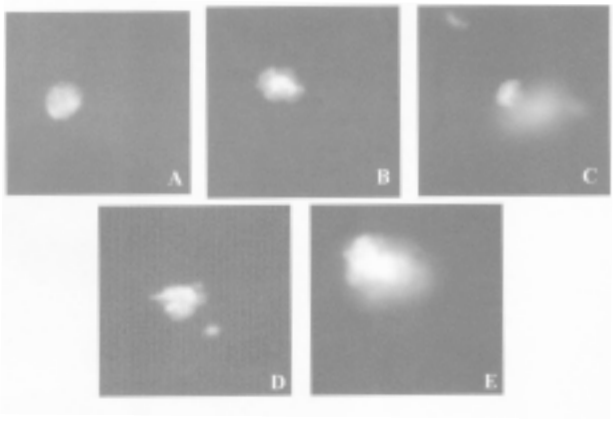

Fig. 1. Micrographs from SCGE of UVC-irradiated barley c. 'Auksiniai II' nuclei isolated from root meristem cells. A – control, B, C – UVC, 3000 J/m^2, D, E – UVC, 6000 J/m^2

The repair of UVC-inducible DNA damage and its kinetics were investigated by defining the amount of damaged nuclei during postirradiational incubation. For that purpose a suspension of isolated nuclei was irradiated with 3000 J/m^2 and 6000 J/m^2 UVC doses. The processes of repair in irradiated nuclei

Table. **The number of UVC damaged nuclei in SCGE experiments**

Experiment No.	UVC dose, J/m^2	Incubation time, h								
		0			2			8		
		Damaged	Undamaged	Significance level *p*	Damaged	Undamaged	Significance level *p*	Damaged	Undamaged	Significance level *p*
1	0	24	74	–	20	39	–	38	79	–
	3000	36	60	0.0623	65	64	0.0406*	41	63	0.3256
	6000	71	82	0.0005*	85	75	0.0145*	59	80	0.1209
2	0	29	75	–	30	76	–	35	74	–
	3000	28	74	1.0	60	62	0.0017*	44	77	0.5783
	6000	21	20	0.0113*	41	85	0.5676	46	69	0.2658
3	0	24	68	–	30	70	–	46	61	–
	3000	28	72	0.5233	56	50	0.0011*	48	68	0.8921
	6000	31	78	0.7524	60	62	0.0008*	48	64	1.0

* Significant difference from control ($p < 0.05$).

as well as in control samples were stopped by exposing the nuclei suspension to a temperature of 4 °C. One part of the suspension was analysed directly after irradiation, and the remaining suspension was incubated at room temperature for 2 and 8 h in the dark. After incubation the nuclei suspensions were placed onto ice and SCGE slides were made. Table shows the absolute calculations and significance level of three experiments. The results of the experiments are summarized in Fig. 2. The number of nuclei with fragmented DNA correlated with UVC dose in the samples that had no reco-

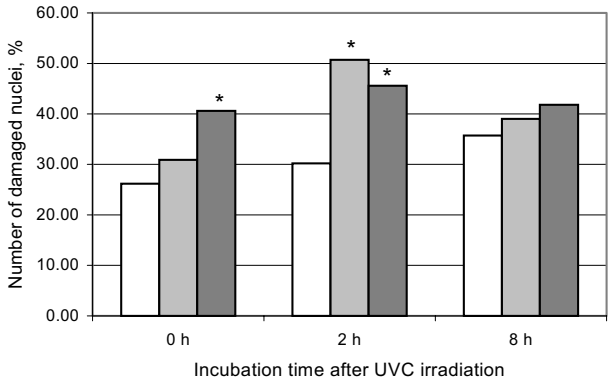

Fig. 2. Dependence of the number of UVC-damaged nuclei on the time of incubation using SCGE (nuclei without distinct outline and comets are attributed to damaged nuclei). □ control, □ 3000 J/m^2, ■ 6000 J/m^2.
* Significant difference from control.

very period. Both UVC doses caused a statisticaly significant increase of DNA fragmentation after 2 h of post-irradiational incubation. Probably the nucleotide and base excision repair is taking place in the nuclei and DNA strand breaks are induced, observed by SCGE as an increase of the quantity of damaged nuclei. The effect is more pronounced in samples irradiated with a 3000 J/m^2 UVC dose. The 6000 J/m^2 dose probably inhibits the processes of repair in UVC-damaged DNA. A decrease of DNA fragmentation was noted after 8 h of recovery. The difference between samples incubated for 2 and 8 h was significant ($p < 0.05$) in nuclei irradiated with 3000 J/m^2 and not significant in those irradiated with 6000 J/m^2. However, after incubation for 8 h DNA damage in irradiated nuclei decreased to the level of control samples incubated at room temperature for the same time. The experiments showed that incubation of nonirradiated (control) nuclei in SB post irradiation induce a slight DNA strand breakage. The procedures of nuclei isolation could evoke the appearance of different cytoplasmic components (nucleases and hydrolytic proteins) which could induce additional DNA strand breaks. Otherwise the nucleus as an experimental model is more sensitive than the intact cell. To eliminate these problems, protoplasts could be used for investigation of DNA repair in the future.

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UVC INDUKUOTŲ DNR PAŽAIDŲ BEI JŲ REPARACIJOS TYRIMAI SCGE METODU MIEŽIUOSE

S a n t r a u k a

Pavienių ląstelių gelio elektroforezė (SCGE), taikoma aptikti DNR pažaidoms, indukuotoms įvairiais fiziniais arba cheminiais agentais *in vivo* ir *in vitro*. Šių tyrimų tikslas – nustatyti UVC indukuojamų DNR pažaidų reparaciją SCGE metodu branduoliuose, išskirtuose iš miežių šaknelių meristemos. DNR reparacijos kinetika tirta SCGE metodu nustatant branduolių su fragmentuota DNR skaičių kaip reparacijos funkciją po UVC apšvitinimo (3000 J/m^2 ir 6000 J/m^2) postradiacinės inkubacijos metu. Po 2 val postradiacinės inkubacijos padidėja DNR trūkių kiekis, tai rodo funkcionuojant ekscizinę reparaciją. DNR fragmentacijos sumažėjimas 'Auksiniai II' miežių branduoliuose, stebėtas po 8 val inkubacijos, susijęs su DNR pažaidų eliminacija.

Raktažodžiai: SCGE, UVC apšvitinimas, DNR reparacija