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

J. Armalytė,
K. Žukas

Department of Botany and Genetics,
Vilnius University,
Čiurlionio 21,
LT-2009 Vilnius, Lithuania
E-mail: Kestutis. Žukas@gf.vu.lt

Investigation of intact nuclei by single cell gel electrophoresis (SCGE) or comet assay from different genotypes of barley showed that the nuclear DNA of mutant tw_3 and genetic line Dorsett was insignificantly fragmented, while the DNA of other genotypes studied was not damaged. UVC (1500 and 3000 J/m^2) induced DNA damage was observed after irradiation of cv. 'Auksiniai II' and Dorsett nuclei. The kinetics of DNA repair was studied by an SCGE assay, measuring the number of nuclei with fragmented DNA as a function of recovery after UVC irradiation (3000 and 6000 J/m^2) time. A decrease of DNA fragmentation in barley cv. 'Auksiniai II' root meristem nuclei appeared after 8 h of incubation. This dependence could reflect the repair process of DNA strand breaks.

Key words: single cell gel electrophoresis, comet assay, DNA repair

INTRODUCTION

SCGE assay can be used to detect DNA damage and to study repair [1]. In the alkaline version of SCGE assay cells with DNA strand breaks and/or alkali labile sites show an increased migration of DNA from the nucleus, generating a 'comet' shape. UVC treatment increased the quantity of DNA strand breaks and alkali-labile sites (als) and X-ray evoked DNA single strand breaks (ssb) induced just after treatment of cells. Individual cell nuclei exposed to a DNA-damaging agent are afterwards alkali-treated for unwinding ectrophoresed at pH >12 and stained with a fluorescent stain to reveal ssb. The comet tail length is in direct correlation with the level of DNA damage. The SCGE can theoretically be applied to every type of eukaryotic cells, including plant cells [2–4]. As a further application of the SCGE method to plant tissues, we have applied this method to barley root cells after treatment with UVC irradiation [5]. The purpose of this study was to determine the kinetics of DNA repair in plants after exposure of barley nuclei from root meristems to UVC.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare)* cultivars and mutant *tw3* were obtained from the Botanical Garden of Vilnius University. 4–5-barley roots were used for the isolation of nuclei as described in [4, 5]. Isolated nuclei were exposed to UVC (БУФ-30, λ_{max} = 254 nm).

Single cell gel electrophoresis was performed according to Navarette et al. and Gichner et al. [1, 4, 5]. Horizontal gel electrophoresis was performed to the slides with nuclei in agarose at pH >12 for 20 min at 0.74 V/cm (26 V, 300 mA). The slides stained with ethidium bromide were analysed using a fluorescense microscope (excitation 520 nm, emission 610 nm). 20–50 nuclei/slide were scored. The SCGE experiment was repeated three times. Data of the experiments were analysed by Fisher's exact test (GraphPad InStat). The micrographs were taken with the 40$^{\times}$objective on Fuji colour film, 1600 ASA.

RESULTS AND DISCUSSION

After SCGE analysis using unirradiated nuclei from different barley genotypes it was detected that the nuclear DNA of mutant tw_3 and line Dorsett was insignificantly fragmented (Fig. 1 C, E), while the nuclei of other genotypes ('Auksiniai II', 'Auksiniai III', Keystone) were not damaged (Fig. 1 A, B, D). DNA fragmentation demonstrated in nuclei from tw_3 and Dorsett genotypes may be influenced by different causes. First, the level of endogenous O_z^- and/or different level of thiolic compounds in various barley genotypes may be different. Second, various genotypes may have different activities of repair.

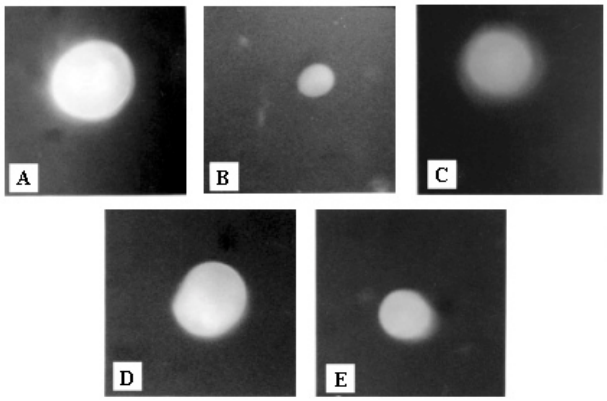

Fig. 1. Micrographs from SCGE of isolated barley nuclei from intact plants. A – 'Auksiniai II', B – 'Auksiniai III', C – tw_3, D – Keystone, E – Dorsett. All micrographs were taken using a 40×objective

Third, various genotypes may have different sesitivity to nuclei isolation procedures. After UVC irradiation of nuclei isolated from cv. 'Auksiniai II' and 'Dorsett', induction of ssb was detected (Fig. 2). From irradiated nuclei of Dorsett only deformation of the halo occurs (Fig. 2 F), at the same time the comet tail whose length is equal to the diameter of nuclei was detected in cv. 'Auksiniai II' (Fig. 2 C).

The repair of UVC-inducible DNA damage and its kinetics were investigated by defining the amount of damaged nuclei during the post-irradiational 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 as well as in control samples were stopped by keeping the nuclei suspension at a temperature of 4 °C. One part of the suspension was analysed directly after irradiation, the remaining suspension was incubated at room temperature for 2 and 8 h in the dark. After incubation the nuclei suspensions were placed into ice and SCGE slides were made. The results of the experiments are summarized in Fig. 3. The number of nuclei with fragmented DNA correlated with the UVC dose in samples that had no recovery period, but the difference from control was significant only for nuclei irradiated with a 6000 J/m^2 dose. Both UVC doses caused a statistically 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 greater 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 is noted after 8 h of recovery. The experiment results show that incubation of nonirradiated nuclei in Sörensen buffer during the post-irradation period induces a slight DNA strand breakage. The procedures of nuclei isolation could cause the appearance of different cytoplasmic components (nucleases and hydrolytic proteins) that can induce additional DNA strand breaks. Otherwise the nucleus as a model for the experiment is more sensitive than the intact cell. To eliminate these problems, protoplasts could be used for investigation of DNA repair in the future.

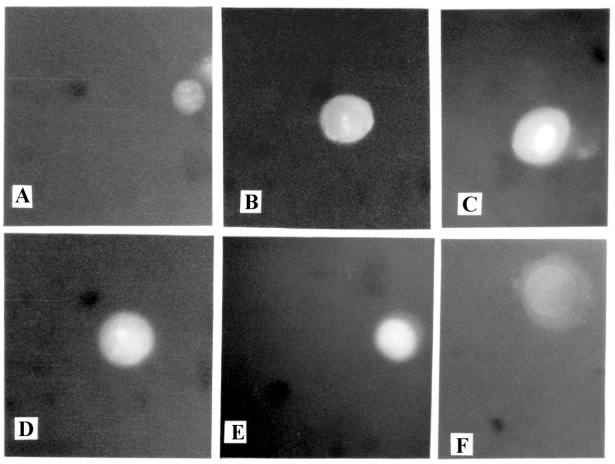

Fig. 2. Micrographs from SCGE of UVC-irradiated barley nuclei. A, B, C, – 'Auksiniai II', A – control, B – UVC 1500 J/m^2, C – UVC 3000 J/m^2. D, E, F – 'Dorsett', D – control, E – UVC 1500 J/m^2, F – UVC 3000 J/m^2

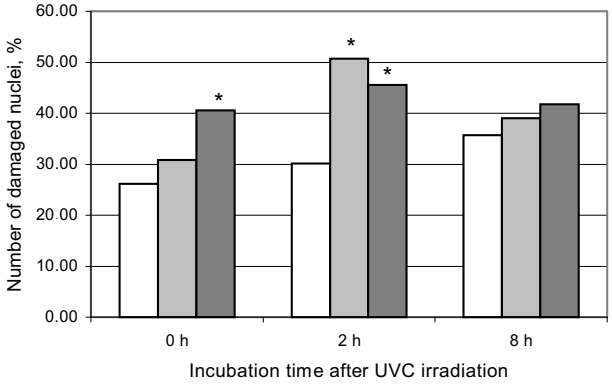

Fig. 3. Dependence of the number of UVC-damaged nuclei upon the time of incubation using SCGE (nuclei without distinct outline and comets are attributed to damaged nuclei)

□ control
□ 3000 J/m^2
□ 6000 J/m^2
* Significant difference from the control.

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UVC INDUKUOTŲ DNR PAŽAIDŲ NUSTATYMAS IR JŲ REPARACIJOS TYRIMAI MIEŽIUOSE SCGE METODU

S a n t r a u k a

Įvairių miežių genotipų intaktinių branduolių analizė SCGE metodu parodė, jog mutanto tw_3 ir linijos Dorsett DNR nedaug fragmentuota, tuo tarpu kitų genotipų DNR trūkių neaptikta. Apšvitinus ‘Auksiniai II’ ir Dorsett branduolius UVC aptinkama DNR trūkių indukcija. Apie DNR pažaidų reparaciją sprendžiama iš DNR fragmentacijos sumažėjimo post-radiacinės inkubacijos metu. DNR fragmentacijos sumažėjimas ‘Auksiniai II’ miežių šaknų meristeminių ląstelių branduoliuose, aptinkamas po 8 val. post-radiacinės inkubacijos sąlygojamas DNR trūkių reparacijos.