Bioassays for cytogenetic risk assessment in irradiated plants

Biotesti za oceno citogenetske ogroženosti obsevanih rastlin

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Abstract. Onion (Allium cepa L.) bulbs were subjected to gamma irradiation with subsequent cytogenetic analysis over prolonged growth periods under field conditions. In root tip meristematic cells, micronuclei and types of chromosomal aberrations were observed and the mitotic index determined. The micronucleus test results in concordance with the chromosome aberration assay results indicated the extent of clastogenic effects of irradiation on plant genetic material. However, the best cytogenetic indicator for radiation hazard assessment was the complete mitotic blockade in root meristems.

Key Words: Allium cepa L., Gamma ray irradiation, Micronuclei, Chromosomal aberrations, Mitotic index

Izvleček. Po obsevanju čebulic čebule (Allium cepa L.) z gama žarki smo gojili poskusne rastline pri zunanjih pogojih in vzorčili korenine za citogenetsko analizo v daljšem času rasti. Analiza meristemskega celica v koreninskih vršičkih je obsegala ugotavljanje frekvence mikronukleusov in tipov kromosomskeh aberacij ter določitev mitotskega indeksa. Pokazala se je skladnost rezultatov mikronukleus testa in analize kromosomskih aberacij glede obsega klastogenih učinkov sevanja na rastlinski genetski material. Toda najboljša citogenetska indikacija vpliva sevanja za oceno ogroženosti rastlin je bila trajna blokada mitotske aktività celica koreninskeg meristema.

Ključne besede: Allium cepa L., obsevanje z gama žarki, mikronukleusi, kromosomske aberacije, mitotski indeks

Introduction

Plant cytogenetic bioassays are commonly used for the detection of clastogenic agents. For rough estimations of the genotoxic effect, the micronucleus assay is usually used. Subsequent detailed analysis of metaphases for chromosomal aberrations is the preferred option (Kihlman 1975, Evans & al. 1980, MA 1982, Rieger & al. 1986, Kanaya & al. 1994), although the analysis of types of structural aberrations is very time-consuming.

Testing of environmental samples in laboratory-based bioassays is routinely performed. Plant cytogenetic assays that monitor the environment in situ and detect genotoxic pollutants are not common, although they are increasingly needed for pollution screening. The Tradescantia micronucleus test is one the rapid bioassays that are convenient and efficient for

For *Allium cepa* and *Vicia faba*, the induction of chromosomal aberrations is detectable at concentrations below those that showed significant increases in frequency of micronuclei (Kergomeaux & al. 1983, Kaushik 1996, Smaka-Kincl & al. 1996). In spite of the general opinion that the micronucleus assay for environmental pollutants lacks the necessary sensitivity, the assay has been found to be a possible alternative to metaphase analysis of chromosomal aberrations (Amphlett & Deelow 1984). However, confirmation in more experimental plants is required, and we present a comparative study using *Allium cepa*.

We irradiated onion bulbs that were subsequently grown for prolonged periods under field conditions. Micronuclei and chromosome aberrations were analysed, as well as the mitotic index in root tips. Using these criteria to monitor damage to plant meristems, the relevance of these cytogenetic bioindicators for the identification of radiation hazard was assessed.

**Materials and methods**

Ten equal-sized onion (*Allium cepa* L.) bulbs were used for each irradiation series and for the control. The bulbs were irradiated with doses of 10, 20, and 30 Gy gamma rays (\(^{60}\)Co) at a dose rate of 1.8 Gy/min.

Irradiated and control bulbs were planted in clay pots and exposed to field growth conditions. The roots were sampled for cytogenetic analysis 6, 8, 11, 15, and 20 days after planting, using two plants for each dose and for the control. Ten roots were taken from each plant, five roots being fixed immediately in 3:1 ethanol-acetic acid mixture and kept at -20°C, the other five being pre-treated in a saturated water solution of α-bromonaphthalene for 24 hours at 4°C before fixing in the same ethanol-acetic acid mixture.

The latter pre-treatment procedure led to an accumulation of cells in metaphase that were analysed for various types of chromosomal aberration. Cytogenetic analysis of mitosis was made immediately after root fixation. For both analyses, squash-slides of onion root tips were prepared using the standard methods: hydrolysis in 3N HCL at 60°C for 3 minutes, staining by the Feulgen method, and maceration in aceto-carmine.

In the analysis of mitosis, the mitotic index (MI) was determined by observing 2000 cells/slide, and the number of micronuclei was scored in 1000 interphase cells/slide for each sampling time, radiation dose, and control. In the metaphase analysis, the types of chromosomal aberrations in at least 200 cells/sample were observed. The differences between irradiation groups and the controls were evaluated using Student's t-test (p<0.05).

**Results**

MI values in the control onion plants varied from 7.6% to 9.4% under field conditions. The average MI in onions irradiated with radiation doses higher than 10 Gy decreased with dosage and time. Doses of 20 Gy and 30 Gy led to a complete mitotic blockade in 15 days after irradiation in all samples analysed (Tab. 1).

The control onion plants had only 0.03% cells with a micronucleus over a 20-day period of sampling in field conditions. In irradiated onion bulbs, the frequency of micronuclei increased with the irradiation dose, the highest values being recorded after eleven days of growth but tending to decrease with time after planting (Fig. 1).
Table 1. Mitotic index (MI) in the control and irradiated bulbs.
Tabela 1. Mitotski indeks (MI) pri kontroli in obsevanih čebulekah.

<table>
<thead>
<tr>
<th></th>
<th>6 days</th>
<th>8 days</th>
<th>11 days</th>
<th>15 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>±sD</td>
<td>P</td>
<td>%</td>
<td>±sD</td>
</tr>
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<td>C*</td>
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<td>0.70</td>
<td>9.41</td>
<td>1.04</td>
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</tr>
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<td>10 Gy</td>
<td>8.45</td>
<td>1.72</td>
<td>NS</td>
<td>7.96</td>
<td>2.09</td>
</tr>
<tr>
<td>20 Gy</td>
<td>6.31</td>
<td>1.86</td>
<td>NS</td>
<td>6.27</td>
<td>0.83</td>
</tr>
<tr>
<td>30 Gy</td>
<td>6.29</td>
<td>0.85</td>
<td>&lt;0.05</td>
<td>6.43</td>
<td>1.61</td>
</tr>
</tbody>
</table>

C* = control

Fig. 1. Number of chromosomal aberrations per cell in the control and irradiated bulbs.
Sl. 1. Število kromosomskih aberacij na celico pri kontroli in obsevanih čebulekah.

Fig. 2. Frequency of micronuclei in the control and irradiated bulbs.
Sl. 2. Frekvenca mikronukleusov pri kontroli in obsevanih čebulekah.
Acentric fragments were found in cells of the controls throughout the sampling period under field conditions. In irradiated bulbs, acentric fragments, dicentric chromosomes, centric and acentric rings, microfragments, chromatide translocations and gaps were observed, the highest number of aberrations per cell being observed at 10 and 20 Gy in 6 days and at 30 Gy in 8 days. The number of aberrations decreased with time of growth (Fig. 2).

**Discussion**

In general, studies on metaphase chromosome aberrations and micronuclei have pointed to an increased frequency of chromosomal aberrations in relation to that of micronuclei in response to genotoxic agents. However, this increase is, at least partially, a consequence of scoring aberrations in metaphases that occur with high frequency after chemical pre-treatment. Similarly, in the case of mitotic delay after clastogenic treatment, the number of micronuclei was lower because of the reduced number of mitotic cells (AMPHLETT & DELOW 1984). Micronuclei represented the dominant indicator of clastogenic damage in *Vicia faba* after seed irradiation, compared to chromosomal aberrations in the mitotic anaphase (AMER & MIKHAEL 1972).

In irradiated onion bulbs grown under field conditions, a gradual decrease in the number of metaphase chromosome aberrations per cell as well as in the frequency of micronuclei was observed with increasing time (Figs. 1 and 2). MI values indicate that cell mitotic activity at 10 Gy was not clearly disturbed in relation to that of the control during the observed growth period (Tab. 1). MI values decreased significantly at 20 and 30 Gy, and with both doses a complete mitotic blockade was found at 15 days. These results suggest that the MI values could reflect clastogenicity and should therefore be considered in assessing radiation hazard.

Mitotic delay in root meristems generally occurs in irradiated roots. If meristematic cells recommence division at normal rates after the delay, the citotoxic effect observed in short-term testing is transient. Depressive effects on mitotic events depend on the radiation dose or on chemical treatment in *Allium cepa* (DALHEIM & al. 1989, PAPES & al. 1989), *Pisum sativum* (SANAEV & LAZARENKO 1989), and *Vicia faba* (GRANT & SALAMONE 1994).

A temporary decrease in MI after treatment should be distinguished from the complete suppression of mitosis when meristematic cells irreversibly lose their ability to divide and subsequently die. As a consequence, in onion bulbs irradiated with 20 or 30 Gy, a complete mitotic blockade was identified as well as obvious growth reduction and bulb decay (PARADIŽ 1998).

Despite the complete mitotic blockade in root meristems, the most common phenomenon in heavily irradiated onion bulbs, our results from the root tip micronucleus assay and from the metaphase chromosome aberration assay suggest that both methods are applicable as bioindicators of the clastogenic effects of irradiation on plants.

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Povzetek


Rezultati so pokazali, da sta obe metodi, mikronukleus test in analiza kromosomskih aberacij, uporabni za indikacijo obsega klastogenih učinkov sevanja na rastline. Pri uporabljenih visokih dozah sevanja je nastopila trajna blokada mitotske aktivnosti celic koreninskega meristema, ki je kot največja stopnja citogenetske poškodbe tudi dokaz za ogroženost obsevanih rastlin.

References


