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Genotoxicity of arsenic evaluated by *Allium*-root micronucleus assay

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Abstract

Arsenic exposure is associated with various diseases and cancers. By using *Allium*-root micronucleus (MN) assay, possible genotoxicity of sodium arsenite (0.3–100 mg/l) and arsenic trioxide (0.05–50 mg/l) was evaluated in this study. Our results showed that arsenic compounds induced MN formation concentration-dependently. Exposure to 0.5–20 mg/l arsenic trioxide or to 1–100 mg/l sodium arsenite caused MN significantly in meristematic cells and daughter cells of *Allium* roots. A time-course study revealed that MN increased significantly after a short term (1 h) exposure to 10 mg/l sodium arsenite, demonstrating an effective rapid response. Arsenic compounds also caused mitotic delay and a concentration-dependent decrease in mitotic index. Results of the present study suggest that *Allium*-root MN assay is a simple, efficient and reproducible method for the genotoxicity monitoring of arsenic water contamination.

Keywords: Genotoxicity; Arsenic; *Allium cepa*; Micronuclei; Mitotic activity

1. Introduction

Arsenic (As), a naturally occurring element widely distributed in the earth's crust, is one of the most toxic pollutants in the environment (National Research Council, 1977; Huysmans and Frankenberger, 1990; Phillips, 1990; Dutré et al., 1998; ATSDR, 2000). Arsenic poisoning has been seen in many parts of the world, generally associated with chronic environmental exposure by contaminated drinking water and industrial pollutants (Evens et al., 2004). Epidemiological evidence has demonstrated that long-term arsenic exposure is strongly associated with increased risks of various diseases and cancers (Chen and Wang, 1990; Chen et al., 1985, 1992; Chiou et al., 1995; Wang et al., 2002; Carter et al., 2003). *In vitro* and *in vivo* studies have shown that arsenic induces chromosome aberrations (CA), sister chromatid exchanges and micronuclei (MN) in animals and humans (Lee et al., 1986, 1988; Jha et al., 1992; Wiencke and Yager, 1992; Wang et al., 1997; Gurr et al., 1993; Wang and Huang, 1994; Gonsebatt et al., 1997; Gebel, 2001). Consequently, it has become important to study any potential genotoxic effects of arsenic on humans.

Plant bioassays, which are more sensitive and simpler than most methods used to detect the genotoxic effect of environmental pollutants, have been validated in international collaborative studies and demonstrated to be efficient tests for monitoring the genotoxicity of environmental pollutants (de Serres, 1994; Ma, 1999; Yi and Meng, 2003; Uhl et al., 2003; Yi et al., 2005; Yi and Si, 2007). *Allium cepa* is one of the plant species commonly used for evaluating the potential genotoxicity of environmental chemicals (Ma et al., 1995; Jos et al., 2005; Grant, 1982;, Fiskesjö 1985; Smaka-Kincl et al., 1996). Using *Allium* bioassay for arsenic genotoxicity

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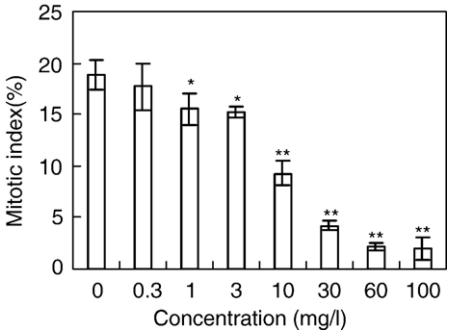

Fig. 1. Mitotic index in *Allium* root tips exposed to sodium arsenite for 12 h.

has been suggested since many plants are known to be injured by arsenic contamination (Carbonell-Barrachina et al., 1997; Liu et al., 2005; Requejo and Tena, 2005), and some plant systems have been suggested as indicators of arsenic exposure (Steinkellner et al., 1998; Kovalchuk et al., 2001). To further understand the genotoxic effects of arsenic on plant cells, the MN assay was performed on root tips of *A. cepa*. In addition, mitotic activity was investigated in *Allium* roots by monitoring mitotic index (MI).

2. Materials and methods

2.1. Chemicals and root tips preparations

Sodium arsenite and arsenic trioxide were purchased from Merck. Small purple bulbs of onions (*A. cepa* L.) were selected as materials. After removal of all dry scales and old roots, the bulbs were suspended in tap water and allowed to germinate at room temperature. When newly emerged roots reached about 2 cm in length, they were used for the tests.

2.2. Treatments

The experiment was performed essentially as described by Ma (Ma et al., 1995). Growing roots were suspended in arsenic solutions for 12 h for treatment, and then maintained in tap water for 24 h recovery. Negative control samples were incubated in tap water. Each group had 6 bulbs. All experimental groups were kept in an incubator at 23±1 °C. For time-course effects test, growing roots were treated with 10 mg/l sodium arsenite for various exposure time (1, 2, 4 and 12 h, respectively), followed by a 24 h recovery period. Thereafter, roots were fixed in a methanol-acetic acid (3:1, v/v) solution and kept at 4 °C overnight. The fixative was replaced with 70% alcohol for long-term storage.

2.3. Mitotic index and micronuclei assays

For slide preparation and microscopic examination, roots were rinsed with distilled water several times, hydrolyzed in 1 M HCl at 60 °C for 8–10 min, and then stained by the Schiff's technique. Meristematic cell and daughter cell (F_1) regions were prepared from each root tip separately by simply cutting the meristem section (first millimeter behind the root cap) and the F_1 section (second 1 mm). These two sections were squashed separately in two different regions on the same slide under two different coverglasses.

Randomly selected views on the slides were monitored to determine the number of dividing cells and micronucleated cells and the total number of scored cells. MI was expressed as percent in the figures, out of approximately 6000 examined cells in M regions taken from 6 separate seedlings for each group. MN frequency was expressed as the number of cells with MN per 1000 scored cells, out of approximately 6000 examined cells taken from 6 separate seedlings for each group.

2.4. Statistical analysis of date

The values of mean and standard deviation (S.D.) were obtained from the results of 6 seedlings for each experimental group. Analysis of variance (ANOVA) and Dunnett's *t* test were used to determine the significant difference (*$P<0.05$, **$P<0.01$) among the negative control and a series of treated groups.

3. Results

3.1. Mitotic index

Figs. 1 and 2 show that As exposure caused mitotic delay and decreased MI in *Allium* root tips. MI

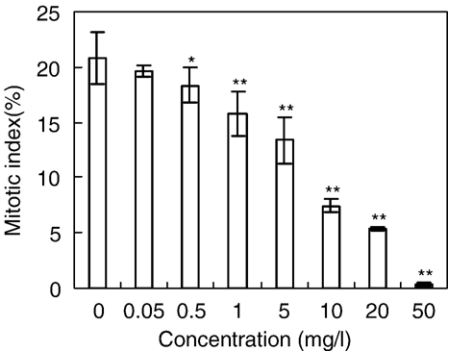

Fig. 2. Mitotic index in *Allium* root tips exposed to arsenic trioxide for 12 h.