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Genotoxicity and growth inhibition effects of aniline on wheat

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HIGHLIGHTS

- Frequency of micronucleus and chromosomal aberrations increased significantly when aniline concentrations >5 mg L^{-1}.
- Aniline significantly inhibits wheat seedling growth at concentrations >25 mg L^{-1}.
- High aniline concentrations in rivers, lakes, and underground water affect crop security.

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ABSTRACT

Aniline is a synthetic compound widely used in industrial and pesticide production, which can lead to environmental pollution. Its high concentration in rivers and lakes is hazardous to aquatic species. Although the mechanism of aniline toxicity has been studied extensively in animals and algae, little is known about its genotoxicity in plants. In this study, we investigated the genotoxicity effects of aniline on wheat root tip cells. The mitotic index of wheat root tip cells decreased when the aniline test concentration was higher than 10 mg L^{-1}. The frequency of micronucleus and chromosomal aberrations increased at aniline concentrations ranging between 5 and 100 mg L^{-1}, and reached 23.3‰ ± 0.3‰ and 8.9‰ ± 0.68‰, respectively, at an aniline concentration of 100 mg L^{-1}. These values were sevenfold higher than those in the control group. The wheat seedlings showed various growth toxicity effects under different concentrations of aniline. The shoot height, root length, fresh weight, and dry weight of wheat seedlings decreased at aniline test concentrations ranging between 25 and 200 mg L^{-1}. At 200 mg L^{-1} aniline, the dry weight was only one-third that of the control group. Overall, the findings of this study provide evidence that aniline is a serious environmental pollutant causing deleterious genotoxic effects on wheat root tip cells and growth toxic effects on wheat seedlings. However, understanding the mechanisms that underlie aniline genotoxicity in plants needs further study.

1. Introduction

Aniline ($C_6H_5NH_2$) is an important intermediate in the organic and fine chemical industry. It has been widely used to produce rubber, organic resin, paint, leather, plastic, and petroleum (Pauluhn, 2004). Furthermore, it plays an important role in the production of a variety of pesticides (fungicides, insecticides, etc.) (Bomhard, 2003). However, its environment-unfriendly nature leads to serious environmental pollution problems (Wang et al., 2014). After entering the natural environment, the molecular structure of aniline becomes very stable. It readily attaches to colloidal organic matter, and changes the physical and chemical properties of water, sediment, and biological populations, resulting in the deterioration of water quality (Zhang et al., 2015). Moreover, aniline is also readily adsorbed by the soil or oxidized to a secondary intermediate product, which is more toxic and difficult to degrade (Chen et al., 2013). Thus, aniline has been listed as a serious organic pollutant that needs to be rigorously controlled in the environment (Suresh et al., 2011). In this regard, investigation of the mechanism of aniline toxicity is both timely and meaningful.

To date, the toxic effects of aniline and nitroaniline have been studied mainly in algae and animals (Batterton et al., 1978; Ward et al., 1996). Bhunia et al.'s research showed that in the human or animal body, aniline could be transformed into a more toxic metabolic intermediate, phenylhydroxylamine (Bhunia et al., 2003). Phenylhydroxylamine contributes to the formation of methemoglobin, which in turn leads to loss of the oxygen-carrying

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capacity of hemoglobin and induces tissue hypoxia, thereby causing multiple damage to the nervous and cardiovascular systems and other organs. Long-term exposure to aniline substantially increases the risk of cancer (such as bladder and prostate cancer) (Ward et al., 1996). Batterton et al. showed that aniline affects the growth and survival of aquatic algae by destroying their structure and inhibiting photosynthesis and respiration (Batterton et al., 1978). Several related studies have shown that the toxic effects of aniline in aquatic animals include digestive dysfunction and interference with foraging behavior (Roberts, 1987; Sun et al., 2013; Dechun et al., 2014). In contrast, the toxic effects of aniline on plants have rarely been reported. Compared to animals, plants are more sensitive, efficient, and convenient models for the rapid screening of the growth and gene toxicity effects of environmental pollutants (Ma et al., 2005). At present, three types of toxicological test are used for assessing the growth of higher plants—seed germination, inhibition of root elongation, and early growth (Burd et al., 2000). These tests have been conducted to evaluate the effects of pollution on plants on the basis of the following indices: root development, degree of biomass reduction, and resistance to pollution. There are currently many established methods used to study genotoxicity in higher plants, including the comet assay, micronucleus assay, chromosome abnormality assay, RAPD, and AFLP, etc. Among these methods, the micronucleus and chromosome abnormality assays are the most basic and the easiest to perform, and have been regarded as sensitive and reliable methods for evaluating chromosomal damage (Guo et al., 2010). Chromosomal abnormality and micronucleus tests have previously been widely used to detect harmful chemical pollutants (Heddle et al., 1991).

In the present study, we used wheat to evaluate the genotoxicity of aniline in plants. The selection of wheat was based on the consideration that cytogenetic analysis of wheat is relatively straight forward, and that the wheat chromosomes are large, and thus appropriate for use in the micronucleus and chromosome abnormality assays. As one of the most important food crops in the world, wheat has been widely cultivated and requires multiple irrigations for its growth (Barbeau et al., 2006). Irrigation water resources generally include rivers, lakes, and underground water (Duda, 1993). High concentrations of aniline in irrigation water resources may have a potentially adverse influence on the growth of wheat. To date, however, studies demonstrating the biological toxicity of aniline on wheat have rarely been conducted. In the present study, wheat seeds were exposed to different concentrations of aniline in order to evaluate the genotoxicity effects of aniline on wheat root tip cells and seedlings. Morphological changes during the growth of wheat seedlings were investigated, and the genotoxicity of aniline on wheat root tip cells was investigated and analyzed using chromosomal aberration and micronucleus assays. This study provides not only a scientific basis for the evaluation of the toxic effect of aniline on crops, but also a technical reference for evaluating the environmental risk of aniline by using plant detection technology.

2. Materials and methods

2.1. The preparation of plant materials and aniline

Wheat (*Triticum aestivum*) variety Long Mai 31, which has stable genetic traits, was provided by the Heilongjiang Academy of Agricultural Sciences. This variety has been widely cultivated in northeastern China. The seeds were surface-sterilized with 5% sodium hypochlorite (NaClO) solution for 15 min, and then washed to remove excess NaClO. Subsequently, the seeds were allowed to germinate for 12 h in a Petri dish containing two pieces of moistened filter paper. The aniline used in this study (purchased from the Tianji Chemical Reagent Factory, Nan Jing, China) was diluted to the required concentrations of 0.1, 5, 10, 25, 50, 100, and 200 mg L^{-1}, and stored in brown bottles until used in the next experimental step.

2.2. Mitotic index and chromosomal aberration in wheat root tip cells

Three hundred wheat seeds were singled out and placed in culture dishes containing two layers of filter paper, which were soaked in distilled water. The seeds were incubated in a plant growth chamber (YI HENG MGC-350BP, China) at 25 °C for 1 d. The seeds were subsequently transferred to new culture dishes containing distilled water or various concentrations of aniline solution (three replicates of each concentration) and incubated until the radicles had elongated to 1 cm. After 24 h of cultivation (12 h light and 12 h dark) at 25 °C, the cultured seeds were rinsed with distilled water for 12 h. The root tips (1–2 cm) were cut and fixed in a solution of ethanol and acetic acid (3:1) for 24 h. Subsequently, the root tips were washed with distilled water and stored in 70% ethanol. For slide preparation, the root apical meristems were immersed in 1 M HCl at 60 °C, and stained with Schiff's reagent after 5 min. All sections were examined microscopically. First, we identified chromosomal aberrations and micronuclei under low magnification, and then used high magnification (× 40 or × 100). Slide preparation procedures and examination processes were carried out in accordance with the standard protocols described by Fenech (1993) and Yi et al. (2005). The frequency of chromosomal aberrations was expressed as the number of cells with chromosomal aberrations per 1000 scored cells, from a total of approximately 10,000 cells. Examined cells were taken from 10 separate seedlings in each group. Each experiment was repeated three times (Guo et al., 2010).

2.3. Germination of wheat seeds and growth of seedlings

The wheat seeds, processed as previously described, were randomly divided into three groups, each consisting of 100 seeds, and transplanted into 12-cm-diameter glass bottles containing two moist filter papers. Seeds were exposed to 25 mL each of the six different concentrations of aniline (Wei et al., 2014). The negative control group was exposed to distilled water for the same period. The treatment was replicated three times. All test groups were incubated at 25 °C under a dark/light cycle (8/16 h) in a plant growth chamber (YI HENG MGC-350BP, China). The treatment solution was replaced every day to maintain a constant concentration (Zhang and Kirkham, 1994). The seed germination rate was defined as the number of germinated seeds (radicle length $\geq$ 0.5 cm) on the 7th day after culture initiation. After 2 weeks of growth, the root lengths, shoot lengths, fresh weight, and dry weight of 100 seedlings in each group were measured. Each experiment was repeated three times.

2.4. Statistical analysis

All data presented are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using the SPSS statistical package version 17.0. Comparisons between the control and treatments were made by one-way analysis of variance (ANOVA). Difference from the control was considered significant at $P < 0.05$ or highly significant at $P < 0.01$.