INTRODUCTION

The atmospheric ozone content has gradually been decreasing every year. Depletion of stratospheric ozone increases the solar ultraviolet radiation in the range of 290–320 nm (UV-B) that reaches the surface of the Earth [1]. Enhanced UV-B radiation directly affects the lives and development of organisms, leading to variations in morphological structure, physiological metabolism, genetic properties, and growth cycle of many animals and plants, and thereby further threatens human beings. Therefore, it is particularly essential to understand the influence of UV-B radiation and the mechanism by which it affects field crops production [2, 3].

Previous studies of UV-B effects on wheat plant have shown abnormal mitosis in somatic cells. Enhanced UV-B could inhibit mitosis frequency and also result in lagging chromosomes, chromosome bridges, free chromosomes, nuclear deformation, partition-bundle division, and other types of aberrations [4]. It was concluded that enhanced UV-B inhibited the DNA replication. The UV-B may also be associated with the chromosome movement pulled by mitotic spindle. However, it would require more investigation to fully understand the effects of UV-B and underlying mechanism.

RCC1 (Regulator of Chromosome Condensation 1) is a candidate enzyme associated with chromatin that could drive spindle assembly. This candidate is the guanine nucleotide exchange factor for the small Ran GTPase (Ran-GTP) and regulates GTP binding and hydrolysis. Localized generation of a high Ran-GTP concentration by chromatin-associated RCC1 in condensed chromosomes has been hypothesized to be essential for mitotic spindle assembly, nucleocytoplasmic transport, and nuclear envelope formation. The phosphorylation of RCC1 in mitosis by Cdc2 kinase is necessary to generate Ran-GTP on mitotic chromosomes in mammalian cells activating a subset of mitotic motors and microtubule associated proteins (MAPs), which in turn are required for spindles assembly and chromosomes segregation [5, 6].

Studies of cell extracts derived from Xenopus laevis eggs support that Ran-GTP is sufficient to promote the assembly of complete spindle-like structures and chromosome-induced spindle formation [7]. Mam-
malian RCC1 contains an NLS (nuclear location signal) at its N-terminus and is imported into the nucleus, where it interacts with chromatin via core histones H2A and H2B [8–11]. Fluorescent recovery after photobleaching (FRAP) experiments demonstrated a highly dynamic interaction between RCC1 fused to green fluorescent protein (GFP) with chromatin. Moreover, the methylated N-terminal tail of RCC1 stabilized its interaction with chromatin in live cells [12–15]. Temperature-sensitive (ts) mutants in the RCC1 gene of BHK cells might fail to maintain correct flow cell cycle. It may prematurely condense chromosomes and enter mitosis at the restrictive temperature without completed S-phase [16]. In tsBN2 cells, spindles form normally even at the nonpermissive temperatures [17, 18]. RanQ69L-GTP disrupts the RCC1-generated Ran-GTP gradient, while flattening the gradient eliminates spindle assembly around chromatin beads [19, 20]. These experiments demonstrated that a Ran-GTP gradient is required for chromatin-dependent spindle assembly in cell extracts from Xenopus eggs.

In this study, the wheat plants were regarded as test objects. We identified and researched the location of RCC1 in the mitotic cells using immunoblotting and immunofluorescence techniques. The results showed the effects of UV-B radiation on the location of RCC1 during mitosis in wheat somatic cells. The mechanism of abnormal mitosis by enhanced UV-B radiation has also been explained.

MATERIALS AND METHODS

Plant material. Wheat seeds (Triticum aestivum L., ML7113) used in this research were supplied by Wheat Research Institute of Shanxi Academy of Agricultural Sciences. The plump seeds of uniform size were selected and rinsed with running water. The seeds were cultured in clean dish paved three layers of wet gauze (1 mL of ice-cold PBS buffer (NaH2PO4—1.1 g; NaCl—4.25 g; NaH2PO4 —0.1 g, pH 7.4; 500 mL), pH 7.9, was added to the sample; centrifuged at 6000 rpm for 5 min at 4°C, and all supernatant was removed. The centrifuge tube containing pellet was put on ice and 5× more buffer A (1 M Hepes, pH 7.9; 1 M KCl; 0.1 M EDTA; 0.1 M EGTA; 0.1 M DTT; 1 mM PMSF; 10% NP-40) was added to the tube. (NP-40 is a commercially available detergent, which is nonyl phenoxypolyethoxylethanol.) The material was re-suspended in the buffer A and incubated on ice for 5 min, gently shaken occasionally, and was centrifuged at 3000 rpm for 5 min at 4°C. Supernatant was again re-suspended in equal volume of buffer B (1 M Hepes, pH 7.9; 5 M NaCl; 0.1 M EDTA; 0.1 M EGTA; 0.1 M DTT; 1 mM PMSF; 25% glycerin) and incubated on ice for 10 min, gently shaken occasionally, and then was centrifuged at 14000 rpm for 5 min at 4°C. Collected supernatant was the nuclear proteins extraction.

Immunoblotting. The protein samples were separated on 12.5% one-dimensional SDS-PAGE. The amount of protein on the gel was quantified using Quantity One. For immunoblotting, the target band was excised from the gel and transferred onto PVDF membrane and probed with rabbit-anti RCC1 polyclone antibody (Shanghai Standard Biotech, China) diluted at a ratio of 1 : 1000. The secondary anti-rabbit HRP conjugate was used in 1 : 1000 dilutions. The chemiluminescence signal was detected by film with ECL kit purchased from Bio-Rad Laboratories, United States.

Immunofluorescence. Wheat root tips (0.01 g) were fixed by 8% paraformaldehyde in PEM buffer (PIPES—2.096 g; MgSO4—1,540 g; EGTA—2.377 g; pH 6.9; 125 mL) in centrifuge tubes for 1 h and then were washed with PEM buffer (pH 6.8) three times. The plant material was digested in PEM with the addition of 2% cellulase and 1% pectinase at room temperature for 40 min and was washed with PEM buffer (pH 6.8) again. It was then treated with a mixture containing Triton X-100, DMSO, BSA, and PBS (pH 7.4) for 2 h. Samples were rinsed with PBS (pH 7.4) three times (10 min each time) and then incubated with primary antibody (1 : 100) overnight at 4°C in dark. After rinsing gently with PBS (pH 7.4) the secondary antibody (1 : 100) was used to incubate samples for 2 h at 37°C. Finally, the samples were washed with PBS (pH 7.4) for four times, and the nuclei were stained with fluorescent dye DAPI for 30 min. The samples were then kept on slides and sealed for visualization by confocal laser scanning microscope (Olympus, Japan).

RESULTS AND DISCUSSION

The Effects of Enhanced UV-B Radiation on RCC1 in Tip Cells of Wheat

The vast majority of RCC1 was located in the nucleus. The same quality materials were prepared for nuclear proteins extraction and used for SDS-PAGE. The SDS-PAGE preliminarily separated RCC1 from...