



Full Length Article

Effects of Enhanced Ultraviolet-B Radiation on Chromosomes and Microtubule Arrays in Wheat Roots

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Abstract

Enhanced ultraviolet-B (UV-B) radiation causes chromosomal aberrations and abnormal microtubule arrays in wheat (*Triticum aestivum*) root cells. Oryzalin induces chromosome doubling through depolymerization of microtubules and results in diploidization. We explored the effect of oryzalin on wheat roots exposed to enhanced UV-B radiation. Treatment with 500 $\mu\text{mol}\cdot\text{L}^{-1}$ oryzalin resulted in chromosome doubling with two equal nuclei. However, exposure to UV-B radiation in combination with oryzalin resulted in two unequal nuclei. The frequency of unequal nuclei caused by treatment with UV-B and oryzalin was higher than that caused by UV-B exposure alone. During the mitotic phase, chromosomal aberrations were indicated to be independent of abnormal microtubule structures. These suggested that chromosomal aberrations caused by enhanced UV-B radiation were not a consequence of abnormal microtubule structures. © 2016 Friends Science Publishers

Keywords: Microtubules; Chromosomes; UV-B; Oryzalin; Wheat

Introduction

Most of the incoming ultraviolet (UV) solar radiation is absorbed by the stratospheric ozone layer, but UV-B (wavelengths 280–315 nm) is a component of solar radiation that reaches the Earth's surface. Intense enhancement of UV-B radiation is one prospective outcome of stratospheric ozone depletion (Taalas *et al.*, 2002). Enhanced UV-B radiation exerts strong impacts on plant growth and development, such as degradation of photosynthetic systems, alteration of secondary metabolism, modification of cell morphology, and impairment of the plant cytoskeleton (Xiong and Day, 2001; Hollósy, 2002; Gao *et al.*, 2003; Zaets *et al.*, 2006).

Microtubules are an extremely important component of the cytoskeleton and are involved in cell division, vesicle transport, cell support, growth and development (Howard and Hyman, 2003; Struk and Dhonukshe, 2014). Disruption of plant microtubules induced by enhanced UV-B radiation is accompanied by cell shrinkage, chromatin condensation, and cytoplasmic vacuolation in both interphase and the mitotic phase (Lytvyn *et al.*, 2010). Plant microtubules also participate in UV-B signaling pathways as a responsive factor (Krasnylenko *et al.*, 2013). Exposure of wheat (*Triticum aestivum*) somatic cells to enhanced UV-B (10.08 $\text{kJ m}^{-2} \text{day}^{-1}$) causes depolymerization of cortical microtubules and aggregation of arrays of spindle microtubules (Guo *et al.*, 2010). In addition, the frequency

of chromosomal aberrations is increased, including chromosome bridges and laggard chromosomes and leads to partition-bundle division (a novel type of chromosomal aberration, in which chromosomes at the transition from anaphase to telophase of mitosis are divided into three, four or six bundles) (Rong *et al.*, 2002; Gao *et al.*, 2010). Damage to the spindle apparatus directly impacts on chromosome behavior. Therefore, the mechanism of UV-B radiation-enhanced chromosomal aberrations probably involves injury to microtubule arrays (Gao *et al.*, 2010; Guo *et al.*, 2010).

Signal transduction in response to exposure to UV-B radiation is a complicated process in plants. A photoreceptor of UV-B radiation has been identified (Jenkins, 2009; Tilbrook *et al.*, 2013). However, downstream signal transduction of UV-B radiation and how it impacts chromosomes remain unknown. Oryzalin, a microtubule inhibitor, blocks microtubule assembly but not ongoing disassembly, causing microtubule depolymerization. Oryzalin is used to induce chromosome doubling in plants by inhibiting mitotic activity and is considered a preferable alternative to colchicine because of its lower toxicity (Kermani *et al.*, 2003; Dhooghe *et al.*, 2011; Rêgo *et al.*, 2011).

In this study we investigated the effects of enhanced UV-B radiation on chromosomes and microtubule arrays in wheat roots. Oryzalin was used to induce microtubule disassembly and we observed the resultant chromosomal

abnormalities. It is hypothesized that chromosomal aberrations caused by UV-B radiation are not caused by abnormalities in microtubule structures.

Materials and Methods

Plant Material

Seeds of wheat (*Triticum aestivum* 'ML 7113') were supplied by the Wheat Research Institute of the Shanxi Academy of Agricultural Sciences, XXXX Shanxi, China. Plump seeds of uniform size were selected and rinsed with running water. After germination, seeds were cultured on moist filter paper in petri dishes.

Treatments

After germination, the seeds were incubated in a growth chamber at 25°C and 60% relative humidity for 1 day. Seeds were treated with UV-B radiation of 10.08 kJ m⁻² day⁻¹. This is equivalent to the UV-B radiation intensity that would result from a 20% decrease in stratospheric ozone concentration and UV-B was enhanced by 40% at Xi'an during a clear day on the summer solstice. In addition, seeds were treated with 500 µmol·L⁻¹ oryzalin. This dosage of oryzalin induces the highest rate of chromosome doubling (Zhang and Li, 2008). Four treatments with three replications each were one hundred seeds applied according to different light/dark photoperiods (Table 1): distilled water without UV-B radiation (control; CK); distilled water with UV-B radiation (B); oryzalin without UV-B radiation (O); and oryzalin in combination with UV-B radiation (OB).

Immunofluorescence Localization of Tubulin and Microscopy

Wheat roots were fixed in 4% paraformaldehyde and 1% glutaraldehyde in microtubule stabilizing buffer (MSB; 50 mM PIPES (Sigma, St. Louis, USA), 5 mM EGTA, 5 mM MgSO₄, pH 7.0, adjusted with KOH) for 1 h at room temperature, then rinsed three times for 10 min each in MSB. The fixed roots were digested with 2% Cellulase R10 and 1% Pectolyase Y-23 (both from Yakult Honsha, Tokyo, Japan) for 30 min at 37°C, then rinsed twice in MSB for 15 min each. The root cell was separated from the wheat root apex, placed on a clean glass microscope slide, and squashed with a coverslip. Samples from each treatment were incubated with mouse anti-β-tubulin antibody (dilution 1:40; Sigma), 1% Triton X-100 and 3% acetylated bovine serum albumin in phosphate-buffered saline (PBS; 3 mM KH₂PO₄, 7 mM K₂HPO₄, 150 mM NaCl) overnight at 4°C. After rinsing gently with PBS, the samples were incubated with anti-mouse IgG-FITC antibody (dilution 1:100; Sigma, St. Louis, USA) in PBS for 2 h at 37°C. Samples were stained with 4'-6-diamidino-2-phenylindole (DAPI) for 30 min and examined with a confocal laser scanning microscope (FV1000, OLYMPUS).

Statistical Analysis

Frequencies of equal and unequal nuclei were counted in at least 1000 nuclei in root cells. All data were statistically analyzed using Student's *t*-test with SPSS software (SPSS 17.0, Inc., Chicago IL, USA). The level of significance applied was 0.001.

Results

Equal and Unequal Nucleus Frequency

Oryzalin treatment (O) induced chromosome doubling and resulted in two equal nuclei within a root cell (Fig. 1A). Oryzalin combined with UV-B radiation treatment (OB) resulted in two unequal nuclei within a root cell (Fig. 1B). Both O and OB treatments induced a significant increase in the frequency of equal nuclei ($P < 0.001$) compared with CK and B. The frequency of unequal nuclei in the OB treatment was significantly increased ($P < 0.001$), but that of the O treatment was not significantly different, compared with the CK and B treatments. Slight but non-significant differences in the frequencies of equal and unequal nuclei were observed between the CK and B treatments (Table 2).

Abnormal Microtubules and Chromosomes in Different Phase

Microtubules formed arrays in the mitotic phase, including a preprophase band (PPB), the spindle apparatus at metaphase and anaphase, and the phragmoplast at telophase, in the CK treatment (Fig. 2A–D). Treatment with UV-B radiation alone induced abnormal microtubule structures, including an asymmetric PPB, asymmetric spindle, and defective phragmoplast (Fig. 2E–H). Spindle arrays of irregular alignment were observed in both O and OB treatments (Fig. 2I–P). Treatment with oryzalin alone resulted in chromosome doubling (Fig. 2K, L). In the OB treatment polykaryocytes and ring chromosomes were observed (Fig. 2M–P). Further examination of the microtubule arrays at telophase showed that the microtubules were arranged at the equatorial plate in the CK and B treatments (Fig. 3A–D), but were arranged irregularly in the O and OB treatments (Fig. 3E–H). Multipolar chromosomal segregation (three-bundle division) was observed in the B and OB treatments, regardless of microtubule depolymerization (Fig. 3C, D, G, H).

Discussion

In a preliminary study, we previously showed that enhanced UV-B radiation could inhibit the frequency of mitotic cell divisions in wheat and cause chromosomal aberrations, including partition-bundle division (Rong *et al.*, 2002).

Table 1: Treatments and the associated photoperiods applied in the study

Treatment	Light (h·d ⁻¹)	Enhanced radiation (h·d ⁻¹)	UV-B Oryzalin (h·d ⁻¹)	Dark culture (h·d ⁻¹)
CK	8	-	-	16
B	8	8	-	16
O	8	-	24	16
OB	8	8	24	16

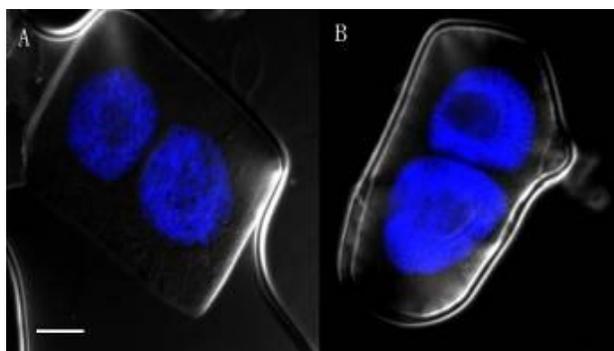
CK: distilled water without UV-B; B: distilled water with UV-B; O: oryzalin without UV-B; OB: combined UV-B and oryzalin

Table 2: Frequency of equal and unequal nuclei in each treatment

Treatment	Equal nucleus	Unequal nucleus
CK	23.67±8.02 c	6.00±2.00 b
B	15.33±4.51 c	19.67±2.52 b
O	207.67±16.86 a	19.33±4.16 b
OB	174.00±6.08 b	106.33±16.20 a

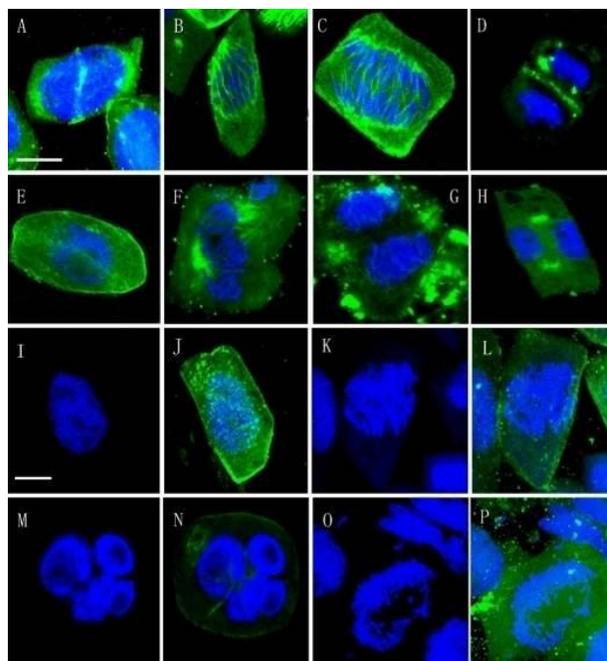
Mean ± standard deviation. Values followed by the same letter are not significantly different ($P > 0.001$)

CK: distilled water without UV-B; B: distilled water with UV-B; O: oryzalin without UV-B; OB: combined UV-B and oryzalin


Fig. 1: Oryzalin-induced doubling of the nucleus in wheat root cells. (A) Equal nuclei, (B) unequal nuclei. Bar = 10 μ m

In order to explain this phenomenon, we have investigated the effects of enhanced UV-B radiation on wheat pertaining to changes in morphology, physiology, and the cytoskeleton (Yang *et al.*, 2013; Huize and Rong, 2014; 2015). Damage to the structure of the spindle apparatus caused by UV-B radiation directly impacts on chromosomes, and changes in microtubule structures influence chromosome distribution. We previously speculated that damage to microtubule structures will readily cause chromosome accumulation or loss in cells and lead to partition-bundle division (Guo *et al.*, 2010).

To study whether the abnormal microtubule structures induce chromosomal aberrations, we used the oryzalin to depolymerize microtubules. We observed that chromosome doubling and unequal nuclei were induced by treatment with oryzalin in combination with UV-B radiation, but not by UV-B radiation treatment alone. This finding showed that UV-B radiation influenced


Fig. 2: Immunostaining of the nucleus and microtubule structures of wheat root cells. Cells were stained with DAPI (blue) and FITC conjugated anti- β -tubulin (green). (A–D) CK treatment: preprophase band (A), metaphase (B), anaphase (C), phragmoplast (D); (E–H) B treatment: asymmetric preprophase band (E), asymmetric spindle (F), defective phragmoplast (G, H); (I–L) O treatment: prophase (I, J), chromosome doubling (K, L); (M–P) OB treatment: polykaryocyte (M, N), ring chromosomes (O, P). Bar = 10 μ m

chromosome polarity, especially if the microtubules were depolymerized, and indicated that microtubules protected chromosome polarity in the presence of UV-B radiation.

To investigate further, we observed the distribution of microtubules and chromosomes in wheat root cells in the mitotic phase. UV-B radiation caused chromosomal aberrations and induced abnormal microtubule arrays, including an asymmetric PPB, asymmetric spindle, and defective phragmoplast. The spindle was not visible in the O and OB treatments, owing to the effect of oryzalin treatment, but polykaryocytes and ring chromosomes were observed. These observations further illustrate that UV-B radiation induced chromosomal aberrations independent of abnormalities in microtubule structures. Three-bundle division, a type of partition-bundle division, was caused by exposure to UV-B radiation. We observed three-bundle division in the B and OB treatments regardless of formation of an equatorial plate by microtubules at telophase. In 3-bundle division, microtubule impacted chromosomes un conspicuous at least in telophase.

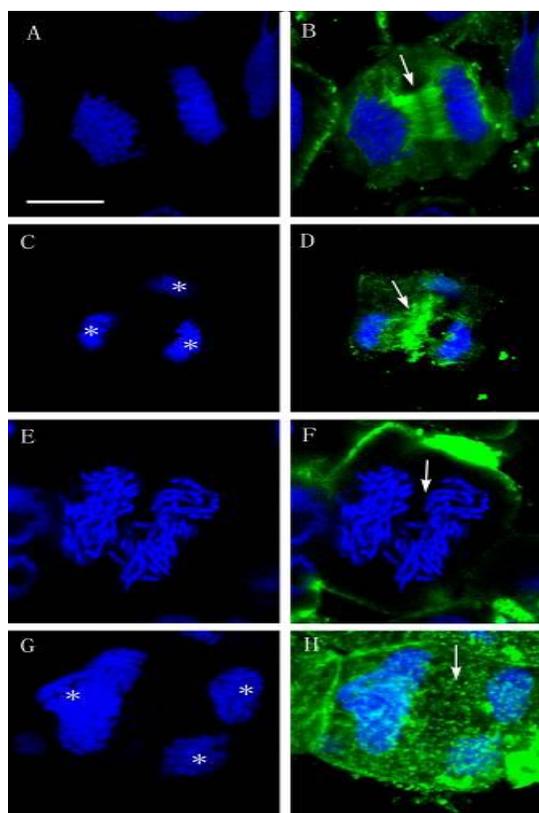


Fig. 3: Telophase of wheat root cells of the CK treatment (A, B), B treatment (C, D), O treatment (E, F), and OB treatment (G, H). Three-bundle division is shown in (C, D, G, H). Asterisks indicate chromosome bundles. Arrows indicate the position of the equatorial plate. Bar = 10 μ m

Conclusion

Considering the present results, we conclude that chromosomal aberrations caused by UV-B radiation are not a consequence of abnormal microtubule structures. That is, UV-B radiation does not influence microtubule structures to affect chromosome behavior. Further research is needed to identify the underlying factors responsible for chromosomal aberrations resulting from exposure to UV-B radiation.

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