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Scientific Research

The Distribution and Morphology Alterations of Microfilaments and Microtubules in Mesophyll Cells and Root-Tip Cells of Wheat Seedlings under Enhanced Ultraviolet-B Radiation

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Abstract

The distribution and morphology alterations of microfilaments and microtubules in the mesophyll cells and root-tip cells of wheat seedlings, which had been radiated by enhanced ultraviolet-B (10.08 KJ·m^{-2}·d^{-1}), were examined through the confocal laser scanning microscope (Model FV1000, Olympus, Japan). Microtubule was labeled with an indirect immunofluorescence staining method, and microfilament was labeled with fluorescein isothiocyanate-phalloidin (FITC-Ph) as probes. The results indicated that microtubules in mesophyll cells, compared with the controls, would be depolymerized significantly, and dispersed randomly showing some spots or short rods in the cytoplasm, under the enhanced UV-B radiation condition. The microtubule bundles tended to be diffused, and the fluorescence intensity of that significantly decreased. The distribution pattern of microfilaments, which usually arranged parallelly in control cells, was broken up by enhanced UV-B radiation. We further investigated the distribution and morphology of microtubules in root-tip cells during every stage of cell division, and found that these aberrant phenomena of microtubules were often associated with abnormal cell division. Our findings suggested that the distribution, morphology and structure of cytoskeleton in mesophyll cells and root-tip cells of wheat seedlings would be affected by enhanced UV-B radiation, which might be related to abnormal cell division caused by enhanced UV-B radiation as an extracellular signal.

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Keywords

Wheat, Microfilament, Microtubule, Enhanced UV-B Radiation, Confocal Laser Scanning Microscope

1. Introduction

With the acceleration development of global industrialization, the environment problem becomes more and more serious, which will increase the Ultraviolet-B (UV-B, the wavelength range of 290 nm - 320 nm) radiation that reaches the surface of the earth [1] [2]. The enhanced UV-B radiation can directly affect the growth and development of many organisms and may further threaten human beings [3] [4]. In the early 1990s', the influence of enhanced UV-B radiation on the growth of living organisms had been extensively concentrated. Recently, lots of researches have indicated that enhanced UV-B radiation can arouse significant effects on living organisms in morphological structures, physiological processes, biochemical metabolism, photosynthesis, gene expression and so on [5]-[7]. Therefore, it is necessary to study further the mechanism of enhanced UV-B radiation damage on organisms, especially crops, in order to obtain some effective protection methods [8].

The microfilament and microtubule are two crucial cytoskeleton components in eukaryotic cells, which play an important role in cell form maintenance, cells or organelles migration, many inorganic or organic substances transport, cell signal transduction, and cell reproduction regulation [9]. However, there are relative few reports about the effects of enhanced UV-B radiation on microfilament and microtubule systems in cells [10], including the relationship between microfilament or microtubule systems and abnormal cell division. Whether the growth change of living organisms is related to the change of cytoskeleton under enhanced UV-B radiation, and how the enhanced UV-B radiation affects microfilament or microtubule. These problems need to be solved as soon as possible [11].

Confocal laser scanning microscope is a kind of essential method for researching cellular or subcellular structures and components, and the application of which establishes a perfect pathway for studying further submicroscopic structures in cells and organelles [12].

During our researches, the influence of enhanced UV-B radiation on microfilament and microtubule systems in mesophyll cells and root-tip cells had been measured through selecting wheat seedlings as experimental materials. The purpose of this study was aimed at exploring the distribution and morphology alterations of cytoskeleton system in cells of wheat seedlings under enhanced UV-B radiation, in order to explain the effects and injury mechanism for enhanced UV-B radiation on growth and development of wheat seedlings [13]. Research achievements of this work may provide theoretical foundation and experimental systems for studying effects of unfavorable environment factors on cytoskeleton system in plant cells.

2. Materials and Methods

2.1. Plant Materials and Culture

Seeds of winter wheat (*Triticum aestium*, cv. 93-4736) were used as materials for this research and obtained from Wheat Research Institute, Shanxi Agricultural Sciences Academy, China. Uniform size and plump seeds were selected and washed repeatedly more than 3 times with sterilized water after being surface sterilized in 0.1% $HgCl_2$ solution for 2 - 3 min, then incubated in Petri dishes (diameter 18 cm) with wet filter paper [14]. There were 3 replications, each containing 50 granules. These seeds in Petri dishes were cultured for 3 d, at 24˚C ± 2˚C, relative humidity 70%, in a plant growth chamber under darkness until germination [14].

2.2. Experimental Methods

Enhanced UV-B radiation was provided by a filter lamp (Qin brand, 30W, Baoji Lamp Factory, Baoji city, China) according to the procedure described by Lydon *et al.* [15]. The UV-B radiation intensity was 10.08 $KJ \cdot m^{-2} \cdot d^{-1}$, which was equivalent to the supplemental level with 20% stratospheric ozone reduction or 40% UV-B radiation enhancement during a clear day [16]. In addition to enhanced UV-B radiation, the visible light radiation was also supplied for 8 h per day at the same time [17]. Wheat seedlings were treated constantly for 7

days, and then were harvested for further being measured.

In our studies, the wheat seedlings were divided into two groups: the control group (C, only visible light radiation, without enhanced UV-B radiation) and enhanced UV-B radiation treatment group (B).

2.3. Protoplasts Preparation and Activities Detection

Fresh leaves (about 5.0 g) of wheat seedlings were immersed in hydrolytic solution and treated about 3 h at 26˚C ± 1˚C under darkness. The hydrolytic solution containing leaf tissues was vibrated one time at interval half an hour. The ratio of leaf tissues and hydrolytic solution was 1:5 (w/v), and the hydrolytic solution was configurated by using standard solution with 1.5% cellulase R-10 (Japan) and 0.1% pectinase (Fluka). When enzyme treatment finished, mesophyll tissue being incompletely digestive were filtered by 20 mesh nylon net. Then desirable protoplasts were collected through centrifuging at 500 r/min and washed 2 times with PBS buffer. Subsequently, these protoplasts were mixed with 25 mg/ml fluorescein diacetate (FDA, Sigma F7378) in proportion to 1:1 (v/v) and placed at room temperature about 10 min for detecting protoplasts activities.

2.4. Microfilament Label in Protoplast of Mesophyll Cells

Microfilaments were labeled with 200 nmol/L FITC-Ph and incubated about 2 h at 26˚C under darkness. At the same time, the comparison group was established and treated under the above circumstance, but in which protoplasts were not labeled with FITC-Ph or labeled with 10% hydroxypropyl-beta-cyclodextrin (Hp-β-CD, Sigma) for 2 h.

2.5. Microtubule Label in Protoplast of Mesophyll Cells and Root-Tip Cells

The microtubules were marked by indirect immunofluorescence antibody localization technology. At first, mesophyll protoplasts in wheat seedlings were fixed with 3.7% polyoxymethylene for 30 min, extracted with 0.5% triton X-100 for 20 min and washed with phosphate buffer saline (PBS) for 3 times, 5 min per time. Then protoplasts were sealed with 3% bovine serum albumin (BSA) for 15 min. The suspension of protoplasts were mixed with mouse monoclonal antibody (Sigma, USA) in accordance with the proportion of 1:160 (v/v) and incubated for 1.5 h at 37˚C, then washed with PBS buffer for 5 times. The suspension of protoplasts were immediately marked with Texas-red IgG (Invitrogen) in accordance with the proportion of 1:200 (v/v) and incubated for 1 h at 37˚C again, washed with PBS buffer for 5 times, 5 min per time. At last, bio-loaded section were sealed immediately with 50% glycerine. In the meanwhile, the control group (C) was set, in which protoplasts were incubated mixed with 3% BSA instead of mouse monoclonal antibody.

Microtubules in root-tip cells were labeled according to the above procedure. The difference was that root-tips should be enzymolyzed (1% cellulose R-10 and 1% pectinase) prior to being extracted with 0.2% triton X-100 and washing buffer was PEM instead of PBS.

2.6. Microfilament and Microtubule Microscopic Observation

The distribution and morphology of microfilaments and microtubules were observed through confocal laser scanning microscope (FV1000, Olympus, Japan), with an excitation wavelength at 488 nm or 543 nm.

3. Results

3.1. Mesophyll Protoplasts of Wheat Seedlings Preparation and Activities Measurement

The desirable mesophyll protoplasts of wheat seedlings were obtained with enzymatic method. There were lots of spherical protoplasts packaged by a thin layer of plasma membrane under the optical microscope, in which many green chloroplasts distributed. These protoplasts were stained with FDA indicator and detected with the confocal laser scanning microscope under 488 nm excitation light. We would find that most of protoplasts still showed green fluorescence brightly, which demonstrated that these protoplasts were high dynamic (**Figure 1(a)**).

3.2. Influence of Enhanced UV-B Radiation on Microfilaments in Mesophyll Cells of Wheat Seedlings

In mesophyll protoplasts of C group, we found that microfilaments system mainly arrayed in parallel rows with

confocal laser scanning microscope (**Figure 1(b)**), the morphology and distribution of which was consistent with the results of Chen *et al.* [18]. However, when being radiated by enhanced UV-B, only few of microfilaments aggregated into microfilament bundles, others depolymerized into dot-like or fragmentary (**Figure 1(c)**). But being treated with CD depolymerization agent, microfilaments system would be destroyed completely (**Figure 1(d)**).

3.3. Influence of Enhanced UV-B Radiation on Microtubules in Mesophyll Cells of Wheat Seedlings

In C group, cortical microtubules in mesophyll cells distributed over the whole cytoplasm and took on three-dimensional network structure (**Figure 1(e)**). While in B group, microtubules system arrayed disorderly and presented punctiform or segment-like (**Figure 1(f)**). As a consequence, cortical microtubules depolymerized significantly under enhanced UV-B radiation.

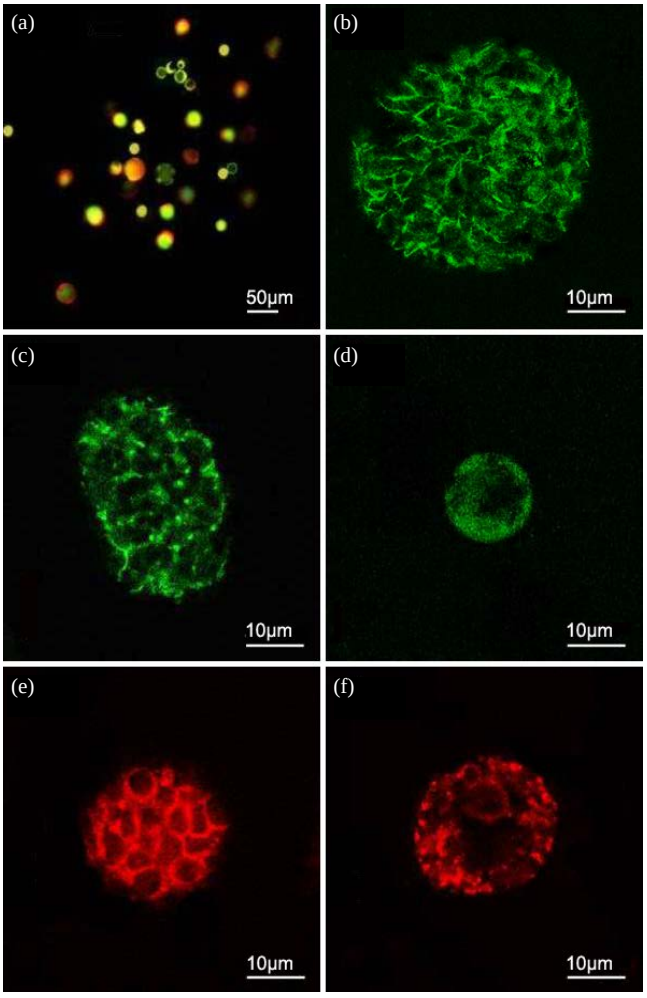

Figure 1. Influence of enhanced UV-B radiation on microfilaments and microtubules in mesophyll cells. (a) Purification and activity assay of wheat mesophyll protoplasts; (b) Microfilaments in wheat mesophyll protoplasts of control group; (c) Microfilaments in wheat mesophyll protoplasts after enhanced UV-B radiation; (d) Microfilaments in wheat mesophyll protoplasts being treated by Hp-*β*-CD; (e) Microtubules in wheat mesophyll protoplasts of control group; (f) Microtubules in wheat mesophyll protoplasts after enhanced UV-B radiation.

3.4. The Distribution and Morphology of Microtubules in Root-Tip Cells during Cell Division

The cytoskeleton of microtubules were labeled with indirect immunofluorescence localization method, and observed through confocal laser scanning microscope. There were different microtubule arrangement types in cell division cycle of C group cells, including cortical microtubules in cell inter-phase (**Figure 2(a)**), pre-prophase band (PPB, **Figure 2(b)**) and perinuclear microtubules (**Figure 2(c)**) in cell prophase, complete spindle body in cell metaphase (**Figure 2(d)**), spindles and polar microtubules (**Figure 2(e)**) in anaphase, phragmoplast microtubules (**Figure 2(f)**, **Figure 2(g)**) in the last phase of cells. And when cell division finished, the phragmoplast

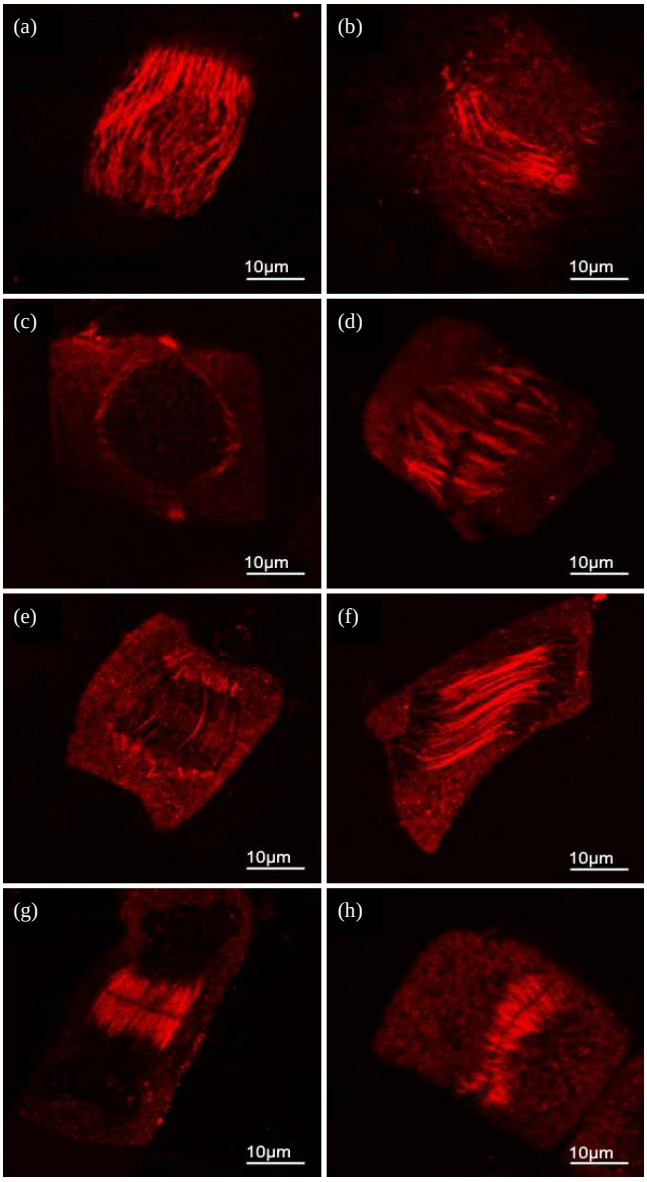

Figure 2. The microtubules distribution and morphology during cell division every stage in root-tip cells of wheat seedlings in C. (a) Cortical microtubules in cell interphase; (b) Preprophase band; (c) Perinuclear microtubules in cell prophase; (d) Complete spindle body in cell metaphase; (e) Spindles and polar microtubules in anaphase; (f), (g) Phragmoplast microtubules; (h) Deposition of cell plate.

microtubules disappeared accompanied with deposition of cell plate (**Figure 2(h)**), the cortical microtubules formed again in cytoplasm.

However, we found that quite a few of microtubules abnormal arrangement phenomenon happened in root-tip cytoplasm, and most of microtubules system would be damaged partially under enhanced UV-B radiation. Compared with the controls, microtubules system displayed periodic arrangement in B group root-tip cells. Microtubules arrayed with network-like (**Figure 3(a)**) or depolymerized into scattered punctuate (**Figure 3(b)**) in inter-phase. When getting into prophase, two pre-prophase bands would form in cytoplasm of B group (**Figure 3(c)**), which also was found in studies of Huang *et al.* [19]. And in cell metaphase, few of microtubules gathered spindle fiber, the intact spindle was invisible (**Figure 3(d)**). Microtubules bundles were unconspicuous in the last phase of cells (**Figure 3(e)**), the phragmoplast microtubules would not come into being (**Figure 3(f)**). Therefore, we speculated that the aberrant phenomenon of microtubules during cell division might be one of the main reasons of cell variation.

4. Discussion

Microfilaments and microtubules system are major components of cytoskeleton in eukaryotic cells, which are essential for lots of physiological processes and biochemical metabolism in living organisms [20]. Previous

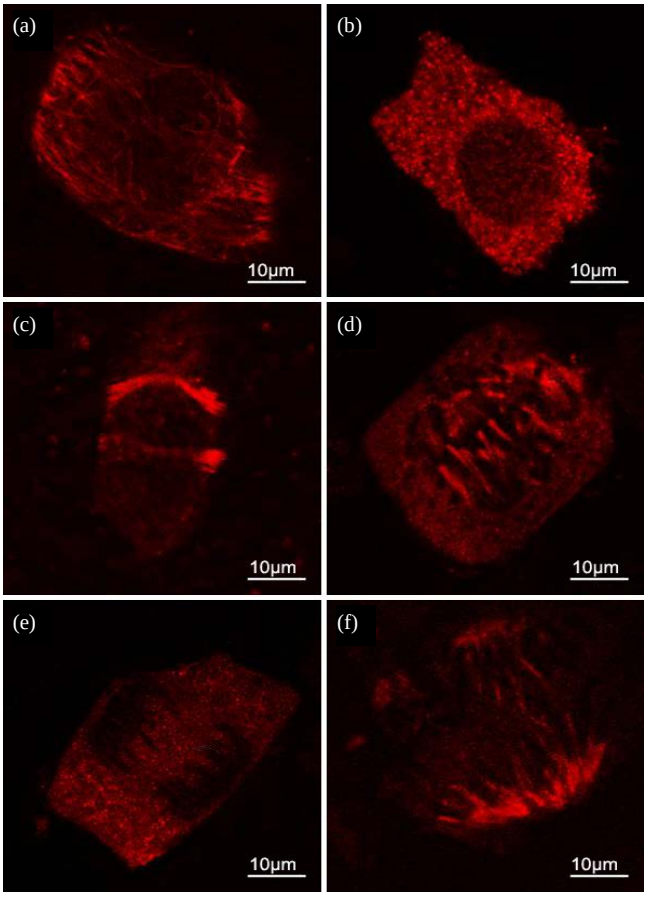

Figure 3. The distribution and morphology of microtubules during cell division every stage of in root-tip cells in B. (a) Microtubules arrayed with network-like in interphase; (b) Other microtubules depolymerized into scattered punctuate; (c) Two preprophase bands in cytoplasm in prophase; (d) Few of microtubules only gathered spindle fiber in cell metaphase; (e) Unconspicuous microtubules bundles; (f) Phragmoplast microtubules were invisible.

studies on cytoskeleton mainly focused on animal cells, and there were rarely researches or reports about cytoskeleton morphological alterations and molecular mechanism using plant cells as materials. Recently, with the continuous improvement of research technology in life sciences, the understandings about microfilaments and microtubules in plant cytoplasm are becoming urgently needed.

Plant tissues or cells are normally able to response on numerous environmental stimulating factors, such as UV-B radiation [11], calcium influx [21], following simultaneously cytoplasmic reorganization rapidly, the occurrence of which usually mediated or regulated by the complex microfilaments and microtubules system in plant cells [22]. Chen *et al*. discovered, selecting wheat-leaf rust interaction system as experimental materials, that the microfilaments could keep complete network-like structure in protoplasts of disease-resistant wheat, however, most of microfilaments in ones of susceptible wheat were in a state of depolymerization [18]. Studies of Wang *et al*. had declared that *Bipolaris maydis* C would bring about protoplast contraction, protoplast leakage and microfilament distribution abnormalities in maize root-cap cells [23]. Lei *et al*. also found that microfilament systems would gather into bundles or take on fragmentary when rice root-cap cells had been treated with bacterial blight toxin [24]. Ren *et al*. researched the effects of nitrogen ion implantation on pollen germination and microfilaments, and revealed that microfilaments would be ring bundle state or concentrated parallel structure induced by low-energy nitrogen ions, which mainly distributed in pollen germination groove [25]. Then, whether does the enhanced UV-B radiation affect microfilaments and microtubules of wheat seedlings as one of external stimulation signals. Few of works on this problem has been done until today.

A large number of studies have shown that enhanced UV-B radiation could lead to some abnormal phenomenon in living organisms, such as bio-membrane damage, membrane surface charge reduction, membrane permeability enhancement, cell electrophoresis rate decline, chromosome morphology alteration and aberrant cell division [26]-[28]. R. Han *et al.* (2002) found that the enhanced UV-B radiation would cause “root-bending” phenomenon when wheat seeds germination and “partition-bundle” division in root-tip cells of wheat seedlings [28]. Cell wall-plasma membrane-cytoskeleton have formed continuum in plant cells [29]. In our study, the morphology and distribution pattern of microfilament and microtubule in wheat seedlings, especially in root-tip cell, were significantly changed under enhanced UV-B stress. Furthermore, the distribution alteration of microtubule at cell division every stage in root-tip cell suggested that the spindle assembling was affected and chromosome separation and movement were hindered, due to the microtubule was the predominant components of the spindle. As a result, one of the main reasons of above abiotic injury including “partition-bundle” division, might be that UV-B radiation participated in signal transduction as a kind of extracellular signal, and caused the corresponding biological reaction or physiological processes, finally led to cytoskeleton morphology and distribution alterations.

5. Conclusion

In the present study, we found that the morphology and distribution of microfilaments and microtubules in mesophyll cells all had greatly changed, such as taking on dot-like or diffused fragments randomly under enhanced UV-B radiation. At the same time, the distribution and morphology of microtubules in root-tip cells also had been remarkably influenced by enhanced UV-B radiation during cell cycle, which might be one of the predominent reasons on the aberrant cell division (such as partition bundle division [27] in root-tip cell) caused by enhanced UV-B radiation.

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