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The Stress Mechanism of Zinc on the Wheat

Zhongliang Su[a,*], Chengwei Liang[a], Yonghong Sun[b], Xueyan Liu[a], Chaoyu Song[b], Jianmei Chen[b], Fangli Sun[a], Yongchao Bao[a], Jinheng Zhang[a]

[a]College of Chemical engineering, Qingdao University of Science &Technology, Qingdao, China, 266042.
[b]Qingdao Academy of Agriculture Science，Qingdao, China, 266100.
albertszhl@126.com

To investigate the stress mechanism of zinc on the wheat, different concentrations of zinc were used to treat wheat, and the ultrastructure of mesophyll cells of wheat was observed by transmission electron microscope (TEM). The results showed that at the tillering stage, the number of chloroplast decreased, and the thylakoid of chloroplast of mesophyll cell was disorder at the concentrations of L4 (1,000 mg/kg). At the jointing stage, the volume of chloroplast of mesophyll cell expanded at the concentrations of L4 (1,000 mg/kg). At the mature stage, under low concentrations of L1 (250 mg/kg), some chloroplast of mesophyll cell disappeared or broke, the number of mitochondrion increased, the cell wall was damaged; and under concentrations of L4 (1,000 mg/kg), the cell wall of mesophyll cell seriously fractured, the cell structure was not intact, the number of chloroplast and mitochondrion decreased significantly, the cytoplasm became turbid. These results showed that the stress of zinc on wheat was significant with the increase of stress concentration and time. If the concentration of Zn reached to a certain extent, cell structure would be destroyed. This is the first report indicating the stress mechanism of zinc on wheat in the view of ultrastructure in whole growth period of wheat.

1. Introduction

China is one of the most populous countries in the world, the yield and security of food is very important to the health of Chinese. With development of China, the yield of food can meet the demand of Chinese. Now, more and more people focus on problem of food security (Singh et al., 2012). With the development of industry, heavy metal becomes a common contamination in environment (Rehman et al., 2015). Excess heavy metal in soil can bring risk to the food security of human being because some of them are essential elements for crop. However, excess heavy metal in soil can affect not only the growth and development of crop, but also the yield and quality (Hafeez et al., 2013). Excessive zinc in soil could result in crop physiological disorders and even death. It could also be harmful to the health of human being through the food chain (Bi, 2013).

Zinc as cofactor of six kinds of enzyme in cells, could involve in the regulation of enzyme activity, the synthesis of auxin and protein, the stabilization of cell wall, the cell differentiation and division (Vallee and Auld, 1990). It could also promote development of reproductive organ and improve the stress resistance of plant (Erenoglu et al., 2000). However, there would be symptoms and sign of poisoning if the level of zinc in plant exceeded a certain extent (Li et al., 2013). In beans, the activity of electron transport photosynthetic phosphorylation could be inhabited by excessive zinc (Li et al., 2015). Grijalbo et al. (2013) studied the stress of excessive heavy metal on maize, they found that the physiological system in leaf cells of maize was destroyed; the number chloroplast in leaf cells of maize decreased. Kaur et al. (2013) reported that Plumbum could cause the roots cell wall of wheat to thin, increase intercellular space. However, compared with other trace elements, there was less toxicity of zinc to crops. The degree of toxicity of zinc to crops depended on crop species, growth stage and environmental factors. Some scientist had ever reported that zinc had a great impact on the carbon metabolism in plant. If plant lacked zinc, the photosynthesis would decrease by 50 % - 70 % (Chen et al., 2012).

Wheat is the primary crops in north China, and heavy metal is a common pollutant in the world. It is necessary of studying the stress mechanism of heavy metal on wheat. In this paper, the stress mechanism of zinc on wheat in field, the structure of organelles in wheat leaves treated with different level of zinc at different developmental stage was observed using transmission electron microscope (TEM). To the best of our

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knowledge, this is the first report of revealing the stress mechanism of excessive zinc on wheat in the view of the structure change of organelles in whole growth period of wheat.

2. Materials and methods

2.1 Material sources and culturing

The wheat seed was Jimai NO.22, planting density was 187.5 kg/ha. There were four levels of zinc as shown in Table 1. Samples were taken at tillering stage, jointing stage, booting stage and mature stage.

Table1: The zinc concentration gradient applied to the experimental field. CK blank; ZnL1 treated by zinc at 250 mg/kg; ZnL2 treated by zinc at 500 mg/kg; ZnL3 treated by zinc at 750 mg/kg; ZnL4 treated by zinc at 1000 mg/kg

	CK	ZnL1	ZnL2	ZnL3	ZnL4
Concentration (mg/kg)	0.00	250	500	750	1000

2.2 Preparation of wheat samples of transmission electron microscope

The wheat leaves were taken from the experimental plot at different growth stage. The samples were immersed into 2.5 % glutaraldehyde solution, and stored at 4 ºC for 24 h. The samples were washed 6 times using 0.1 mol/L phosphate buffer solutions for 15 min per time, and then the samples were fixed using 0.1 mol/L OsO_4 for 4 h. The samples were washed 3 times with 0.1 mol/L phosphate buffer solution, 15 min for each time. Then the samples were gradually dehydrated using 30 %, 50 %, 70 %, 85 %, 90 %, 95 % and 100 % ethanol, 15 min for each ethanol concentration. The samples were soaked in 100 % propylene oxide and ethanol for 12 h. After soaking, the samples were washed two times using epoxy propane, 30 min for each time. Then samples were immersed sequentially into propylene oxide and embedding medium with the ratio of 2: 1, 1: 1 and 1: 2. The samples were soaked in pure embedding medium for 3 - 4 h. After that, the samples were embedded again in embedding medium, and baked sequentially at 27 ºC, 45 ºC and 60 ºC. Finally, the samples were sliced and stained with uranyl acetate and lead citrate. The subcellular structure was observed using transmission electron microscope (TEM).

3. Results and analysis

3.1 The ultrastructure of wheat mesophyll cell at tillering stage

As shown in Figure 1a, at tillering stage, the shape of chloroplast was fusiform, cytoplasm was transparent and the distribution of grana thylakoids was along the longitudinal axis of chloroplasts in blank sample of Wheat. However, the number of chloroplast in mesophyll cells of L4 sample was lower than in the blank sample, and the distribution of thylakoid in chloroplast was disorder (Figure 1b, 1c and 1d).

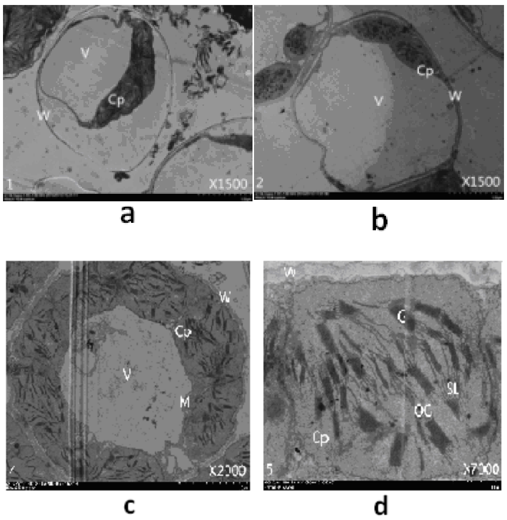

Figure 1: The ultrastructure of wheat mesophyll cell at tillering stage. a) blank; b), c) and d) treated by zinc at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.2 The ultrastructure of wheat mesophyll cell at jointing stage

At the jointing stage, in blank sample, the shape of chloroplasts in mesophyll cells was spherical, close to the cell wall, the arrangement of grana was along the longitudinal axis of chloroplasts (Figure 2a). However, in L4 samples, the volume of chloroplast in mesophyll cell was expansive and was larger than in blank samples (Figure 2b).

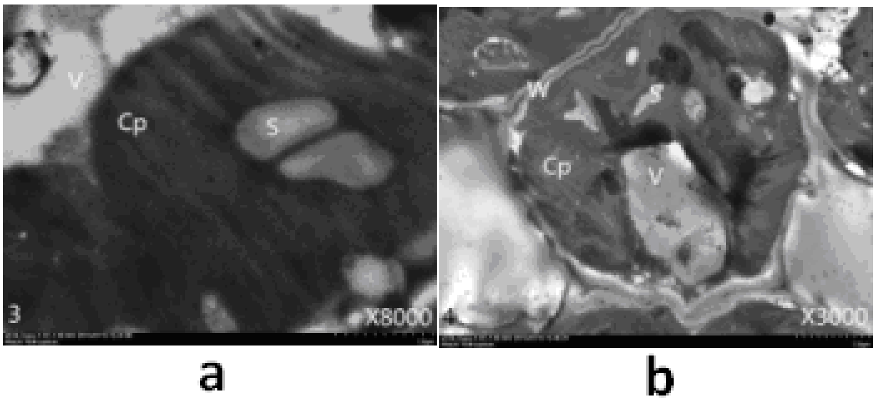

Figure 2: The ultrastructure of wheat mesophyll cell at jointing stage. a) blank; b) treated by zinc at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.3 The ultrastructure of wheat mesophyll cell at low level of zinc at mature stage

At this stage, in blank samples, the chloroplast in mesophyll cells of wheat was in the form of fusiform, close to the cell wall, the distribution of grana was along the long axis of chloroplast, and there appeared a small amount of starch grains and osmiophilic granules (Figure 3a). In the mesophyll cell of L1 samples, the chloroplasts in some cells disappeared, and the cell was severely deformed. Moreover, the number of mitochondria increased, the cell wall was damaged seriously (Figure 3b, 3c and 3d).

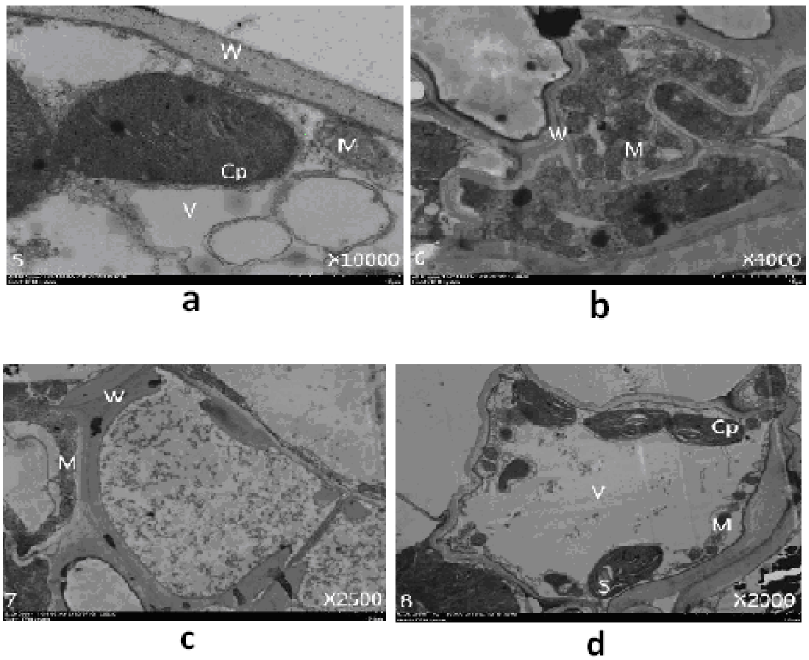

Figure 3: The ultrastructure of wheat mesophyll cell treated by zinc at concentration of zero and L1 at mature stage. a) blank; b), c) and d) treated by zinc at level of L1. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.4 The ultrastructure of wheat mesophyll cell at high level of zinc at mature stage

At mature stage, the wheat was stressed more seriously by high concentration of zinc (L2, L3 and L4) than low concentration of zinc (L1). At level of L2, in the mesophyll cell, the cell wall damaged. The number of chloroplast decreased, and part of chloroplast was expansive and they occupied most of the cell space (Figure 4a, 4b and 4c). In L3 samples, the cell wall damaged seriously; the cytoplasm was feculent; the chloroplast in some mesophyll cell disappeared. The number of mitochondria in mesophyll cell reduced, the volume of some mitochondria dramatically expanded and they occupied most of cell space. There were a lot of broken organelles in some mesophyll cell (Figure 4d, 4e and 4f). In L4 samples, the cell deformed seriously, the cell wall damage severely, the chloroplast disappeared, and the number of mitochondria decreased obviously (Figure 5g, 5h, 5i and 5j).

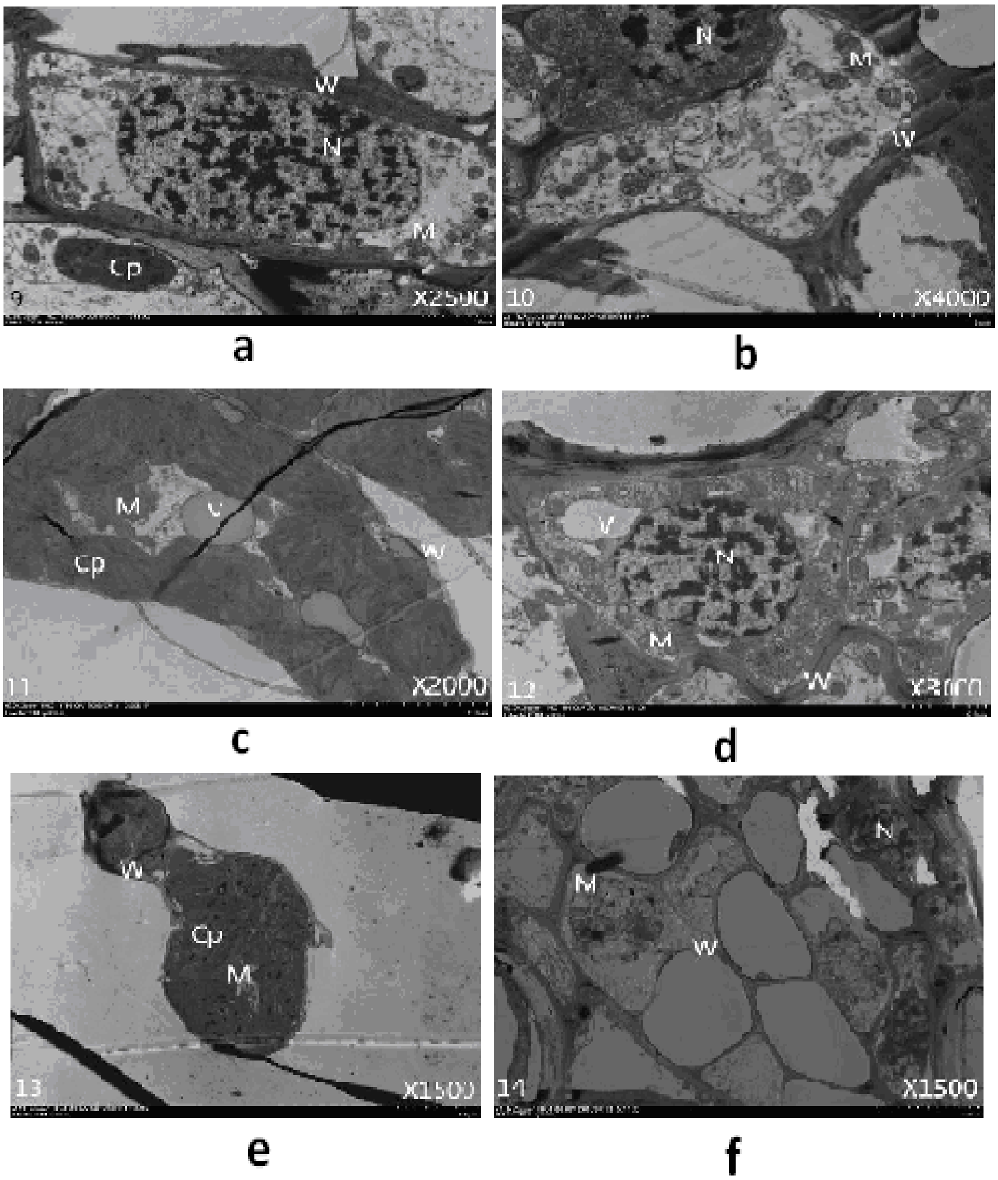

Figure 4: The ultrastructure of wheat mesophyll cell treated by zinc at concentration of L2 and L3 at mature stage. a), b) and c) treated by zinc at level of L2; d), e) and f) treated by zinc at level of L3. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

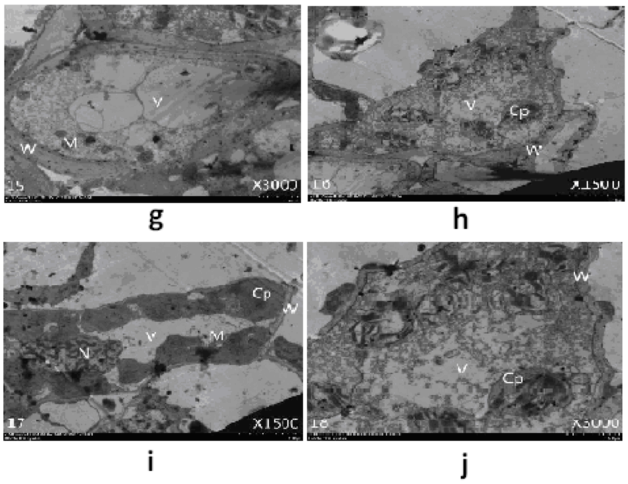

Figure 5: The ultrastructure of wheat mesophyll cell treated by zinc at concentration of L4 at mature stage. g), h), i) and j) treated by zinc at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

4. Discussion

Chloroplasts of wheat leaves were the most sensitive organelle under the stress of heavy metal. The low concentration of heavy metal could cause the chloroplast in cells of wheat leaves to expand, increasing the number of chloroplasts (Blaylock et al., 1997). In addition, it could lead to the arrangement of thylakoid to be out of order. Moreover, the low concentration of heavy metal also reduced the degree of grana stacking. The chloroplast membrane would rupture and the thylakoid would escape from chloroplast under high concentration of heavy metal (Shi et al., 2003). With the extension of heavy metal stress time, the number of mitochondrionis in the cells of wheat leaves would change. And the mitochodrial cristae became indistinct. The structure of mitochodrial membrane would be destroyed (Mitchell and Barre, 1995). Heavy metal could also destroy the structure of cell nucleus and cell wall, which could lead to the abnormal cell (Yu et al., 2010). Excess zinc could generate great stress on wheat. It could not only generate toxicity to chloroplasts and mitochondria in mesophyll cells of wheat, but also cause great damage to the cell wall (Wu et al., 2012).

In our study, we found the same results. The chloroplast was the first damaged organelles. The structure and number of chloroplast in mesophyll cells were changed under different concentration of zinc (Figure 2b). The mitochondria was the second damaged organelle, the number of mitochondria changed seriously (Figure 3b, 3c and 3d), and the volume of some mitochondria dramatically expanded (Figure 4d, 4e and 4f). The results above indicated that the changes in chloroplast and mitochondria were one of important stress mechanisms of zinc to wheat (Fan et al., 2011). With the increase of zinc concentration and the extension of zinc stress time, the mesophyll cell became to deform and the cell wall damaged seriously (Figure 4d, 4e and 4f), which caused some of the mesophyll cell to break (Figure 5g, 5h, 5i and 5j).

The stress of zinc to wheat was not significant during the tillering stage. This was due to that at this growth stage, because of the low temperature, the intensity of wheat photosynthesis was not strong, the growth of wheat was very slow, and the number of chloroplasts in mesophyll cells was small, so that the stress of zinc to wheat was not obvious (Wang and Jin, 2009). With the growth of wheat, the damaged degree caused by zinc to organelle in mesophyll cells became significant because there were so many chloroplasts and mitochondrial in mesophyll cells (Wang and Jin, 2009).

5. Conclusions

There was different stress degree at different growth stage of wheat under the same zinc level. The stress of zinc on wheat was not significant at tillering stage. However, the chloroplast became expansion under the stress of zinc at the jointing stage; the stress of zinc on wheat was more significant at mature stage. Chloroplasts in wheat mesophyll cell were the most sensitive organelle under the stress of zinc. The low level of zinc resulted in the expansion of chloroplast and the high level of zinc resulted in the rupture of chloroplast. Mitochondria in wheat mesophyll cell were the other sensitive organelle. The mitochondria in mesophyll cells became unstable. At low concentration of zinc, the number of it increased. While at high concentration of zinc, the mitochondria raptured. With the increase of level of zinc and the extension of stress time, cell nucleus and cell wall of mesophyll cell were damaged, which resulted in some mesophyll cells disintegrate and led to the death of some wheat.

In this paper, some of stress mechanism of zinc to wheat was investigated. The heavy metal could cause the chloroplasts and mitochondria in wheat mesophyll cells to break to beatk at high concentration, and then led to the death of wheat. The chloroplasts and mitochondria in wheat mesophyll cells were so sensitive to the stress of heavy metal that they could be used to indicate the stress degree of wheat at subcellular level. However, the damage mechanism of the chloroplasts and mitochondria in wheat mesophyll cells caused by heavy metal had not elucidated yet. In addition, the molecules involved this process was still unclear. Therefore, we would focus on the questions above in the further study.

Acknowledgements

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