Dynamic change of wheat eco-physiology and implications for establishing high-efficient stable agro-ecosystems under Hg stress

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**Article info**

**Abstract**

Heavy metal-poisoning exerts the serious influence on crops growth, yield and quality. This research has focused on the impacts of HgCl\(_2\) with different concentrations on the dynamic trends of photosynthesis, transpiration and water use efficiency (WUE) by using different wheat varieties as materials. The results showed that under 100 \(\mu\)M HgCl\(_2\) treatment, wheat leaf photosynthetic rate (Pn) and transpiration rate (Tr) exhibited significant changes, but photosynthetic characteristics presented no obvious regularity, and this kind of impact exerted the critical effects on different Hg\(^{2+}\) concentrations. Similar changes sometimes appeared under low concentration and concentrations. WUE changed more regularly, and WUE of each wheat variety tended to drop after Hg\(^{2+}\) treatments, except the individual concentration treatment. This change indicated that HgCl\(_2\) treatment changed normal transpiration and photosynthesis, which led to the changes in leaf water use efficiency and related wheat eco-physiological parameters. All these results provide valuable information for establishing high-efficient stable agro-ecosystems in abiotic-stress area.

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**1. Introduction**

Throughout their life cycle, the growth and development of agricultural crops can be affected by a myriad of adverse stress, such as drought stress (Pei et al., 2010), salt stress (Wang and Wang, 2012) and heavy metal stress (Feng et al., 2002; Ko et al., 2013; Song et al., 2013). With the rapid development of modern industrial and agricultural production and increasing population, a growing number of heavy metal pollutants enter into ecological systems (Stom et al., 1991; Ye et al., 2013; Yanez-Arancibia et al., 2014). Environmental effects and biological consequences that could occur due to possible changes in water chemistry, would lead to deterioration of water quality and reduction of its biological productivity and destruction of ecosystem functions (Hao et al., 2013; Zhang et al., 2009; Weinstein and Day, 2014). Moreover, heavy metal in water would pollute the agricultural lands in the immediate vicinity of the river or lake (Zimmer et al., 2011; Spasovski et al., 2012; Zheng et al., 2013; Palmea and Davies, 2014). Among all the heavy metal ions polluting the ecosystems, mercury ion (Hg\(^{2+}\)) holds the highest toxicity (Comino et al., 2009; Zhang and Shao, 2013; Zuo et al., 2013; Salem et al., 2014). As one of the essential factors for optimum crop growth, soil can rapidly absorb and fix over 95% of Hg\(^{2+}\) (Jing et al., 2006; Liu et al., 2012). Some researchers showed that after entering into crop fields, mercury ions mainly deposit in the surface layers of soils, further influencing agro-ecosystems (Sparke et al., 2011; Mitsch, 2013); and because of their small mobility, most mercury ions entering soil in the form of HgCl\(_2\) can be absorbed and adhered by organic matters and clay minerals (Zuo et al., 2013; Shang et al., 2000; Duong and Han, 2011), thus bringing about lasting toxicity in crops. The toxicity exerted by mercury ions on plants leads to the inhibition of cell division and plant growth, and the stimulation and suppression of some enzymatic activities (Yang et al., 2002; Anna et al., 2013; Gupta et al., 2012; Muddarisna et al., 2013; Wu et al., 2012). In addition, mercury ions can also affect chlorophyll content, activities of root systems, and free proline content in crops (Liu et al., 2004; Deng et al., 2013; Debeljak et al., 2013; Górska-Czekaj and Borucki, 2013; Verbruggen et al., 2009). Plants have evolved...
diverse-defensing mechanisms to cope with heavy metals, such as extrusion, chelation, vacuolar sequestration and regulation of distribution (Verbruggen et al., 2009; Singh et al., 2010; Demim et al., 2013; Huang et al., 2000). Other researches also indicated that mercury ions can bring about severe effects on water transportation in individual plants and plant organs through influencing water channel proteins from a molecular perspective (Daniels et al., 1996; Ager et al., 1998; Zhang et al., 2012); and under the existence of sub-mM levels’ HgCl2 concentrations, water conductance in plant root systems can be 60–90% lower than that of controlled group (Martre et al., 2001; Quiñones et al., 2013). The above-mentioned researches revealed to a certain extent that mercury ions affect the physiological characteristics of various types of crops, such as wheat (Li et al., 2013); Medicago truncatula (García de la Torre et al., 2013), and maize (Gupta et al., 2012; Muddarisa et al., 2013; Liu et al., 2004). However, only a few researches concern on the effects of mercury ions on crop WUE and its relevant physiological characteristics were analyzed so as to lay a solid foundation for relevant further study concerning the physiological, toxicological and molecular mechanisms of Hg effects for establishing a high-stable agro-ecosystem.

2. Materials and methods

2.1. Experimental materials

Four typically representative winter wheat varieties, namely Shi4185 (S4), Jinmai47 (JM), Shijiazhuang8 (S8) and Xifeng20 (XF) were selected as experimental materials, and measurements of relevant physiological indexes were conducted. Varieties S4 and S8 represent water land varieties, which possess such quality characteristics as half-winter, middle-ripped, seedlings with half-creeping stems and strong tillering abilities. Variety S4 generates relatively high yields under the conditions of high water and fertilizer supply with low a planting density, while variety S8 possesses excellent resistance, comprehensive disease-resistance, and widespread adaptations, constituting a combined-typed variety. Varieties XF and JM belong to drought-resistance varieties, holding such characteristics as creeping seedlings, compact plant types and strong tillering, drought-resistance, cold-resistance and lodging-resistance, and adapting to sowing in dry lands.

2.2. Methods for wheat cultivation

Wheat seeds were selected, were soaked in 0.1% HgCl2, were sterilized under 25 °C, absorbed water for 16 h, and then were placed in incubators for 2-day germination. The germinated seeds were placed into vermiculite-filled white plastic boxes for soil culture. After growing into the two-leaf stage, seedlings with consistent growing conditions, seedling weight and stem and leaf length were selected and placed into triangular flasks. The bases of the seedlings were sponge-bound or cotton-bound, a small hole was left for seedling leaves to pass through, and the outside of each triangular flask was bound by black plastic bags for light avoidance. The seedlings were cultured through applying 1/2 Hoagland nutrient solution, which was replaced every 3d. Then, the seedlings were cultured in artificial climatic chambers (26 °C day/22 °C night, with an illuminating time of 14-h/d) with an illumination intensity of 400 mmol m⁻² s⁻¹. When the seedlings grew into the three-leaf stage, experiments for Hg treatment were conducted.

2.3. Experiments for Hg treatment

Six treatments were set in this experiment: CK (Hoagland nutrient solution), 25-HgCl2 (Hoagland nutrient solution +25 μM HgCl2), 50-HgCl2 (Hoagland nutrient solution +50 μM HgCl2), 100-HgCl2 (Hoagland nutrient solution +100 μM HgCl2), 300-HgCl2 (Hoagland nutrient solution +300 μM HgCl2), and 500-HgCl2 (Hoagland nutrient solution +500 μM HgCl2). Five seedlings were remained in every triangular flask and every treatment had 6 replications. The experiments were conducted in 9:00 in the morning, each triangular flask was added with prepared solution, the seedlings were placed into the triangular flasks and the triangular flasks were cultured in artificial climatic chambers.

2.4. Methods for the calculation of Pn, Tr and WUE in seedling leaves

Wheat seedlings were treated under different HgCl2 concentrations at different times (0, 15, 30, 45 and 60 min), and the measurements of such indexes as photosynthetic rate (Pn, μmol/m² s) and transpiration rate (Tr, mmol/m² s) were implemented in flag leaves through utilization of Li-6400 Portable Photosynthetic System manufactured by LI-COR 6400, Large Vista Group Ltd, USA. For each measurement, 3 seedlings were randomly chosen and 3 replications were conducted under the following conditions: gas flow amount: 500 μmol/s, CO2 concentration: 385 ± 5 mmol/dm⁻³, and room temperature: 26 ± 2 °C. An internal light source (with an illumination intensity of 500 μmol/m² s⁻¹) was set for the measurements, and the instant leaf WUE (WUE, μmol/mmol) was calculated according to the following formula:

\[ WUE = \frac{Pn}{Tr} \]  

(1)

3. Results

3.1. Changes in Pn for different varieties with concentrations of HgCl2 treatments

Pn (photosynthetic rate) values of varieties S4, S8, JM and XF were measured under different concentrations of HgCl2 treatment at different times, and the results for the measurements were shown in Fig. 1. According to Fig. 1, under different concentrations for treatment at different times, Pn values were different, but these values generally fluctuated around Pn value of CK (Pn₀).

As for variety S4, within 15 min after HgCl2 treatments, compared with such values of CK (Pn₀), Pn₂₅ (Pn value under 25 μM HgCl₂ treatment), Pn₅₀ (Pn value under 50 μM HgCl₂ treatment) and Pn₁₀₀ (Pn value under 100 μM HgCl₂ treatment) of this variety declined significantly, while Pn₁₀₀ (Pn value under 100 μM HgCl₂ treatment) and Pn₃₀₀ (Pn value under 300 μM HgCl₂ treatment) of this variety enhanced significantly. From 15 to 30 min after HgCl₂ treatments, Pn₅₀ and Pn₅₀₀ started to increase, while Pn₁₀₀ and Pn₃₀₀ started to decrease until the end of 60 min measurement, and 30 min after HgCl₂ treatments, Pn₅₀₀ basically remained increase, while Pn₂₅ and Pn₅₀ increased until 45 min
Fig. 1. \( P_n \) of different varieties at different times under different \( \text{HgCl}_2 \) concentrations. Note: In this figure, S4 stands for variety Shijiazhuang8, JM represents variety Jinmai47, S8 refers to variety Shijiazhuang8, and XF stands for variety Xifeng20. The solid diamond represents CK, the solid square represents \( P_n \) under 25 \( \mu \text{M} \) \( \text{HgCl}_2 \) treatment, the solid triangle represents \( P_n \) under 50 \( \mu \text{M} \) \( \text{HgCl}_2 \) treatment, the multiplication represents \( P_n \) under 100-\( \text{HgCl}_2 \) treatment, the asterisk represents \( P_n \) under 300-\( \text{HgCl}_2 \) treatment, and the cross represents \( P_n \) under 500-\( \text{HgCl}_2 \) treatment.

after treatments and then began to decrease to a value lower than \( P_n_{\text{ck}} \).

As for variety JM, after \( \text{HgCl}_2 \) treatments, changes in \( P_n_{50} \) and \( P_n_{100} \) remained basically the same trend, whereas the value of \( P_n_{50} \) was lower than that of \( P_n_{\text{ck}} \). Within 15 min after \( \text{HgCl}_2 \) treatments, \( P_n_{100} \) value displayed a significant enhancement and then began to gradually decline. Within 45 min after \( \text{HgCl}_2 \) treatments, changes in \( P_n_{25} \) and \( P_n_{100} \) remained relatively consistent, whereas 15 min after treatments, \( P_n_{25} \) and \( P_n_{50} \) declined significantly, among which, \( P_n_{100} \) declined gradually, whereas \( P_n_{50} \) enhanced once again, while \( P_n_{25} \) witnessed a large-extent increase and then an apparent decrease. In a word, after 60 min under low concentrations of \( \text{HgCl}_2 \) treatments, \( P_n \) values of variety JM exhibited a general trend of improvement, indicating that adaptations to slight \( \text{HgCl}_2 \) inhibition may have been created in seedlings; however, after 60 min under high concentrations of \( \text{HgCl}_2 \) treatments, \( P_n \) values dropped apparently.

As for variety S8, changes of \( P_n \) values under different concentrations of \( \text{HgCl}_2 \) treatments showed different trends. In a word, within 15 min after \( \text{HgCl}_2 \) treatments, \( P_n_{25} \) and \( P_n_{100} \) exhibited significantly increased, whereas \( P_n_{300} \) and \( P_n_{500} \) increased insignificantly. From 15 to 30 min after \( \text{HgCl}_2 \) treatments, \( P_n_{300} \) and \( P_n_{500} \) radially declined again, and \( P_n_{25} \) declined remarkably. After that, \( P_n_{300} \) and \( P_n_{500} \) continued to drop, whereas \( P_n_{25} \) increased and then witnessed a change trend of increase and decrease. \( P_n_{50} \) declined gradually, experienced the minimum value at 30 min after treatment, and then showed a trend of linear enhancement. \( P_n_{100} \) showed an unapparent magnitude of change, whereas values were around those of \( P_n_{\text{ck}} \). However, 45 min after \( \text{HgCl}_2 \) treatments, \( P_n_{100} \) exhibited a significant trend of decline. According to the experimental results shown in Fig. 1, low concentrations of \( \text{HgCl}_2 \) treatments exerted promotive effects on \( P_n \) of variety S8.

As for variety XF, a drought-resistant wheat variety, it can be seen from Fig. 1 that under low concentrations of \( \text{HgCl}_2 \) treatment, and dynamic changes in \( P_n \) values of this variety exhibited two different kinds of conditions. After treatments, \( P_n_{50} \) improved gradually, and \( P_n_{25} \) declined significantly at 15 min after \( \text{HgCl}_2 \) treatments, and then showed repeated changes of increase and decrease till approached such values of \( P_n_{\text{ck}} \) at 60 min after \( \text{HgCl}_2 \) treatments. Within 15 min after \( \text{HgCl}_2 \) treatments, \( P_n_{100} \) and \( P_n_{300} \) consistently drop and then showed different trends of change; \( P_n_{300} \) enhanced significantly, whereas \( P_n_{500} \) continued to drop but enhanced within 30–45 min after \( \text{HgCl}_2 \) treatments, and 45 min after \( \text{HgCl}_2 \) treatments both \( P_n_{300} \) and \( P_n_{500} \) dropped apparently and their values were both lower than those of \( P_n_{\text{ck}} \).

3.2. Changes in \( T_r \) for different varieties with concentrations of \( \text{HgCl}_2 \) treatments

Changes in \( T_r \) of different varieties with different concentrations of \( \text{HgCl}_2 \) treatments were shown in Fig. 2. According to Fig. 2, \( T_r \) changes exhibited irregular trends among different varieties. As for variety S4, within 15 min \( \text{HgCl}_2 \) treatments, \( T_r \) values increased in all treatments, whereas 15 min after \( \text{HgCl}_2 \) treatments, except that \( T_r_{500} \) (\( T_r \) value under 500 \( \mu \text{M} \) \( \text{HgCl}_2 \) treatment) continued to increase, \( T_r \) values in other treatment groups began to drop. Under low concentrations of \( \text{HgCl}_2 \) treatments, namely under \( T_r_{25} \) (\( T_r \) value under 25 \( \mu \text{M} \) \( \text{HgCl}_2 \) treatment) and \( T_r_{50} \) (\( T_r \) value under 50 \( \mu \text{M} \) \( \text{HgCl}_2 \) treatment), 30 min after \( \text{HgCl}_2 \) treatments, \( T_r \) values increased significantly, whereas 45 min after \( \text{HgCl}_2 \) treatments, \( T_r \) values declined significantly, whereas 45 min after \( \text{HgCl}_2 \) treatments, \( T_r \) displayed no significant change, whereas 60 min after \( \text{HgCl}_2 \) treatments both \( T_r_{300} \) and \( T_r_{500} \) dropped apparently and their values were both lower than those of \( T_r_{\text{ck}} \).

As for variety S4, within 15 min \( \text{HgCl}_2 \) treatments, \( T_r_{25} \) showed relatively large magnitude of change with no significant regularity.
**Fig. 2.** Tr of different varieties at different times by treatments with different concentrations of HgCl₂. Note: In this figure, S4 stands for variety Shi4185, JM represents variety Jinmai47, S8 refers to variety Shijiazhuang8, and XF stands for variety Xifeng20. The solid diamond represents CK, the solid square represents Pn under 25 μM HgCl₂ treatment, the solid triangle represents Pn under 50 μM HgCl₂ treatment, the multiplication represents Pn under 100 μM HgCl₂ treatment, the asterisk represents Pn under 300 μM HgCl₂ treatment, and the cross represents Pn under 500 μM HgCl₂ treatment.

**Tr50** exhibited a trend of change similar to that of Tr0, while the values of Tr50 were lower than those of Tr0. Tr100 and Tr500 showed relatively consistent trends of change, while Tr100 values exhibited an overall trend of decline. Tr500 improved significantly after treatments, and 15 min after treatment, Tr500 declined significantly, and amounted to its minimum value at 60 min after treatment. In a word, Tr values exhibited a trend of decline under high concentrations of HgCl₂ treatments (Tr100, Tr300 and Tr500).

As for variety S8, Tr values exhibited different trends of change under different concentrations of HgCl₂ treatments. Tr25 changes of this variety showed a similar trend with that of variety JM, although with a relatively large magnitude of change; and Tr25 values were generally greater than Trck values. Tr50 increased slowly after HgCl₂ treatments, and it was apparently greater than Trck after 60 min HgCl₂ treatments. Tr100 improved extremely apparent after 15 min HgCl₂ treatments, declined extremely apparent after 30 min HgCl₂ treatments, and declined to a value around that of Trck after 60 min HgCl₂ treatments. Tr300 and Tr500 showed relatively consistent trends of change, after 15 min HgCl₂ treatments, both values increased to their peaks, then declined gradually, and after 45 min HgCl₂ treatments, both values were lower than that of Trck. From the experimental results, it can be clearly seen that higher concentrations of HgCl₂ treatments exerted relatively obvious effects. It can be clearly observed from Fig. 1 that Tr changes in variety S8 shared certain similarity with those in variety S4.

As for variety XF, Tr values exhibited different trends of change under different concentrations of HgCl₂ treatments. Tr25, Tr50 and Tr100 values generally enhanced and were far greater than Trck value, whereas after HgCl₂ treatments, Tr300 and Tr500 increased unapparent and both values declined and were lower than Trck value. Therefore, low and moderate concentrations of HgCl₂ stresses exerted no relatively considerable effects on transpiration.

### 3.3. Analysis on changes in leaf WUE for different wheat varieties

According to Fig. 3, as for variety S4, after HgCl₂’s inhibition of aquaporins, leaf WUE of this variety exhibited a general trend of decline.

As for variety JM, leaf WUE also showed a general trend of drop, whereas at 50 μM and 300 μM HgCl₂ treatments, leaf WUE showed no trend of decline and there was no significant difference between both values and that of CK. However, at 25 μM and 500 μM HgCl₂ treatments, leaf WUE exhibited relatively significant trends of decline.

As for variety S8, within 30 min after HgCl₂ treatments, leaf WUE showed general trends of decline from 25 μM to 100 μM HgCl₂ treatments, whereas at high concentrations of HgCl₂ treatments, namely 300 μM and 500 μM, leaf WUE showed trends of enhancement during the process of measurements.

### 4. Discussions

In plants, metals exert their toxic action mostly by damaging chloroplast and disturbing photosynthesis (Anjali et al., 2012). In higher plants, photosynthesis is reduced indirectly by heavy metal accumulation in leaves which influences the stomata performance (Yu et al., 2014a,b). In addition, Hg²⁺ ions affected both light and dark reactions of photosynthesis and strongly inhibited the photosynthetic electron transport chain, photosystem (PS) II being the most sensitive target (Asztalos et al., 2012). In this research, the absorption and accumulation of heavy metal ion Hg²⁺ led to the drop in Pn of wheat seedlings to some extent.
extent at different times. Meanwhile, the accumulation of Hg\textsuperscript{2+} also brought about relatively large magnitudes of Tr fluctuation, because the root systems of plants exerted certain inhibitive effects on the uptake of heavy metal ions (Niu et al., 2013), which partly resulted in the enhancement of Pn and Tr values in wheat seedlings (Figs. 2 and 3). At cellular level, the continuous inhibitive effects of Hg\textsuperscript{2+} damage the structure and function of cell membranes. Because Hg\textsuperscript{2+} can exert inhibitive effects on plant aquaporin, some membrane proteins relating to water uptake in root systems, such as AQPs, can be inhibited. The rapid accumulation of Hg\textsuperscript{2+} in leaves and root systems of wheat seedlings disrupted normal processes of water transpiration and photosynthesis, thus leading to Pn and Tr reduction. Meanwhile, the sustaining effects of Hg\textsuperscript{2+} probably induced or stimulated some proteins or their family members (such as AQPs) to improve water permeability, thus promoting the progression of photosynthesis and transpiration. Studies have suggested that plants subjected to moderate drought or metal stress might enhance the WUE of leaves [37]. In this research, the results showed that different wheat varieties responded differently to Hg\textsuperscript{2+} inhibition, and leaf WUE of all varieties exhibited general trends of decline after HgCl\textsubscript{2} treatments. However, WUE of varieties with relatively strong drought-resistances partly enhanced under certain Hg\textsuperscript{2+} concentrations. All these results provide good implications for establishing a stable agro-ecosystem under increasing global climate change.

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