

## The alleviative effect of salicylic acid on the physiological indices of the seedling leaves in six different wheat genotypes under lead stress

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### Abstract

In this research, relevant physiological indices were measured in seedling leaves of six different wheat genotypes growing at three-leave stage. The seedlings were cultured under hydroponic cultivation and treated by different lead ion ( $Pb^{2+}$ ) treatments (namely 10, 50, 100, 200 mg L<sup>-1</sup>  $Pb^{2+}$  stresses, respectively) and  $Pb^{2+}$ -SA (salicylic acid) jointed-treatments (namely 200 mg L<sup>-1</sup> SA-alleviated 10, 50, 100, 200 mg L<sup>-1</sup>  $Pb^{2+}$  stresses, respectively). The results showed that stress induced by the application of  $Pb^{2+}$  triggered significant inhibitory effects on indices such as chlorophyll content, ascorbate peroxidase (APX) activity, catalase (CAT) activity, malonic dialdehyde (MDA) content and proline content. Moreover, application of SA exerted certain alleviative effects on these indices in seedling leaves of all genotypes. Peroxidase (POD) activity, superoxide dismutase (SOD) activity and soluble sugar content were significantly affected by inhibitory effects of  $Pb^{2+}$  stress, while SA exerted limited alleviative effects on these parameters. For all physiological indices, SA had the most apparent alleviative effects on seedling leaves of all genotypes treated by the maximum  $Pb^{2+}$  concentration, namely, 200 mg L<sup>-1</sup>  $Pb^{2+}$  stress. No significant differences were observed in response of all genotypes to stress, in terms of the change in physiological indices values, indicating that hydroponic cultivation at same nutrition conditions minimized the differences in stress tolerance or resistance of all genotypes. These data provide a basic study on physiological mechanism of wheat resistance (tolerance) to heavy metal stress.

**Keywords:** Seedling Leaf, Wheat Genotype, Lead Stress, SA, Physiological Index, Breeding.

**Abbreviations:** APX\_ascorbate peroxidase; CAT\_catalase; MDA\_malic dialdehyde; PCD\_programmed cell death; POD\_peroxidase; ROS\_reactive oxygen species; SA\_salicylic acid; SOD\_superoxide dismutase.

### Introduction

With the rapid development in worldwide agricultural production a great attention has been paid to the heavy metal pollution. In many arid and semi-arid areas, the toxic effects of heavy metals have caused a considerable reduction in wheat yield and human health. Among the heavy metals causing environmental pollution and toxicities on soil fertility, Lead (Pb) is one of the main ones which severely affect wheat production. Lead causes a considerable amount of inhibitory and toxic effects on seedling growth and development of plants, which constitute serious threats to the improvement of wheat production.  $Pb^{2+}$  causes accumulation of reactive oxygen species (ROS), leading to the change of the permeability of cellular membranes, which in turn, results in various degree of damages in cellular organelles such as nuclei, chloroplasts and mitochondria in plant cells (Clemens, 2001; Fargasova, 1994; Mishra and Choudhuri, 1999). As a major food crop in the world, wheat sustains the survival of more than half of the world population. However, numerous researches have shown

that wheat seedlings are vulnerable and sensitive to  $Pb^{2+}$  stress (Clemens, 2001; Fargasova, 1994; Mishra and Choudhuri, 1999; Wang et al., 2010). The accumulation of  $Pb^{2+}$  in wheat seedlings not only seriously affects seedling growth and wheat yield, but also exerts toxic effects on human health by food chain. Therefore, researches concerning the toxic effects of metals on wheat seedling growth and development are of vital importance for the clarification of the physiological and molecular mechanisms for  $Pb^{2+}$ -caused stress on wheat production. As a novel endogenous plant hormone, salicylic acid (SA) is considered to have beneficial physiological effects on plants. As a sort of organic acid, SA is believed to exert alleviative effects on plants growing under  $Pb^{2+}$ -stressed environment. SA can promote fluorescence process, improve plants disease-resistance, reduce transpiration and inhibit ethylene synthesis. Under ozone and osmotic stress, SA can induce the generation of some stress proteins. The results of numerous researches showed that SA can alleviate the stress

effects of many heavy metal elements for a variety of crops (Bush, 1996; Chen et al., 1993; Mur et al., 1996). In recent years, SA has been found to exert some positive effects on the improvement of plant's resistances to some abiotic stresses, such as salt, drought, cold and heat stresses (Gaffney et al., 1993; Guo et al., 2007). Since the discovery of SA's protective roles in safeguarding pathogen-infected plants, a myriad of researches have been conducted in SA-induced both locally and systematically acquired resistances of plants (Bush, 1996; Chen et al., 1993; Gaffney et al., 1993; Guo et al., 2007; Mur et al., 1996; Neuenschwander et al., 1995). Numerous researches showed that SA serves as a critical signal molecule in inducing systematically-acquired resistances of plants. A growing number of experimental researches also indicated that SA can induce plants resistances to biotic stresses caused by fungi, bacteria and viruses, and certain plant's resistances (or tolerances) to abiotic stresses resulted from heavy metals, ozone, ultraviolet radiation, low temperature, heat shock, water deficit, salt and so forth (Gaffney et al., 1993; Guo et al., 2007; Mur et al., 1996; Neuenschwander et al., 1995). Some researches have been carried out in SA-induced tolerance to abiotic stresses and the results showed that SA exerts positive roles in maintaining and enhancing such resistances (or tolerances). These results also indicated that SA may involve in the induction of plants' protective effects on cross resistances in response to various abiotic stresses. In addition, other kinds of stresses (such as thermal stress and salt stress) can also exert inhibitive and even damaging effects on plant growth and development (Yan et al., 2011; Zhang et al., 2011), which can all be alleviated to certain extents by SA. Numerous researches have confirmed that under various stresses, SA may promote plants resistance to various stresses through many possible mechanisms, including ROS (Reactive Oxygen Species) participation into SA-mediated signal transduction pathways, SA's involvement into PCD (Programmed Cell Death), SA's accession into  $\text{Ca}^{2+}$ -mediated signal transduction pathways and protein phosphorylation and dephosphorylation during SA-induced anti-stress processes. In this research, we aimed at studying the physiological mechanism for the growth of wheat seedling leaves under different concentrations of  $\text{Pb}^{2+}$  treatments and SA's alleviative effects on  $\text{Pb}^{2+}$  stress. This study provides some theoretical and experimental overview for the study of wheat growth in response to unfavorable heavy metal polluted environments, and for the breeding practices of wheat for the selection of wheat cultivars with high tolerance or resistance to heavy metal ions.

## Results

### ***The effects of $\text{Pb}^{2+}$ treatments and $\text{Pb}^{2+}$ -SA jointed treatments on chlorophyll content***

According to Table 1, all the wheat genotypes suffered significant changes in their chlorophyll contents under different treatments. From  $T_1$  to  $T_4$  treatment, all genotypes exhibited gradually decreasing of chlorophyll content with the increase of  $\text{Pb}^{2+}$  concentration, indicating that greater concentrations of  $\text{Pb}^{2+}$  exerted relatively greater adverse effects on chlorophyll generation in seedling cells. All genotypes presented the minimum chlorophyll content at  $T_4$  treatment, showing that high concentrations of  $\text{Pb}^{2+}$  significantly inhibited chlorophyll synthesis in seedling cells. Among all genotypes, genotype 4 (Shijiazhuang8) showed the lowest chlorophyll content at  $T_4$  treatment, which was 34.92% of chlorophyll content of the same genotype at  $T_0$  treatment (CK). There were extremely

significant difference between values ( $P<0.01$ ), demonstrating that chlorophyll production of this wheat genotype (Shijiazhuang8) was most sensitive to high concentration of  $\text{Pb}^{2+}$  stress. From  $T_5$  to  $T_8$  treatment, all genotypes exhibited certain recovery effects exerted by SA, albeit under  $T_5$  treatment, all genotypes except genotype 2 (Jinmai47) possessed slightly smaller values of chlorophyll content than such values under  $T_1$  treatment. Chlorophyll contents of all genotypes at  $T_6$ ,  $T_7$ , and  $T_8$  treatments were higher than values at  $T_2$ ,  $T_3$  and  $T_4$  treatments, respectively, showing that SA with a concentration of 200 mg L<sup>-1</sup> exerted certain alleviative effects on different higher concentrations of  $\text{Pb}^{2+}$  stress. From the comparison of chlorophyll content values of all genotypes at  $T_4$  treatment and such values at  $T_8$  treatment, it can be clearly seen that extremely significant differences ( $P<0.01$ ) occurred between such values at both treatments, showing that SA effectively alleviated seedling growth of all genotypes at high concentration of  $\text{Pb}^{2+}$  stress, albeit chlorophyll content values of all genotypes were lower at  $T_8$  treatment than such values of CK. No significant differences were observed among chlorophyll content values in seedling leaves of different wheat genotypes, except that at  $T_2$  treatment, genotypes 5 and 6 possessed lower chlorophyll content values and at  $T_5$  treatment, genotype 6 held a lower chlorophyll content value, which all amounted to extremely significant differences ( $P<0.01$ ), indicating that all genotypes exhibited generally the same trends of chlorophyll content changes in their seedling leaves in response to both  $\text{Pb}^{2+}$  stress and SA alleviative effects.

### ***The effects of $\text{Pb}^{2+}$ treatments and $\text{Pb}^{2+}$ -SA jointed treatments on anti-oxidative enzymatic activities***

According to Fig.1, all genotypes exhibited the maximum APX activity at  $T_2$  treatment, and presented their minimum APX activity at  $T_4$  treatment, indicating that low concentrations of  $\text{Pb}^{2+}$  stress promoted APX activity to certain extents in different genotypes, whilst high concentrations of  $\text{Pb}^{2+}$  stress exerted certain inhibitory effects on APX activity. Among all genotypes, genotype 3 (Xifeng20) showed the maximum APX activity at  $T_2$ , which amounted to 6.53 U g<sup>-1</sup> FW, demonstrating that this genotype is more adaptive to hydroponic cultivation than any other genotypes selected for this research in terms of changes in APX activity. At  $T_1$  and  $T_6$  treatments, certain differences ( $P<0.05$ ) were observed among APX activities in seedling leaves of different genotypes, indicating that different genotypes held different responses to  $\text{Pb}^{2+}$  stress at concentration of 10 mg L<sup>-1</sup>. In contrast, the SA induced different alleviative effects on different genotypes treated at 50 mg L<sup>-1</sup>  $\text{Pb}^{2+}$  stress. Among all genotypes, genotype 4 (Shijiazhuang8) showed the minimum APX activity at  $T_4$  treatment, which reached to 3.37 U g<sup>-1</sup> FW, showing that this genotype was sensitive to high concentration of  $\text{Pb}^{2+}$  stress. Compared with other genotypes, genotype 6 (Zhousai18) held a more changeable trend in APX activity, and significant differences ( $P<0.05$ ) were observed among the values of APX activity of this genotype at different  $\text{Pb}^{2+}$  concentrations and SA-alleviated  $\text{Pb}^{2+}$  stresses, demonstrating that this genotype is more susceptible to  $\text{Pb}^{2+}$  stress and hydroponic cultivation than any other studied genotypes, in term of change in APX activity. Compare with  $T_2$  and  $T_6$  treatments, it can be clearly seen that all genotypes held lower values of APX activity at  $T_6$  treatment than at  $T_2$  treatment, indicating that SA induced limited or little alleviative effects at 50 mg L<sup>-1</sup>  $\text{Pb}^{2+}$  stress. Compare with  $T_3$  and  $T_7$  treatments, genotypes 1, 2, 3, and 6 possessed lower values of APX activity at  $T_7$  treatment than at  $T_3$  treatment,

**Table 1.** Chlorophyll contents of seedling leaves of different wheat genotypes under different Pb<sup>2+</sup> treatments and Pb<sup>2+</sup>-SA jointed treatments (mg g<sup>-1</sup>)

Treatments	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
T <sub>0</sub> (CK)	2.47±0.03 <sup>aA</sup>	2.38±0.02 <sup>bA</sup>	2.39±0.04 <sup>bA</sup>	2.52±0.12 <sup>aA</sup>	2.49±0.08 <sup>aA</sup>	2.43±0.06 <sup>aA</sup>
T <sub>1</sub>	2.31±0.02 <sup>bA</sup>	2.25±0.03 <sup>bA</sup>	2.28±0.11 <sup>bA</sup>	2.33±0.03 <sup>bA</sup>	2.29±0.06 <sup>bA</sup>	2.35±0.07 <sup>bA</sup>
T <sub>2</sub>	2.02±0.09 <sup>cB</sup>	1.98±0.01 <sup>cB</sup>	1.92±0.05 <sup>cB</sup>	2.12±0.11 <sup>cB</sup>	1.89±0.08 <sup>dC</sup>	1.86±0.04 <sup>dC</sup>
T <sub>3</sub>	1.72±0.08 <sup>eC</sup>	1.69±0.12 <sup>ID</sup>	1.67±0.06 <sup>eC</sup>	1.78±0.02 <sup>ID</sup>	1.76±0.04 <sup>eC</sup>	1.65±0.08 <sup>eC</sup>
T <sub>4</sub>	1.02±0.03 <sup>ID</sup>	0.98±0.01 <sup>ID</sup>	1.11±0.05 <sup>ID</sup>	0.88±0.02 <sup>ID</sup>	1.12±0.09 <sup>ID</sup>	0.92±0.06 <sup>ID</sup>
T <sub>5</sub>	2.22±0.03 <sup>bA</sup>	2.26±0.08 <sup>bA</sup>	2.19±0.12 <sup>cA</sup>	2.18±0.03 <sup>cA</sup>	2.16±0.06 <sup>cA</sup>	1.93±0.03 <sup>cB</sup>
T <sub>6</sub>	2.18±0.02 <sup>cA</sup>	1.98±0.09 <sup>cB</sup>	2.03±0.03 <sup>cB</sup>	2.11±0.11 <sup>cB</sup>	2.19±0.03 <sup>cA</sup>	2.25±0.04 <sup>bA</sup>
T <sub>7</sub>	1.96±0.11 <sup>cDB</sup>	2.01±0.02 <sup>cB</sup>	1.98±0.12 <sup>cB</sup>	2.03±0.03 <sup>cB</sup>	2.05±0.07 <sup>B</sup>	1.99±0.02 <sup>cB</sup>
T <sub>8</sub>	1.85±0.07 <sup>dC</sup>	1.77±0.03 <sup>eC</sup>	1.86±0.01 <sup>dC</sup>	1.89±0.01 <sup>dC</sup>	1.72±0.03 <sup>cC</sup>	1.71±0.05 <sup>cC</sup>

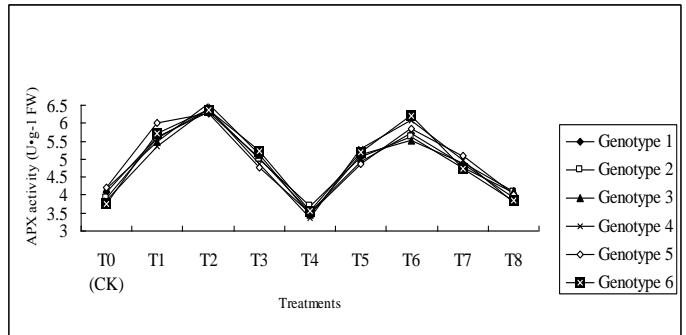
Note: The different small and capital letters indicate significant difference among different treatments at P < 0.05 and P < 0.01 levels, respectively.

**Table 2.** MDA contents of seedling leaves of different wheat genotypes under different Pb<sup>2+</sup> treatments and Pb<sup>2+</sup>-SA jointed treatments (μmol g<sup>-1</sup>)

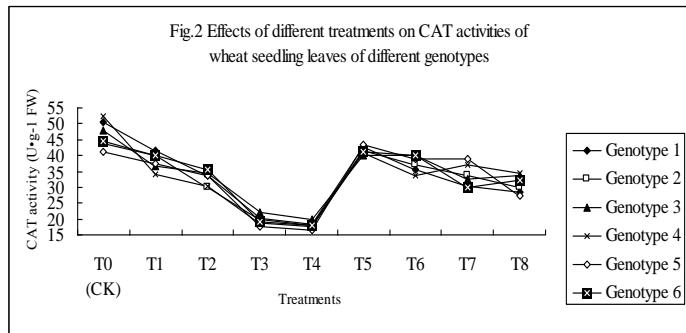
Treatments	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
T <sub>0</sub> (CK)	2.82±0.08 <sup>aA</sup>	3.11±0.12 <sup>aA</sup>	2.97±0.02 <sup>aA</sup>	3.28±0.08 <sup>bA</sup>	2.89±0.12 <sup>aA</sup>	2.95±0.02 <sup>aA</sup>
T <sub>1</sub>	2.98±0.11 <sup>aA</sup>	3.08±0.02 <sup>aA</sup>	3.22±0.03 <sup>bA</sup>	3.43±0.04 <sup>bAB</sup>	2.96±0.01 <sup>aA</sup>	2.88±0.05 <sup>aA</sup>
T <sub>2</sub>	5.89±0.04 <sup>cB</sup>	6.22±0.09 <sup>cB</sup>	6.33±0.02 <sup>cDB</sup>	6.08±0.02 <sup>cB</sup>	6.17±0.08 <sup>cB</sup>	5.92±0.12 <sup>cB</sup>
T <sub>3</sub>	8.92±0.03 <sup>dC</sup>	9.08±0.05 <sup>dC</sup>	8.86±0.01 <sup>dC</sup>	9.27±0.03 <sup>dC</sup>	10.06±0.01 <sup>dC</sup>	9.98±0.11 <sup>dC</sup>
T <sub>4</sub>	11.72±0.12 <sup>eD</sup>	12.83±0.06 <sup>eD</sup>	11.98±0.05 <sup>eD</sup>	13.68±0.03 <sup>eD</sup>	12.92±0.02 <sup>eD</sup>	12.28±0.03 <sup>eD</sup>
T <sub>5</sub>	2.89±0.02 <sup>aA</sup>	2.99±0.03 <sup>aA</sup>	3.01±0.06 <sup>aA</sup>	3.14±0.03 <sup>abA</sup>	3.22±0.07 <sup>bA</sup>	2.93±0.05 <sup>aA</sup>
T <sub>6</sub>	3.07±0.08 <sup>bA</sup>	3.86±0.07 <sup>bA</sup>	3.29±0.04 <sup>bA</sup>	4.33±0.05 <sup>bcB</sup>	4.02±0.03 <sup>bbB</sup>	3.52±0.02 <sup>bbB</sup>
T <sub>7</sub>	4.21±0.01 <sup>bcB</sup>	4.39±0.08 <sup>bcB</sup>	4.18±0.09 <sup>bcB</sup>	4.77±0.04 <sup>bcB</sup>	4.52±0.12 <sup>bcB</sup>	4.03±0.02 <sup>bbB</sup>
T <sub>8</sub>	5.82±0.04 <sup>cB</sup>	6.08±0.12 <sup>cB</sup>	5.73±0.02 <sup>cB</sup>	6.27±0.07 <sup>cB</sup>	5.92±0.03 <sup>cB</sup>	6.05±0.05 <sup>cB</sup>

Note: The different small and capital letters indicate significant difference among different treatments at P < 0.05 and P < 0.01 levels, respectively.

whilst genotypes 4 and 5 held higher values of APX activity at T<sub>7</sub> than T<sub>3</sub> treatment, indicating that SA exerted limited or little alleviative effects on genotypes 1, 2, 3, and 6 treated at 100 mg L<sup>-1</sup> Pb<sup>2+</sup> stress, while triggered some alleviative effects on genotypes 4 and 5. Compared with T<sub>4</sub> and T<sub>8</sub> treatments, all genotypes held significantly higher values (P<0.05) of APX activity at T<sub>8</sub> than T<sub>4</sub> treatment, demonstrating that SA exerted obvious alleviative effects on all genotypes treated at 200 mg L<sup>-1</sup> Pb<sup>2+</sup> stress. Fig. 2, shows that from T<sub>0</sub> (CK) to T<sub>4</sub> treatments, all genotypes experienced a significant decline in their values of CAT activity, in line with the increase of Pb<sup>2+</sup> concentration, showing that different concentrations of Pb<sup>2+</sup> exerted certain repressive effects on CAT activities in seedling leaves of different genotypes. In other words, the inhibitory effects induced by high Pb<sup>2+</sup> concentrations were high than those caused by low Pb<sup>2+</sup> concentrations. Except genotype 5 (Shi4185), all genotypes possessed the maximum values of CAT activity at T<sub>0</sub> treatment (CK), and genotype 5 exhibited its maximum value of CAT activity at T<sub>5</sub> treatment, demonstrating that SA exerted more positive effects on genotype 5 than on other genotypes. All genotypes held their minimum values of CAT activity at T<sub>4</sub> treatment, indicating that the highest concentration of Pb<sup>2+</sup> stress exerted the strongest inhibitory effects on CAT activity. Through the comparison of T<sub>2</sub> and T<sub>3</sub> treatments, it can be apparently seen that there is extremely significant differences (P<0.01) between the values of CAT activity of all genotypes at T<sub>2</sub> and T<sub>3</sub> treatments. The results indicated that application of 200 mg L<sup>-1</sup> Pb<sup>2+</sup> stress caused more striking effects on CAT activity of seedling leaves than 100 mg L<sup>-1</sup> Pb<sup>2+</sup> on different wheat genotype. From T<sub>5</sub> to T<sub>8</sub> treatments, genotypes 1, 2, and 5 exhibited gradual declines in their CAT activity, while genotype 3 held higher value at T<sub>6</sub> treatment than T<sub>5</sub>. Genotype 4 exhibited higher value of CAT activity at T<sub>7</sub> and T<sub>8</sub> treatments than T<sub>6</sub> and genotype 6 presented higher value of activity at T<sub>8</sub> than T<sub>7</sub> treatment, indicating that SA triggered different alleviative effects on CAT activities of seedling leaves of different wheat genotypes.



**Fig 1.** Effects of different treatments on APX activities of wheat seedling leaves of different genotypes.



**Fig 2.** Effects of different treatments on CAT activities of wheat seedling leaves of 6 different genotypes.

Comparison of treatments T<sub>1</sub> and T<sub>5</sub>, T<sub>2</sub> and T<sub>6</sub>, T<sub>3</sub> and T<sub>7</sub> and T<sub>4</sub> and T<sub>8</sub> shows that the values of CAT activity of all genotypes under T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub> are all greater than such values under T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> treatments, respectively. This indicates that SA with a concentration of 200 mg L<sup>-1</sup> induces some alleviative effects on seedling growth, in term of changes in CAT activity. The values of CAT activity of all genotypes under T<sub>8</sub> treatment were significantly greater than those under

$T_4$  treatment, and the differences between CAT values of all genotypes treated under  $T_4$  and  $T_8$  treatments reached to levels of extreme significance ( $P<0.01$ ). This indicates that SA induces extremely significant effect in mitigating  $200\text{ mg L}^{-1}\text{ Pb}^{2+}$  stress, which is at its high toxic level effects. According to Fig 3, it can be clearly seen that different genotypes exhibited their maximum values of POD activity at different treatments, albeit all genotypes held their minimum values of POD activity at  $T_4$  treatment. Genotypes 1 and 4 exhibited their maximum values of POD activity at  $T_3$  treatment. Genotypes 2 and 6 showed their maximum values of POD activity at  $T_7$  treatment, while genotypes 3 and 5 presented their maximum values of POD activity at  $T_2$  and  $T_6$  treatments, respectively, indicating that different genotypes held different responses to  $\text{Pb}^{2+}$  treatments and  $\text{Pb}^{2+}$ -SA jointed treatments, in term of POD activity. Among all genotypes, genotype 4 (Shijiazhuang8) possessed the maximum value of POD activity at  $T_3$  treatment, which reached to  $252.31\text{ U g}^{-1}\text{ FW}$ . The average values of POD activity of this genotype were greater than those of other genotypes, and this genotype held maximum values of POD activity at four treatments such as  $T_0$  (CK),  $T_3$ ,  $T_6$  and  $T_7$ , indicating that under all treatments, genotype 4 generally performed better in its POD activity than other genotypes. From  $T_0$  to  $T_2$  treatment, all genotypes exhibited a trend of increase in their POD activities, indicating that lower concentrations of  $\text{Pb}^{2+}$  stress promoted POD activities of all genotypes to a certain extent. From  $T_2$  to  $T_3$  treatment, genotypes 1, 4 and 5 exhibited a trend of slight increase in their POD activities, while other genotypes presented a trend of slight decrease in their POD activities. This suggests that application of  $50\text{ mg L}^{-1}\text{ Pb}^{2+}$  and  $100\text{ mg L}^{-1}\text{ Pb}^{2+}$  basically induced similar effects on POD activities of all genotypes. From  $T_3$  to  $T_4$  treatment, all genotypes presented a significant decrease ( $P<0.05$ ) in their POD activities, indicating that  $200\text{ mg L}^{-1}\text{ Pb}^{2+}$  caused significantly adverse effects on POD of seedling leaves of all genotypes. It can be clearly seen that no significant differences exist between POD activities of different genotypes under  $T_1$  and  $T_5$ ,  $T_2$  and  $T_6$ , and  $T_3$  and  $T_7$  treatments, respectively, showing that SA exerted limited effects on the alleviation of  $10$ ,  $50$ , and  $100\text{ mg L}^{-1}\text{ Pb}^{2+}$  stresses. It also can be seen that there are no significant differences ( $P<0.05$ ) between POD activities of different genotypes treated under  $T_4$  and  $T_8$  treatments. According to Fig 4, all genotypes exhibited a tendency of gradual increase in their SOD activities from  $T_0$  towards  $T_2$  treatment, indicating that lower concentrations of  $\text{Pb}^{2+}$  stress exerted certain positive effects of SOD activities in seedling leaves of different genotypes. From  $T_2$  to  $T_4$  treatments, all genotypes encountered a sharp decline in their SOD activities, demonstrating that higher concentrations of  $\text{Pb}^{2+}$  stress exerted certain inhibitory effects on SOD activities in seedling leaves of different genotypes. Genotypes 1, 2, 3 and 6 possessed higher values of SOD activity at  $T_5$  treatment than at  $T_1$ , while other 2 genotypes exhibited lower values of SOD activity at  $T_5$  treatment, showing that SA exerted certain alleviative effects on some genotypes treated at  $T_1$  treatment, whilst it exhibited no effects on other genotypes. There were certain differences among genotypes responses to  $\text{Pb}^{2+}$  stress and SA-alleviated  $\text{Pb}^{2+}$  stress. Similar results can also be seen in the comparisons between  $T_2$  and  $T_6$ , and  $T_3$  and  $T_7$  treatments. All genotypes possessed significantly higher values of SOD

activity at  $T_8$  treatment than  $T_4$  ( $P<0.05$ ), demonstrating that SA exerted significantly apparent alleviative effects on all genotypes under high concentrations of  $\text{Pb}^{2+}$  ( $200\text{ mg L}^{-1}$ ), and such effects were significantly striking than the effects of SA on all genotypes under other concentrations of  $\text{Pb}^{2+}$  stress.

#### ***The effects of $\text{Pb}^{2+}$ treatments and $\text{Pb}^{2+}$ -SA jointed treatments on MDA contents***

From Table 2, it can be clearly seen that from  $T_0$  (CK) to  $T_1$  treatment, all genotypes basically witnessed no significant changes in their MDA contents. From  $T_2$  (CK) to  $T_4$  treatment, all genotypes experienced extremely significant changes ( $P<0.01$ ) in their MDA contents. MDA accumulation under unfavorable growth conditions has been long held as a reaction of plants in response to adverse biotic and abiotic stresses (Shao et al., 2005b; Shirasu et al., 1997). The high MDA content in plants has been believed as an indication of stresses and as a weak resistance or tolerance to stresses (Shao et al., 2005b; Cao et al., 2011). Therefore, such obvious changes of MDA content in seedling leaves of all treated genotypes indicated that  $\text{Pb}^{2+}$  stress exerted dramatically negative effects on growth of all genotypes. From  $T_5$  to  $T_8$  treatment, all genotypes experienced a striking enhancement in their MDA contents, showing that SA exerted weaker alleviative effects on higher concentrations of  $\text{Pb}^{2+}$  stress. Through the comparison between  $T_1$  and  $T_5$  treatments, it can be apparently seen that all genotypes witnessed little changes in their MDA contents between both treatments, showing that SA exerted little alleviative effects on MDA contents in seedling leaves of all genotypes at  $T_1$  treatment. There are extremely significant differences ( $P<0.01$ ) between MDA contents of all genotypes treated under  $T_2$  and  $T_6$ ,  $T_3$  and  $T_7$ , and  $T_4$  and  $T_8$  treatments, respectively, showing that SA exerted significant alleviative effects on MDA contents in seedling leaves of all genotypes treated by  $T_2$ ,  $T_3$  and  $T_4$  treatments.

#### ***The effects of $\text{Pb}^{2+}$ treatments and $\text{Pb}^{2+}$ -SA jointed treatments on proline contents***

According to Table 3, it can be clearly seen that from  $T_0$  (CK) to  $T_4$  treatment, all genotypes experienced a dramatic gradual increase in their proline contents, showing that  $\text{Pb}^{2+}$  stress exerted certain effects on proline accumulation in seedling leaves of all genotypes. Proline accumulation has long been held as an indication of plant's resistance or tolerance in response to adverse growth conditions (Cao et al., 2011; Shao et al., 2006) and high proline contents in plant cells have been considered as marks of weak resistance or tolerance to unfavorable conditions (Cao et al., 2011; Shao et al., 2006). Therefore, it can be clearly concluded that different concentrations of  $\text{Pb}^{2+}$  exerted considerably negative effects on seedling leave growth of all genotypes, especially that at  $T_4$  treatment, in which the negative effects reached to the highest level. From  $T_5$  to  $T_8$  treatment, all genotypes also witnessed a dramatic gradual increase in their proline contents. Through the comparisons between  $T_1$  and  $T_5$ ,  $T_1$  and  $T_5$ ,  $T_2$  and  $T_6$ ,  $T_3$  and  $T_7$ , and  $T_4$  and  $T_8$  treatments, it can be clearly observed that SA exerted more significant alleviative effects of lowering proline

**Table 3.** Proline contents of seedling leaves of different wheat genotypes under different Pb<sup>2+</sup> treatments and Pb<sup>2+</sup>-SA jointed treatments( $\mu\text{g g}^{-1}$ ).

Treatments	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
T <sub>0</sub> (CK)	50.82±0.08 <sup>aA</sup>	46.78±0.22 <sup>aA</sup>	62.13±0.08 <sup>aA</sup>	50.62±0.13 <sup>aA</sup>	48.92±0.12 <sup>aA</sup>	58.63±0.07 <sup>aA</sup>
T <sub>1</sub>	70.45±0.12 <sup>bA</sup>	72.88±0.14 <sup>bA</sup>	68.98±0.03 <sup>bA</sup>	73.46±0.09 <sup>bA</sup>	69.97±0.03 <sup>bA</sup>	75.88±0.16 <sup>bA</sup>
T <sub>2</sub>	100.83±0.08 <sup>cB</sup>	108.98±0.09 <sup>cB</sup>	110.33±0.08 <sup>cB</sup>	99.86±0.08 <sup>cB</sup>	117.55±0.21 <sup>cB</sup>	102.43±0.19 <sup>cB</sup>
T <sub>3</sub>	150.38±0.09 <sup>dB</sup>	149.98±0.08 <sup>dB</sup>	147.72±0.18 <sup>dB</sup>	152.98±0.21 <sup>dB</sup>	148.42±0.06 <sup>dB</sup>	155.66±0.02 <sup>dB</sup>
T <sub>4</sub>	228.46±0.15 <sup>eC</sup>	237.72±0.07 <sup>eC</sup>	243.52±0.17 <sup>eC</sup>	237.88±0.08 <sup>eC</sup>	229.96±0.12 <sup>eC</sup>	233.57±0.03 <sup>eC</sup>
T <sub>5</sub>	66.76±0.03 <sup>bA</sup>	72.52±0.07 <sup>bA</sup>	70.67±0.03 <sup>bA</sup>	69.87±0.12 <sup>bA</sup>	74.65±0.07 <sup>bA</sup>	59.78±0.04 <sup>aA</sup>
T <sub>6</sub>	83.59±0.07 <sup>cB</sup>	89.88±0.04 <sup>cB</sup>	92.58±0.11 <sup>cB</sup>	87.69±0.03 <sup>cB</sup>	90.18±0.02 <sup>cB</sup>	85.46±0.08 <sup>cB</sup>
T <sub>7</sub>	90.58±0.02 <sup>cB</sup>	88.92±0.03 <sup>cB</sup>	93.62±0.04 <sup>cB</sup>	89.94±0.05 <sup>cB</sup>	92.38±0.12 <sup>cB</sup>	93.62±0.18 <sup>cB</sup>
T <sub>8</sub>	120.33±0.03 <sup>cdB</sup>	132.28±0.15 <sup>cdB</sup>	129.86±0.14 <sup>cdB</sup>	111.77±0.07 <sup>cdB</sup>	123.32±0.23 <sup>cdB</sup>	128.92±0.17 <sup>cdB</sup>

Note: The different small and capital letters indicate significant difference among different treatments at P < 0.05 and P < 0.01 levels, respectively.

**Table 4.** Soluble Sugar contents of seedling leaves of different wheat genotypes under different Pb<sup>2+</sup> treatments and Pb<sup>2+</sup>-SA jointed treatments (mg mL<sup>-1</sup>).

Treatments	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
T <sub>0</sub> (CK)	2.28±0.04 <sup>aA</sup>	2.32±0.02 <sup>aA</sup>	1.98±0.03 <sup>aA</sup>	2.08±0.02 <sup>aA</sup>	2.13±0.01 <sup>aA</sup>	1.96±0.05 <sup>aA</sup>
T <sub>1</sub>	2.11±0.07 <sup>aA</sup>	2.27±0.01 <sup>aA</sup>	1.99±0.02 <sup>aA</sup>	2.03±0.04 <sup>aA</sup>	2.23±0.03 <sup>aA</sup>	2.19±0.08 <sup>aA</sup>
T <sub>2</sub>	1.98±0.01 <sup>aA</sup>	2.05±0.05 <sup>aA</sup>	1.87±0.03 <sup>abA</sup>	1.76±0.06 <sup>bb</sup>	1.82±0.02 <sup>abA</sup>	1.97±0.03 <sup>aA</sup>
T <sub>3</sub>	1.78±0.02 <sup>abAB</sup>	1.68±0.04 <sup>BB</sup>	1.83±0.02 <sup>abA</sup>	1.88±0.01 <sup>abA</sup>	1.72±0.06 <sup>bb</sup>	1.92±0.01 <sup>aA</sup>
T <sub>4</sub>	1.66±0.05 <sup>BB</sup>	1.52±0.03 <sup>BB</sup>	1.63±0.03 <sup>BB</sup>	1.58±0.09 <sup>BB</sup>	1.69±0.01 <sup>BB</sup>	1.53±0.03 <sup>BB</sup>
T <sub>5</sub>	2.12±0.03 <sup>aA</sup>	2.01±0.01 <sup>aA</sup>	1.94±0.04 <sup>aA</sup>	1.96±0.02 <sup>aA</sup>	1.89±0.08 <sup>abA</sup>	1.92±0.07 <sup>aA</sup>
T <sub>6</sub>	2.23±0.06 <sup>aA</sup>	2.17±0.08 <sup>aA</sup>	2.19±0.04 <sup>aA</sup>	2.20±0.03 <sup>aA</sup>	2.03±0.07 <sup>aA</sup>	2.21±0.02 <sup>aA</sup>
T <sub>7</sub>	2.16±0.04 <sup>aA</sup>	2.09±0.07 <sup>aA</sup>	2.08±0.05 <sup>aA</sup>	2.23±0.06 <sup>aA</sup>	1.99±0.08 <sup>aA</sup>	2.22±0.11 <sup>aA</sup>
T <sub>8</sub>	2.33±0.08 <sup>aA</sup>	2.36±0.12 <sup>aA</sup>	2.18±0.08 <sup>aA</sup>	2.25±0.06 <sup>aA</sup>	2.19±0.01 <sup>aA</sup>	2.11±0.07 <sup>aA</sup>

Note: The different small and capital letters indicate significant difference among different treatments at P < 0.05 and P < 0.01 levels, respectively.

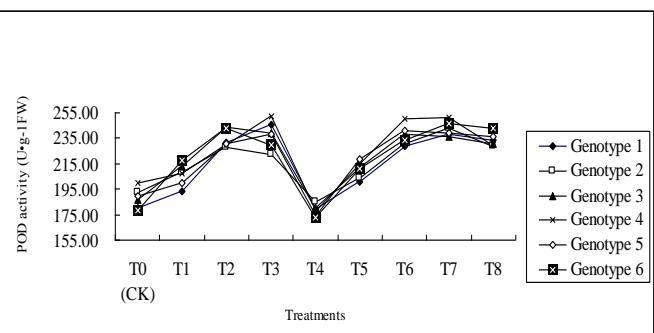
contents at higher concentrations of Pb<sup>2+</sup> stress. The difference between proline contents at T<sub>4</sub> and T<sub>8</sub> treatment reached to extremely significant level (P<0.01), showing that SA exerted extremely significant alleviative effects on seedling leaves of all genotypes treated under T<sub>4</sub> treatment.

#### The effects of Pb<sup>2+</sup> treatments and Pb<sup>2+</sup>-SA jointed treatments on soluble sugar contents

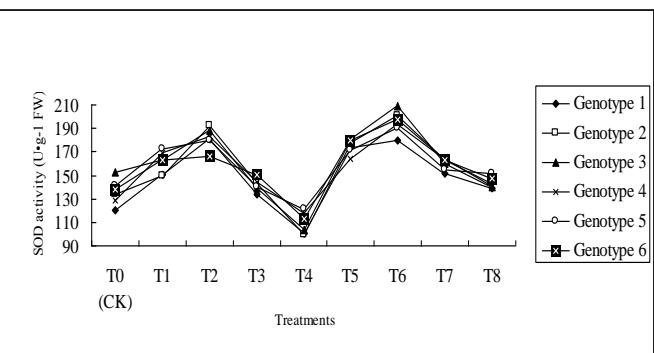
From Table 4, it can be clearly observed that from T<sub>0</sub>(CK) to T<sub>4</sub> treatment, all genotypes showed a tendency of decline in their soluble sugar contents, and all genotypes exhibited their minimum values of soluble sugar content at T<sub>4</sub> treatment. This indicates that higher concentrations of Pb<sup>2+</sup> stress are unfavorable for the accumulation of soluble sugar in seedling leaves. From T<sub>5</sub> to T<sub>8</sub> treatment, no significant difference can be observed among soluble sugar contents in all genotypes, and at all Pb<sup>2+</sup>-SA jointed treatments (namely T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>8</sub> treatment), except for genotype 5 (Shi4185) under T<sub>5</sub> treatment, which had the minimum values of soluble sugar content under all Pb<sup>2+</sup>-SA jointed treatments. This represented a significant difference (P<0.05) when compared with other values of soluble sugar contents. Such results demonstrate that SA exerted nearly similar alleviative effects on soluble sugar contents in seedling leaves of all genotypes.

#### Discussion

Numerous researches have been conducted on the issues of heavy metal pollution, which cause a series of adverse consequences on plant growth, and directly lead to aggravated environmental deterioration (Atici et al., 2005; Fargasova 1994; Mukherji and Maitra, 1977; Wierzbicka and Obidzinska, 1998; Xiong 1997, 1998). In addition, heavy metal pollution causes extremely unfavorable effects on human health and food production. With their toxicity, heavy metal ions can exert certain degrees of inhibitory effects to plant growth through



**Fig 3.** Effects of different treatments on POD activities of wheat seedling leaves of 6 different genotypes.



**Fig 4.** Effects of different treatments on SOD activities of wheat seedling leaves of 6 different genotypes.

destroying or repressing plant resistances or tolerances to disadvantageous growth conditions. It was reported that heavy metals in high concentrations inhibit seed germination, plant growth and development and disrupt many physiological and biochemical processes (Clemens, 2001). High concentrations of heavy metal ions can also result in decreased photosynthesis

rates, breakdown of protein syntheses and can affect the activities of anti-oxidative enzymes, such as APX, CAT, POD and SOD, thus causing lowered plant resistances or tolerances to both biotic and abiotic stresses (Atici et al., 2005; Hao et al., 2006). It was also reported that hyper-accumulation of heavy metals by plants in the food chains is extremely dangerous to human health and animal growth and development (Wierzbicka and Obidzinska, 1998; Sanita and Gabbielli, 1999). Like other heavy metal elements, Pb (lead) belongs to nonessential element for plant growth and development. There have been a number of reports on the inhibitory and toxic effects of lead in the germination of plants (Atici et al., 2005; Fargasova 1994; Mukherji and Maitra, 1977; Wierzbicka and Obidzinska, 1998; Wozny et al., 1982; Xiong 1997, 1998). There have also been many researches which were conducted on the effects of lead on chlorophyll synthesis (Lichtenthaler, 1987; Shao et al. 2005b; Wei et al., 2009; Rau et al., 2007), showing that lead stress with certain concentrations exerted inhibitory effects on chlorophyll content in plant leaves. In this research, our experimental data also indicated that lead caused certain degrees of negative effects on chlorophyll synthesis with further deterioration of leaf growth. As a novel kind of endogenous plant hormone, SA was reported to have some beneficial physiological effects on plant growth and development (Dat et al., 1998; Scott et al., 1999). The results of this research also confirmed that SA caused certain alleviative effects on leaves of different wheat genotypes under lead stress. We also found that SA had the best alleviative effects on the relevant physiological indices of wheat seedlings stressed by the maximum lead concentration ( $200 \text{ mg L}^{-1}$ ), perhaps indicating that SA's alleviative effects were more effective in lead concentrations at this concentration. However, whether the alleviative effects of SA will still work at  $\text{Pb}^{2+}$  concentrations of greater than  $200 \text{ mg L}^{-1}$  needs to be further investigated. As an important defensive system in plant cells, the anti-oxidative enzymes play important roles in resisting or tolerating stresses caused by many unfavorable factors, including heavy metal ions (Atici et al., 2005; Fargasova 1994; Liu et al., 2009; Liu et al., 2009; Mukherji and Maitra, 1977; Wierzbicka and Obidzinska, 1998; Wozny et al., 1982; Xiong 1997, 1998; Song et al., 2006). Anti-oxidative enzymes, such as APX, CAT, POD and SOD, have been long viewed as important indices for stress resistance or tolerance of plants (Cao et al., 2011; Dat et al., 1998; Dong et al., 2006; Liu et al., 2009; Liu et al., 2009; Wang et al., 2008; Wang et al., 2010). Results of anti-oxidative enzymatic activities of seedling leaves of different wheat genotypes were shown in Figs 1-4. From the figures, it can be apparently seen that all genotypes exhibited basically the same trends in terms of anti-oxidative enzymatic activity changes under different treatments, although at some treatments, significant differences ( $P < 0.05$ ) or extremely significant differences ( $P < 0.01$ ) observed among the seedling leaves of different genotypes. According to these figures, from the comparisons between  $T_1$  and  $T_5$ ,  $T_2$  and  $T_6$ ,  $T_3$  and  $T_7$ , and  $T_4$  and  $T_8$  treatments, it can be obviously seen that SA with a concentration of  $200 \text{ mg L}^{-1}$  induced some recovery effects on seedling growth, in term of changes in anti-oxidative enzymatic activities, although the pattern of such alleviative effects were variable in different genotypes. Such results were basically in line with the results acquired by many researches (Cao et al., 2011; Dat et al., 1998; Dong et al., 2006). However, our results did not find significant differences among anti-oxidative enzyme contents in seedling leaves of different wheat genotypes treated under different  $\text{Pb}^{2+}$  treatments and  $\text{Pb}^{2+}$ -SA jointed treatments. Such phenomenon was probably because that under hydroponic cultivation systems all genotypes were affected by the similar effects of cultivation environment,

which bears no resemblance with such environments for pot cultivation and field cultivation. Therefore, the mechanism for less difference among anti-oxidative enzymatic activities of all genotypes needs to be further investigated and discussed, and the mechanism for wheat seedling leaves' resistance or tolerance to  $\text{Pb}^{2+}$  stress and SA-alleviated  $\text{Pb}^{2+}$  stress under pot cultivation and field cultivation also needs to be further clarified. As important indicators for plant resistance or tolerance, such indices like MDA and proline content were reviewed for plant tolerance to unfavorable environmental stresses (Romero et al., 2008). In this research, MDA content and proline content presented a dramatic increase along with the increase of  $\text{Pb}^{2+}$  concentrations, showing that both of these factors are sensitive to  $\text{Pb}^{2+}$  stress. SA exerted apparent alleviative effects in lowering MDA content and proline content in seedling leaves of genotypes, showing that SA plays an important role in mitigating  $\text{Pb}^{2+}$  caused stresses, which were beneficial for the growth of seedling leaves during early stage of seedling period. Soluble sugar content was also believed as a critical index for plant responses to a myriad of environmental stresses (Cao et al., 2011; Dat et al., 1998; Dong et al., 2006; Shao et al., 2006). In this research, we found that  $\text{Pb}^{2+}$  stress induced little effects on soluble sugar content and SA exerted little alleviative effects in mitigating soluble sugar content in seedling leaves of all genotypes, perhaps indicating that no significant processes of sugar synthesis occur in seedling leaves during the early stage of wheat seedling growth. However, the detailed mechanism for such results needs to be further studied. Apart from plant leaves, root systems also play an important part in resisting or tolerating environmental heavy metal pollution. Many researches concerning the effects of heavy metal pollution on root systems were also conducted, and some researches indicated that heavy metal pollution exerted more apparent inhibitory effects on root systems than on leaves (Liu et al., 2009; Liu et al., 2009; Mittler, 2002). In this research, we merely implemented relevant experiments on seedling leaves, and the data acquired from such experiments can serve as references for the experiments concerning the effects of  $\text{Pb}^{2+}$  stress on seedling root system. However, the detailed physiological and molecule mechanisms for such effects need also to be further researched.

## Materials and methods

### Plant materials

Six different types of wheat genotypes (namely Hanyou118, Jinmai47, Xifeng20, Shijiazhuang8, Shi4185 and Zhoumai18) labeled as genotype 1, 2, 3, 4, 5 and 6, respectively, were obtained from Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. Among them, genotypes 1 and 3 represented wheat varieties with high drought-resistance, genotype 2 and 6 represent moderate, and genotypes 4 and 5 low drought-resistance.

### Seed sterilization and germination

Seeds of these 6 wheat genotypes were surface-sterilized through utilizing  $0.1\%$   $\text{HgCl}_2$  for 10 min, then washed by flushing water and placed into darkness for 24h germination.

### Hydroponic cultivation and $\text{Pb}^{2+}$ and $\text{Pb}^{2+}$ -SA jointed

#### Treatments of seedlings

The germinated seeds were planted into vermiculite-covered

white ceramic plates for initial stage of seedling growth, and the plates were then placed into an incubator with a set illumination time of 12 h/d and a temperature of around 25 °C. At single-leave stage, the seedlings of different genotypes were placed into plastic pots with the addition of half-strength Hoagland solutions for further cultivation. At three-leave stage, the seedlings were treated by different concentrations of Pb<sup>2+</sup> and SA-alleviated Pb<sup>2+</sup> stresses for 7d, respectively. The treatments were labeled as T<sub>0</sub>(CK, with no Pb<sup>2+</sup> addition), T<sub>1</sub> (10 mg L<sup>-1</sup> Pb<sup>2+</sup>), T<sub>2</sub> (50 mg L<sup>-1</sup> Pb<sup>2+</sup>), T<sub>3</sub> (100 mg L<sup>-1</sup> Pb<sup>2+</sup>), T<sub>4</sub> (200 mg L<sup>-1</sup> Pb<sup>2+</sup>), T<sub>5</sub> (10 mg L<sup>-1</sup> Pb<sup>2+</sup> + 200 mg L<sup>-1</sup> SA), T<sub>6</sub> (50 mg L<sup>-1</sup> Pb<sup>2+</sup> + 200 mg L<sup>-1</sup> SA), T<sub>7</sub> (100 mg L<sup>-1</sup> Pb<sup>2+</sup> + 200mg L<sup>-1</sup> SA), T<sub>8</sub> (200 mg L<sup>-1</sup> Pb<sup>2+</sup> + 200mg L<sup>-1</sup> SA), respectively. All the above-mentioned treatments were conducted through the addition of PbCl<sub>2</sub>, whilst Pb<sup>2+</sup> concentrations were recorded and calculated as pure Pb element concentrations.

#### **Detection of physiological indices for Pb<sup>2+</sup> stress-resistance detection of chlorophyll content**

The chlorophyll contents of seedling leaves were measured under different treatments following the procedure described by Shao et al. (2005a) and Wei et al. (2009) with a little modification. The acquired absorbance values were used for the calculation of chlorophyll content according to Shao et al. (2005a) and the unit of such content was represented as mg g<sup>-1</sup>.

#### **Detection of anti-oxidative enzymatic activities**

The activities of such anti-oxidative enzymes as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) of both untreated and treated seedlings of all genotypes were detected respectively, according to the methods described as following:

APX activity was measured according to the method of Nakano and Asada (1981) with a little modification. A unit of APX activity was calculated in reference to the method described by Wei et al. (2009), and exhibited as U g<sup>-1</sup> FW.

CAT activity was measured according to the method of Aebi (1984) with a slight modification. A unit of CAT activity was calculated as reference to the method described by Shao et al. (2005a), and presented as U g<sup>-1</sup> FW.

POD activity was measured in reference to Rao's guaiacol method (1996) with a slight modification. A unit of POD activity was calculated according to the method described by Shao et al. (2005a) and Wei et al. (2009), and presented as U g<sup>-1</sup> FW.

SOD activity was measured in reference to the method of NBT photochemical reduction described by Shao et al. (2005a) with a little modification. A unit of SOD activity was calculated as enzyme demand for 50% NBT reductive inhibition, and exhibited as U g<sup>-1</sup> FW.

#### **Measurement of malonic dialdehyde content**

Malonic dialdehyde (MDA) content was measured according to the methods described by Aravind and Prasad (2003; 2005) and Shao et al. (2005a) with a slight modification. The unit of MDA content was presented as μmol·g<sup>-1</sup>.

#### **Measurement of proline content**

Measurement of proline content was conducted in reference to the procedures described by Wei et al. (2009) with a slight modification. The unit of proline content was calculated according to the method described by Wei et al. (2009), and

presented as μg·g<sup>-1</sup>.

#### **Measurement of soluble sugar content**

Measurement of soluble sugar content was conducted in reference to the procedures described by Wei et al. (2009) and Shao et al. (2005a) with a slight modification. The unit of soluble sugar content was calculated and presented as mg mL<sup>-1</sup>.

#### **Statistical analysis**

The results presented as mean± SD (standard deviations) obtained from at least three replicates. Significant differences between the treated (under different concentrations of Pb<sup>2+</sup> and under SA-alleviated Pb<sup>2+</sup> stresses) and control group of wheat seedlings were determined using ANOVA test (P≤ 0.05 and P ≤ 0.01). Data analysis was conducted through the utilization of SPSS 18.0 Software. All treatments held at least 3 replicates. After being treated for 7d, all the samples were taken for measurements of relevant physiological indices.

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