ARBUSCULAR MYCORRHIZA IMPROVES GROWTH, PHOTOSYNTHESIS AND PROTECTS PHOTOSYSTEM OF *MALUS ROYALTY* IN Pb-CONTAMINATED SOIL

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Abstract. The effect of inoculating arbuscular mycorrhizal fungi (*Glomus caledonium*) on the growth, chlorophyll content, photosynthetic gas exchange parameters, and chlorophyll fluorescence characteristics of *Malus royalty* in the lead (Pb) contaminated soil was studied. The results showed that in Pb contaminated soil, the root activity of *Malus royalty*'s decreased significantly. Moreover, as the Pb amount increased, the chlorophyll content, particularly the chlorophyll a content, fell dramatically. The PSII activity, especially *Malus royalty* leaves' the receptor side function (PSII receptor side), and photosynthetic carbon assimilation ability were significantly inhibited under Pb stress. The interaction rate of the root system of *Malus royalty* by *G. caledonium* was high. On inoculation, *G. caledonium* showed an increased mycorrhizal infection rate of 50-70%. The mycorrhizal interaction led to increased root activity in *Malus royalty*, which alleviated chlorophyll degradation in its leaves in Pb contaminated soil. *G. caledonium* infection showed improvement in the stomatal limitation of *Malus royalty* leaves in Pb contaminated soil, along with a rise in the tolerance of photosynthetic apparatus' to Pb and other non-stomatal factors to improve photosynthetic capacity. During Pb stress, the interaction improved the capacity of PSII for photosynthetic electron transport in *Malus royalty* leaves. It has been demonstrated that it somewhat increases the PSII receptor side OEC activity and Q_A to Q_B electron transfer ability in *Malus royalty*. Thus, it ensured relatively high activity of PS II in the inoculated leaves under Pb stress. Therefore, inoculation with *G. caledonium* can improve *Malus royalty* leaves' Pb tolerance and reduce the toxicity effect on photosynthetic function and morphological characteristics.
Keywords: *Malus royalty, AMF, Pb, G. caledonium, photosystem, photosynthesis*

Introduction

Heavy metal pollution of Urban soil, especially lead (Pb) pollution, is becoming a matter of concern. One of the most dangerous components of heavy metal pollution is lead (Soffianian et al., 2014; Sauliute et al., 2015). The main sources of the soil pollutants are industrial production and transportation, which is dangerous for the health of people, which poses a threat to human health. It can accumulate in the cause lead poisoning or plumbism in the human body (Shu et al., 2012; Ogbomida et al., 2018). Pb pollution has become a hot topic of research in environmental science (Hall, 2002; Pallara et al., 2013; Chen et al., 2017a). Pb stress can inhibit plant growth, especially in roots development (Jiang and Liu, 2010; Arena et al., 2017). Moreover, the toxicity slows down the activity of the photosynthetic system or harms it. Also, various plants have varying levels of Pb tolerance. For instance, super-accumulating plants have a higher level of Pb tolerance than sensitive plants (Xu et al., 2012; Yang et al., 2015; Figlioli et al., 2019). How to repair Pb contaminated soil has become a research hotspot of scientists all over the world. Phytoremediation is green and sustainable, but the process is slow. Therefore, improving

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the remediation efficiency of contaminated soil, increasing the stability of heavy metals or removing heavy metals from soil is an important direction for researchers to explore. AMF can be widely distributed in all kinds of heavy metal contaminated soils, whether single heavy metal pollution or combined heavy metal pollution (Sorrention et al., 2018). AMF can affect the absorption, accumulation and transfer of heavy metals in host plants (Zhang et al., 2007; Xue and Gao, 2017).

In the contaminated soils, arbuscular mycorrhizal fungi (AMF) are present. AMF can create a symbiotic relationship with plant roots known as arbuscular mycorrhiza, which can hasten plant restoration, increase restoration effectiveness, and boost heavy metal stability. AMF mycelial network can expand the volume of host plant roots in the soil so that the roots are capable of absorbing more heavy metals (Rozpądek et al., 2016; Xue and Gao, 2017). It can provide phosphate, microelements, and water to the host plant, so as to improve its nutritional level. Similarly, AMF can transfer the heavy metals absorbed by it to the plants through its mycelium. Therefore, in some cases, *mycorrhizal* plants absorb more heavy metals and improve the transport efficiency of root crown, while in some cases, AMF can promote the fixation of heavy metals in soil (Qu et al., 2009; Rizwan et al., 2017). AMF can be widely found in all kinds of heavy metal contaminated soil, whether single or complex heavy metal pollution. When the concentration of heavy metals increases, there is an increase in the adsorption of heavy metals by the host, which results in inhibited plant growth due to the heavy metal stress (Chen and Zhao, 2009; Chen et al., 2017b, c). AMF interaction, however, either stimulates plant development or reduces the toxicity of heavy metals to plants, or it has no impact at all. Therefore, it is of practical significance great and theoretical value to study the combinatorial mechanism of AMF and plant for remediation of heavy metal contaminated soil, thus accelerating this process.

Malus royalty is a deciduous shrub or sub-tree. Because of its strong resistance, wide planting range, and good durability, it was gradually introduced into the Northern provinces and cities of China. It has important ecological value in the local area (Sun et al., 2019). According to the needs of cities around the country, resources have been introduced and varieties with strong resistance have been cultivated over time. However, due to the geographical and climatic reasons in northern cities of China, the garden green space is often polluted by heavy metals in the land, and there is still a lack of economic and feasible remediation technology at present. In addition, the contradiction between more people and less green gardens in our country is becoming increasingly prominent. Therefore, it is necessary to find economic and effective methods to reduce the heavy metal content of soil to ensure the environmental safety of urban green Spaces.

Several studies have been conducted recently on the potential of AMF to increase plants' resistance to heavy metals. Nevertheless, there are not many studies on *Malus royalty'*s growth index and photosynthetic traits in Pb-contaminated soil. To supply some fundamental information for enhancing *Malus royalty*'s Pb stress resistance mechanism.

Materials and methods

Experiment materials

The experiment was conducted in the laboratory of soil science of Harbin University (Harbin, Heilongjiang Province, China) from March to July 2022. The culture medium was composed of organic fertilizer, perlite and yellow soil (6.0:3.0:0.5:0.5). The culture medium was sterilized at high temperature (121℃) for 2 h in an autoclave to kill the

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indigenous mycorrhiza and other microorganisms in the soil. The tested mycorrhizal strain was glomus caledonium, and the inoculum was purchased from China arbuscular mycorrhizal fungi germplasm resource bank, numbered "BGCBJ04A". The mycorrhizal agent used for inoculation contains spores, hyphae and mycorrhizal segments, and each gram of mycorrhizal agent contains 50-60 spores.

Malus Royal was planted in our laboratory, and the annual seedlings were provided by Northeast Agricultural University. The plant height of the tested materials was about 20 cm. According to the method of Bao Shidan et al. (2000), the physicochemical properties of sterilization matrix were determined pH 7.66 (soil/water = 1:2.5, W/V), organic matter 16.85 $g \cdot kg^{-1}$, ammonium nitrogen 7.37 $mg \cdot kg^{-1}$, nitrate nitrogen 25.77 $mg \cdot kg^{-1}$, available phosphorus 11.48 $mg \cdot kg^{-1}$, available potassium 108.96 $mg \cdot kg^{-1}$, total Pb 3.58 $mg \cdot kg^{-1}$.

Two factor test AMF × Pb was arranged in complete random block. The appropriate amount of Pb $(NO_3)_2$ solution was added to the culture medium and mixed evenly, so that the Pb content in the soil Pb test medium was set at 4 gradients of 0, 50, 200 and 400 $mg \cdot kg^{-1}$, respectively. The control group was added with the same amount of distilled water, and each Pb level was treated with inoculation of *Glomus caledonium* (+AMF) and no inoculation of arbuscular mycorrhizal fungi (CK). The treatment of inoculating mycorrhizal fungi was to add 50 g of soil containing bacteria per kilogram of culture medium, and the treatment of not inoculating mycorrhizal fungi was to add the same amount of sterilized soil. Each treatment was repeated in 5 bowls, a total of 40 bowls. The pot was cultured in an artificial climate box with temperature of 25℃, light intensity of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Photoperiod of 12/12 h (light/dark) and relative humidity of about 75%.

Test items and methods

Determination of growth parameters

After measuring the plant height, the plant was taken out of the culture medium, the roots were washed, and then the root length was measured for a single plant. After absorbing the water on the surface of the root system with the absorbent paper, the aboveground and underground parts were put into aluminum boxes for killing (105°C, 30 min), drying (60°C, 30 h) to constant weight, and then weighing the biomass. The average aboveground biomass, underground biomass, and root crown ratio of each plant were calculated as follows:

$$\text{Root shoot ratio} = \text{biomass in underground} / \text{aboveground}$$

Determination of mycorrhizal interaction rate, chlorophyll content, and root activity

The infection rate of *mycorrhiza* was determined by section staining. The root system was washed under tap water, put into 1 N KOH solution at 80°C for 1 h. It was then taken out, washed with distilled water, put into 20% H_2O_2 solution for 30 min, and then into 1 N HCl for 2 min, and finally dyed with 0.5% acid fuchsin. The stained cells were observed under the microscope. The content of soil organic matter was determined by K_2CrO_7–H_2SO_4 oxidation method. The pH value of soil was determined by the electrode method, and the field water holding capacity of the soil was determined by the drying method. The interaction rate was calculated as follows:

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$$\text{The mycorrhizal interaction rate (\%)} = \frac{\text{The number of root segments}}{\text{The number of root segments measured}} \times 100$$

The content of chlorophyll was determined by the acetone method and the root activity was determined by the TTC method (Bao Shidan et al., 2000).

Determination of photosynthetic gas exchange parameters

The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of the second fully expanded functional leaf of *Malus royalty* were measured at 9:00-11:00 a.m. using Li-6400 photosynthetic measurement system (Licor company, USA). The PFD was provided by the instrument's light source at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and CO_2 concentration was fixed at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using a CO_2 cylinder. The process was repeated 5 times.

Determination of carboxylation efficiency (CE)

The PFD was fixed at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with the light source of the Li-6400 photosynthetic measurement system, and then the CO_2 concentrations of 400, 300, 200, 100, 50, 700, 1300, and 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were provided by the CO_2 cylinder. P_n value of the *Malus royalty* leaves was measured and the Pn-CO_2 response curve was drawn. The slope of 50-300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the curve was used as the carboxylation efficiency (CE).

Chlorophyll fluorescence parameters (Φ_{PSII}, ETR and NPQ)-determination of light response curve

The light response curves of chlorophyll fluorescence to different PFD were measured by FMS-2 portable modulation fluorometer (Hansatech, UK). The maximum fluorescence (F_m) was measured after dark adaptation of 30 min before the measurement, and then different PFD (100, 200, 400, 600, 800, 1000, and 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) values were applied to the leaves by the built-in light source of FMS-2. The maximum fluorescence (F_m') and steady-state fluorescence (F_s) were measured after 3 min of adaptation at each PFD. The actual photochemical efficiency of *Malus royalty* leaves at different PFD was determined by the following equation:

$$\Phi_{PSII} = (F_m' - F_s) / F_m'$$

$$ETR = 0.5 \times 0.85 \times \Phi_{PSII} \times PFD$$

$$NPQ = (F_m/F_m')/F_m'$$

Each treatment was repeated 5 times.

Determination of OJIP curve and 820 nm optical reflection curve (MR_{820nm})

After dark adaptation, the OJIP curve and 820 nm light reflection curve were measured by Multi-Function Plant Efficiency Analyser (M-PEA). The fluorescence signal was induced by 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ pulse red light, and each treatment was repeated 5 times. The activity of PSII is expressed by the maximum photochemical efficiency of PSII (F_v/F_m), where $F_v = F_m - F_o$, F_m, and F_o which are the relative

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fluorescence intensities at 0.01 and 1000 ms on the OJIP curve, respectively. The activity of PSI is reflected by the relative fall of 820 nm light reflection curve (MR_{820nm}) signal, $\triangle I/I_o$, where I_o is the maximum value of reflection signal in mr820nm curve, and $\triangle I$ is the difference between the maximum and the minimum value of reflection signal in mr820nm curve.

Based on $V_{O\text{-}P} = (F_t\text{-}F_o)/(F_m\text{-}F_o)$ $V_{O\text{-}J} = (F_t\text{-}F_o)/(F_J\text{-}F_o)$, O-P and O-J curves of OJIP were standardized to obtain $V_{O\text{-}P}$ and $V_{O\text{-}J}$ curves, where F_J is the relative fluorescence intensity at 2.0 on the OJIP curve, and F_t is the relative fluorescence intensity at each time point on the OJIP curve. The relative variable fluorescence of J point at 2 ms on the $V_{O\text{-}P}$ curve is expressed in V_J, and the relative variable fluorescence of K point at 0.3 ms on the $V_{O\text{-}J}$ curve is expressed in V_K. The curve of *Malus royalty* leaves to $V_{O\text{-}P}$ and $V_{O\text{-}J}$ and CK under different treatments were used as the difference value, which was expressed by $\triangle V_{O\text{-}P}$ and $\triangle V_{O\text{-}J,}$ respectively. The change amplitude of each characteristic point on the curve was analyzed.

Determination of chlorophyll content, ROS metabolism and other physiological indexes

The chlorophyll content was determined by the ethanol extraction spectrophotometry method. Chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Chla+b) and chlorophyll a/b (Chla/b) were calculated respectively. $O_2^{\bullet}$production rate and H_2O_2 content was measured using the method of Bao (2000). The malondialdehyde (MDA) content was and the activity of ascorbic acid peroxidase (APX) was determined by the Bao et al.'s method.

Data processing methods

Excel and SPSS software (Version. 22) were used to conduct statistical analyses on the measured data. The data in the figure was denoted as mean ± standard deviation (SD). Two-way ANOVA was used to detect the significant differences of heavy metal concentration, AMF inoculation and their interaction on all variables.

Results and analysis

Growth characteristic interaction rate and root activity

Increased Pb content in the soil showed a decrease in the plant height, root length and biomass in *Malus royalty*. However, no significant change was observed in the plant height and biomass of each part. The root shoot ratio y also tended to increase. When the Pb content (400 $mg \cdot kg^{-1}$), *Malus royalty* inoculated with *G. caledonium* showed increased plant height, root length, total biomass, aboveground biomass and underground biomass which was recorded as 13.34%, 27.34%, 20.69%, 44.44% and 17.39%, respectively, as compared to those without inoculation ($P < 0.05$), and the root infection rate dropped by 83.88% ($P < 0.05$). Although *Malus royalty* roots' activity decreased as the amount of Pb in the soil increased, the root vigor of *Malus royalty* treated with *G. caledonium* increased at different degrees compared with the control due to the infection (*Fig. 1; Table A1*).

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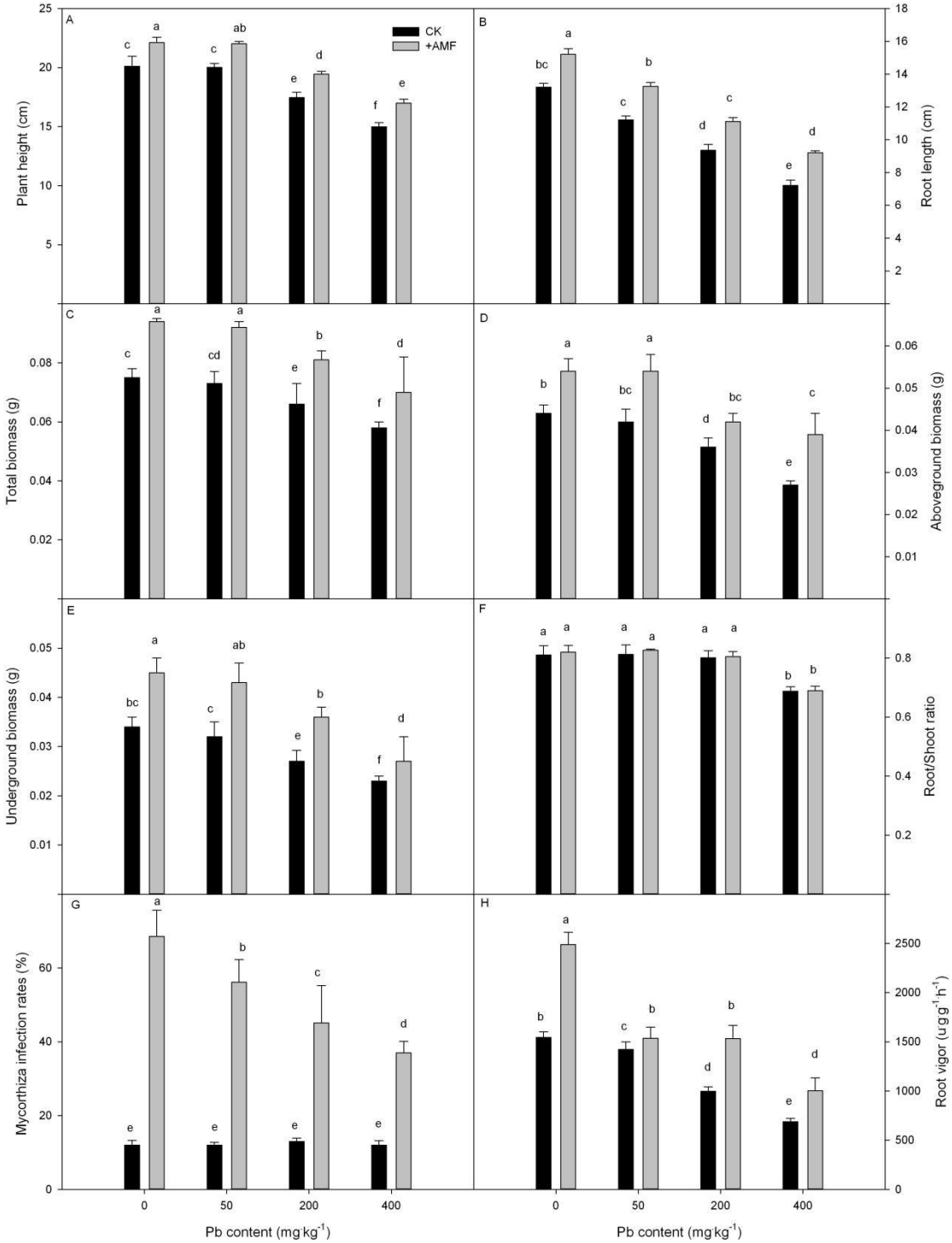

Figure 1. *Growth characteristics, interaction rate and root activity of Malus royalty mycorrhizae in Pb contaminated soil. CK: CK + 0 AMF contents; + AMF: CK + various concentrations of arbuscular mycorrhizal fungi (AMF). The data in the picture are the mean and standard error (SE); values shown by various lowercase letters denote significant differences ($P < 0.05$)*

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Underground and aboveground parts

With the rising Pb concentration in soil, Pb contents of *Malus royalty* showed significant growing tendencies both underground and aboveground. The roots of *Malus royalty* absorbed more lead thanks to AMF. Except in the treatments lacking Pb, the Pb level in the underground plant portions of the + AMF treatment was significantly higher than that of the plants not inoculated (*Fig. 2; Table A2*).

Chlorophyll content and antioxidant enzymes activities

Malus royalty leaves contained significantly less chlorophyll a, chlorophyll b, and total chlorophyll as the amount of Pb in the soil increased. Chlorophyll a concentration declined more than chlorophyll b content did. Research demonstrated that when the amount of Pb in the soil increased, the value of chlorophyll a/b similarly reduced. With the increase in concentration of Pb, there was a rise in the rate of production of superoxide (O2•-), malondialdehyde (MDA) content and electrolyte permeability. The SOD activity was lower under 400 $mg \cdot kg^{-1}$ Pb stress, compared with of 200 $mg \cdot kg^{-1}$ ($p < 0.05$). The activity of ascorbate peroxidase (APX) decreased as the concentration of Pb increased (*Fig. 3; Table A3*).

Gas exchange parameters

The high levels of Pb in the soil had a substantial impact on the gas exchange characteristics of *Malus royalty leaves*. The P_n, G_s, and T_r values of *Malus royalty leaves* declined as the amount of Pb in the soil rose. Their numbers significantly improved after being vaccinated with *G. caledonium*. The P_n, G_s, and T_r values in plants inoculated with *G. caledonium* increased by 99.94% and 91%, respectively, when the soil's Pb concentration rose to 400 $mg \cdot kg^{-1}$ ($P < 0.05$) (*Fig. 2; Table A4*).

OJIP curve

With an increase in Pb level in the soil, the relative fluorescence intensity of the O-point dramatically increased. *Malus royalty* (+AMF) leaves experienced OJIP curve changes that were noticeably less than those of the CK ($P < 0.05$). When the Pb level of the soil was 200 and 400 $mg \cdot kg^{-1}$, there was a significant difference in the relative fluorescence intensity (F_o and F_m) of O-point and P-point ($P < 0.05$) (*Fig. 3; Table A5*).

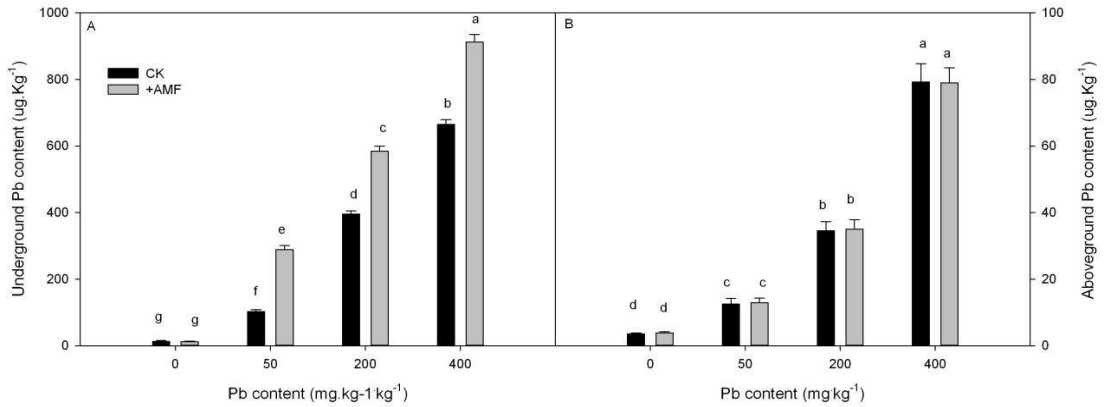

Figure 2. *Growth characteristics, infection rate and the Pb content in the underground and aboveground parts*

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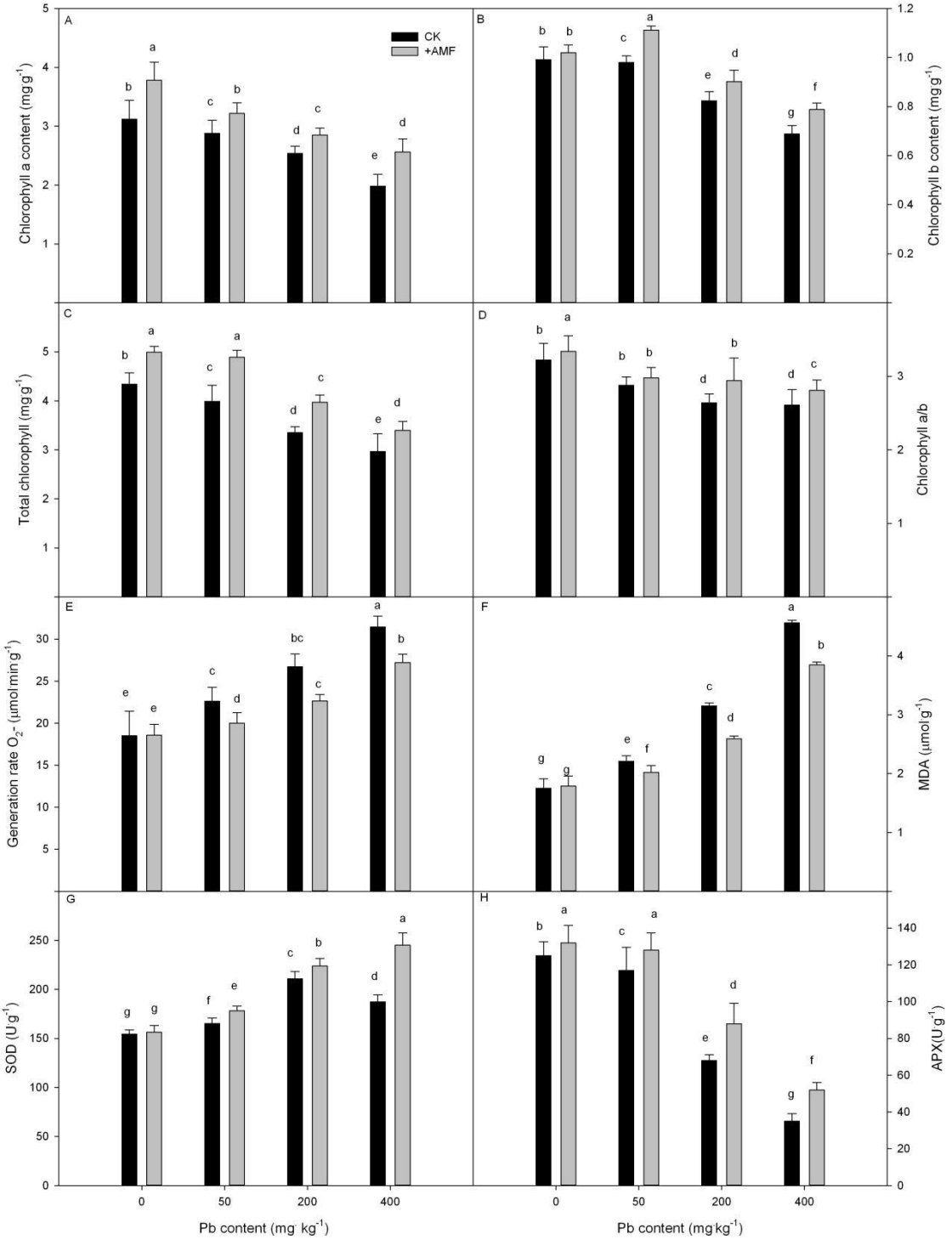

Figure 3. *Effect of Glomus caledonium on the chlorophyll content and the activities of antioxidant enzymes of Malus royalty leaves in Pb-contaminated soil*

PSII and PSI photochemical activity

With an increase in Pb content in the soil, the relative variable fluorescence V_J of J point at 2 ms on the standardized OJIP curve of *Malus royalty* leaves rose. With the increase in the V_J value for the *Malus royalty* leaves treated with *G. caledonium* was lower than that of

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the control without inoculation ($P < 0.05$). Quantitative analysis of V_J and V_K in *Malus royalty* leaves also increased significantly ($P < 0.05$). V_J and V_K of *Malus royalty* leaves (+AMF) were significantly lower than those of the non-inoculated leaves ($P < 0.05$) (*Fig. 4; Table A6*).

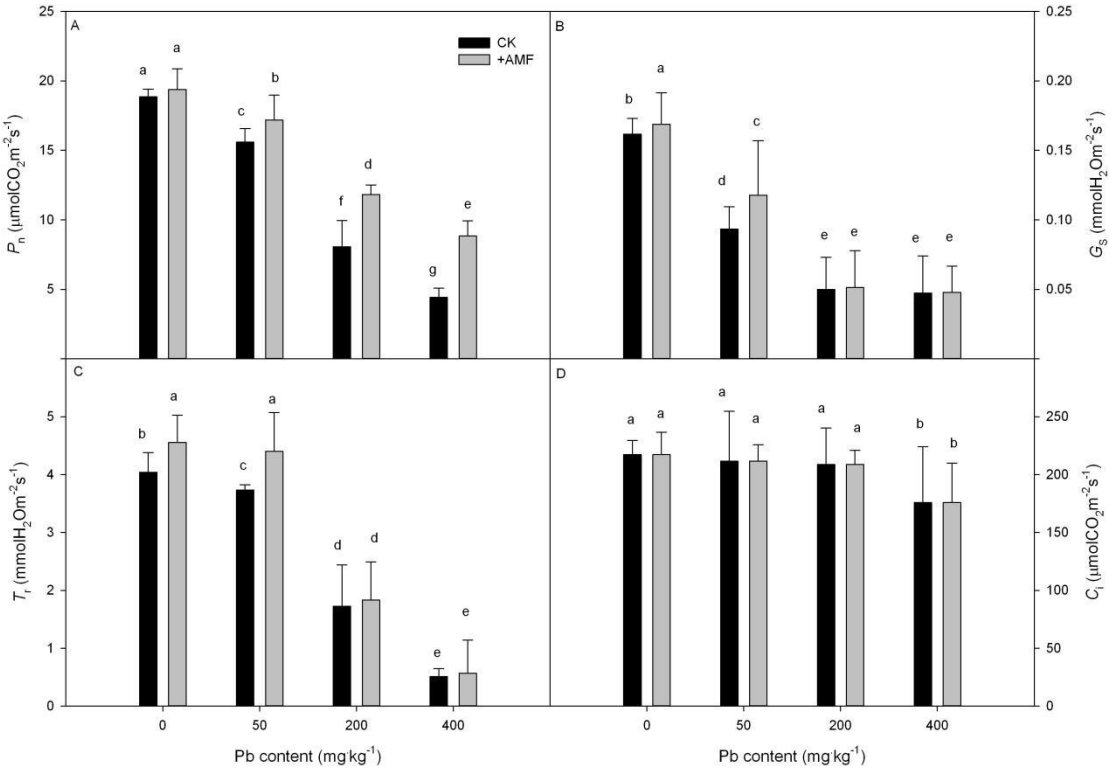

Figure 4. *The photosynthetic characteristics of Malus royalty leaves in Pb contaminated soil*

With the increase in concentration of Pb to 400 mg·kg^{-1}, a decrease in Φ_{PSII} in *Malus royalty* leaves was observed, and the increase of ETR was significantly lower than CK. In addition, the NPQ of *Malus royalty* leaves increased significantly compared with that of the CK under the 400 mg·kg^{-1} Pb stress. *Malus royalty* leaves (+AMF) had a PI_{ABS} that was 16.68% ($P < 0.05$) higher in soil devoid of Pb pollution than uninoculated leaves ($P < 0.05$). The PI_{ABS} of *Malus royalty* leaves drastically decreased as soil Pb content rose. The F_v/F_m and $\triangle I/I_o$ of *Malus royalty* leaves tended to decrease with the increase in soil Pb concentration. However, the change in amplitude of F_v/F_m of *Malus royalty* leaves under different concentrations of Pb stress was smaller. All remained above 0.8, while the decrease in amplitude of $\triangle I/I_o$ was much larger than that of F_v/F_m (*Fig. 4; Table A6*).

Discussion

In order to maintain regular water intake via the root system and the physiological processes' ability to function under these circumstances, plants must adapt to environmental stress in terms of physiological functions in order to maintain growth and nutrient accumulation (Del et al., 1999a, b; Dong et al., 2008). The growth characteristics of *Malus royalty* in Pb-contaminated soil demonstrated a considerable

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inhibition in our investigation. But with increased Pb stress, there was a gradual decrease in the ratio of root to shoot, which indicated hindrance to the growth and development of *Malus royalty* root system, which might be caused by the accumulation of Pb in the roots of *Malus royalty*. Under different levels Pb pollution, the influence of *G. caledonium* interaction, the root activity of *Malus royalty* was significantly higher than that of the non-inoculated roots (*Fig. 5; Table A7*).

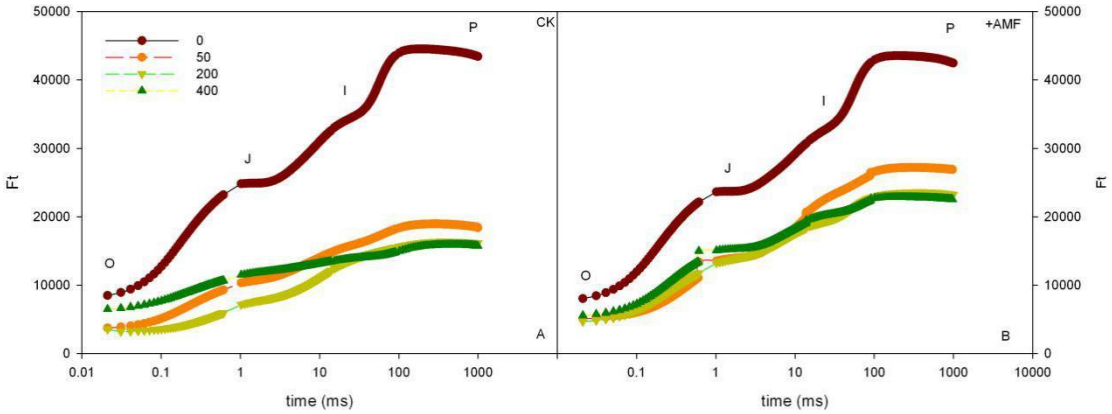

Figure 5. *The OJIP curve of Malus royalty leaves in Pb contaminated soil*

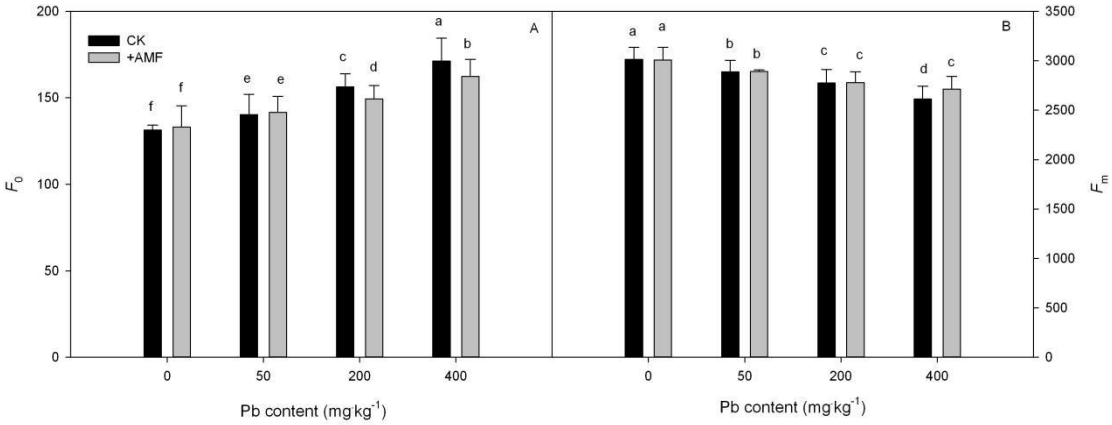

Figure 6. *The F_o and F_m of Malus royalty mycorrhiza in soil contaminated with Pb*

Pb stress also leads to disorder of the stacking of basal grains, the influences chloroplast function, and the disappearance of matrix lamellae (Kurpa et al., 1999; Liu et al., 2008; Bah et al., 2010; Semane et al., 2010; Hossain et al., 2012; Kalaji et al., 2016; Liu et al., 2017). In this experiment, the amount of Chla and Chlb in *Malus royalty* leaves drastically dropped as Pb concentration rose. According to the research findings (Romanowska et al., 2006; Tukaj et al., 2007; Marmiroli et al., 2013), Chla was discovered to be more sensitive to Pb stress. Moreover, we discovered that Chla was more susceptible to Pb stress than Chlb. *Malus royalty* leaves' capacity to capture light energy and to use that energy can both be diminished by heavy metals. The effect of inoculating *G. caledonium* on the content of chlb in *Malus royalty* leaves was not significant, but it could increase the content of chla, which showed that the ratio of chla/b in the treated leaves of *Malus royalty* was higher than that of the non-inoculated leaves. It also

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improved the efficiency of the reaction center and ensured higher photosynthesis (*Fig. 6; Table A7*).

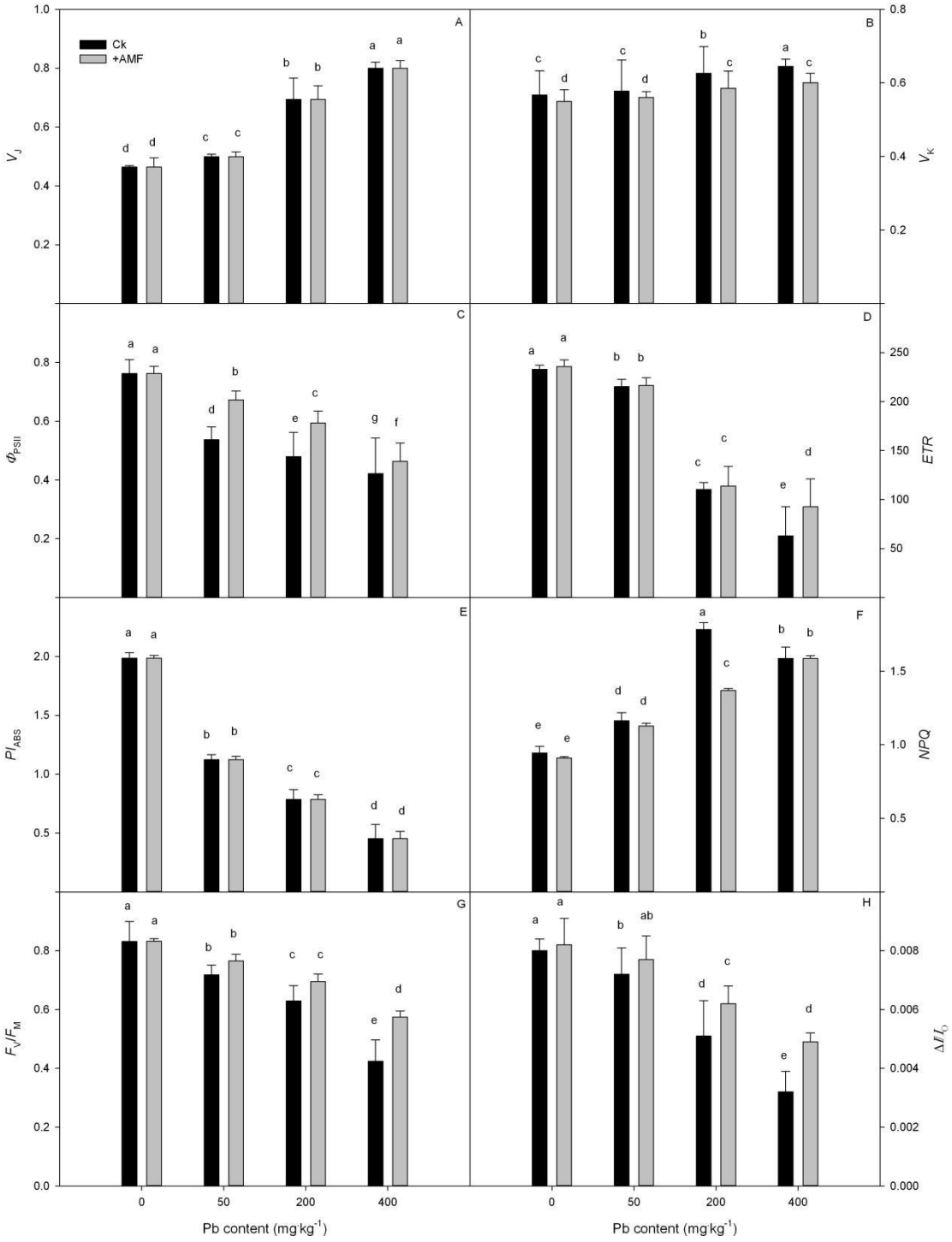

Figure 6. *The PSII and PSI photochemical activity of Malus royalty leaves in Pb contaminated soil*

When the plant under environment stress, the utilization of excitation energy by photosynthetic apparatus is reduced (Ahmed et al., 2009). The excess electrons in the photosynthetic electron transfer chain will attack free O_2, resulting in the production of

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superoxide anion (O2•-). Superoxide dismutase (SOD) will catalyze the disproportionation of $O2•^-$ and H_2O_2 to form H_2O_2. In plants, an increase in ROS such as $O2•^-$ and H_2O_2 will upset the redox balance, lead to membrane peroxidation, and result in oxidative damage to cell structure and components. Oxidative damage induced by ROS under Pb stress is one of the most important toxic characteristics (Gill and Tuteja et al., 2010; De Silva et al., 2012). In our study, the O2•- production rate and H_2O_2 content of *Malus royalty* leaves increased significantly with the increase of Pb concentration, and MDA content and electrolytic decomposition permeability increased obviously. ROS in chloroplast can cause oxidative damage of photosynthetic apparatus, leading to photoinhibition of PSI and PSII. Especially in the photoinhibition of PSI is mainly related to the ROS around it. Under Pb stress, an increase in ROS can drive the production of SOD, POD, CAT, and glutathione (GSH), which in turn helps plants remove excess ROS (Malecka et al., 2001; Qureshi et al., 2007; Skórzyńska-Polit et al., 2010). Some studies found that in a certain range of Pb concentration, Pb treatment could maintain the balance of active oxygen by enhancing the activities of SOD and cat in maize leaves. Our study has similar results to those studies, 50 $mg \cdot kg^{-1}$ Pb stress slightly increased the activity of SOD, while the activity of APX showed significant decrease. Under 200 $mg \cdot kg^{-1}$ Pb stress, the activities of SOD and APX in *Malus royalty* leaves were significantly decreased.

The amount of chlorophyll directly influences a plant's ability to photosynthesize as well as its ability to grow (Gupta et al., 2010). Under Pb stress, there might be a reduction in the number of chloroplasts, destruction of its structure, and accelerated degradation of chlorophyll, which might affect the photosynthetic capacity (Liu et al., 2008; Zhang et al., 2019a, b). In our work, the Pb stress led to decrease of P_n, G_s and T_r values in leaves of *Malus royalty*. *Malus royalty* leaves' stomatal restriction was greatly enhanced by AMF. Following this, P_n and T_r were increased, which was beneficial for the nutrient accumulation. Under Pb stress (200 $mg \cdot kg^{-1}$), the C_i of the leaves of *Malus royalty* treated with *G. caledonium* continued to decrease as compared to the untreated leaves. Results suggested that Pb stress (200 $mg \cdot kg^{-1}$) might be connected to a decrease in CO_2 utilization capacity, even when stomatal conductance is lowered, which would impair the photosynthetic system of *Malus royalty* leaves. However, the C_i content of *Malus royalty* leaves inoculated with *G. caledonium* was lower than that of the non-inoculated leaves when the Pb content was 200 $mg \cdot kg^{-1}$. Results suggested that infected leaves could enhance the photosynthetic machinery of *Malus royalty* leaves and protect their physiological function. Also, by boosting the ability of the photosynthetic system to withstand Pb stress, *G. caledonium* inoculation can enhance photosynthetic capacity. We came to the conclusion that both stomatal and non-stomatal limiting factors contributed to the decrease in photosynthetic capability of *Malus royalty* leaves produced by Pb stress (Malecka et al., 2001; Qureshi et al., 2007; Skórzyńska-Polit et al., 2010).

Chlorophyll fluorescence technology is usually to study the function of the photosynthetic system (Khalid Al-aghabary et al., 2005; Mani et al., 2015). In our experiment, F_o in the OJIP curve of *Malus royalty* leaves increased obviously, while F_m decreased with the increase of Pb content in the soil. The parameters F_v/F_m and PI_{ABS} reflecting the photochemical activity of PSII in *Malus royalty* leaves decreased significantly, but F_v/F_m and PI_{ABS} of inoculated *Malus royalty* leaves were lower compared to non-inoculated ones. With *G. caledonium* treatment could alleviate the decrease of PSII photochemical activity in *Malus royalty* leaves in Pb contaminated soil. Further research revealed that under stress, blocked sites in the photosynthetic

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electron transfer chain could be seen on the electron acceptor and electron donor sides of the reaction center of PSII, and that the transfer of Q_A to Q_B is the primary site of inhibition of the photosynthetic system. (Zhang et al., 2019a, b). On the OJIP curve, the rised V_K was usually considered as a specific marker for the injury of OEC activity, and the injury on the donor side of PSII. Some studies also reported that when *Maize* leaves under Pb stress, the main cause of PSII photoinhibition was related to the damage of OEC (Xu et al., 2018; Zhang et al., 2018). Under Pb stress, we considered the main reason for the decrease of PSII photochemical activity in *Malus royalty* leaves was proportional to high Pb concentration that inhibited the transfer of electrons from Q_A to Q_B and reduced the activity of OEC. However, the rise of V_J and V_K in the leaves of *Malus royalty* treated with *G. caledonium* (+AMF) was obviously lower than that of the non-inoculated treatment, which indicated that the leaves activity of PSII under Pb stress could be improved by stabilizing the electron transport between PSII's donor and receptor sides, PSII under Pb stress could be enhanced.

As light response's core component, plant PSI is also sensitive to stress. Stress often leads to the decrease of activity of the even photoinhibition, deactivation or PSI reaction center. Heavy metal stress often leads to a decrease in the PSI activity (Xu et al., 2018). Pb stress' effect on *Malus royalty* leaves' PSI function was analyzed by the chlorophyll fluorescence technique. The results that were showed that, $\triangle I/I_o$ in leaves of *Malus royalty* showed a decreasing trend. The results demonstrated that under Pb stress, PSI and PSII's photochemical activities in *Malus royalty* leaves decreased, and PSII's linear electron transfer was blocked. Additionally, the reduction of $\triangle I/I_o$ in *Malus royalty* leaves under Pb stress was significantly greater than that of F_v/F_m, indicating that PSI in *Malus royalty* leaves was more severely damaged by Pb than PSII.

Conclusion

Pb pollution will first influence *Malus royalty* roots, resulting in root vigor's decline and the decrease of underground biomass accumulation as well as the decline of chlorophyll content and photosynthetic capacity of leaves. The reduction of *Malus royalty* leaves' photosynthetic carbon assimilation capacity was the result of both stomatal and non-stomatal factors' limitation. Some of the important non-stomatal factors focused in this study are as follows: decrease in PSI and PSII activity, carboxylation efficiency and oxidative damage of ROS. The sensitivity of PSI in *Malus royalty* leaves to Pb stress was higher than that of the PSII. Besides, the PSI donor side's damage was higher than that of the PSII *Malus royalty* leaves' photochemical activity leaves can also be improved in different degrees. receptor side. Therefore, the inoculation of *G. caledonium* can improve *Malus royalty* leaves' Pb tolerance from photosynthetic function and morphological characteristics.

Data availability. The following information was supplied regarding data availability: The research in this article did not generate any raw data.

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APPENDIX

Table A1 demonstrates that the effect of AMF inoculation on the root/shoot ratio of *Malus royalty* was minor, but that it had a substantial ($P < 0.01$ impact on underground biomass and other growth metrics. *Malus royalty*'s growth characteristics were significantly impacted by soil Pb ($P < 0.01$), but there was no evidence of a significant interaction with AMF inoculation. The rate of mycorrhizal infection and root vigor of *Malus royalty* were significantly impacted by AMF inoculation and soil Pb. Moreover, the rate of mycorrhizal infection was significantly impacted by the interaction between these two variables, but not by root vigor ($P > 0.05$). *Malus royalty*'s subsurface Pb content was significantly affected by AMF inoculation ($P < 0.01$, but its aboveground Pb content was unaffected ($P > 0.05$).

Table A1. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (+AMF × Pb) on plant height, root length, total biomass, aboveground biomass, underground biomass and root/shoot ratio, mycorrhizal infection rate, root vigor*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
Plant height	34.14	< 0.01	22.32	< 0.01	21.32	0.53
Root length	42.23	< 0.01	77.34	< 0.01	8.43	0.32
Total biomass	54.88	< 0.01	23.21	< 0.01	3.48	0.34
Aboveground biomass	55.92	< 0.01	12.34	< 0.01	5.34	0.35
Underground biomass	42.83	< 0.01	14.43	< 0.01	12.32	0.65
Root/shoot ratio	14.37	< 0.01	25.24	< 0.01	12.32	0.54
Mycorrhizal infection rate	76.33	< 0.01	26.37	< 0.01	23.13	< 0.01
Root vigor	56.32	< 0.01	34.26	< 0.01	13.22	0.12

Malus royalty's subsurface Pb content was strongly impacted by AMF inoculation, as indicated in *Table A2* ($p < 0.05$), although its aboveground Pb level was not significantly impacted. Both the subsurface and the surface Pb content were significantly influenced by soil Pb ($p < 0.05$). The subsurface Pb content was significantly impacted by the relationship between AMF inoculation and soil Pb, but not the aboveground Pb content.

Table A2. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (+AMF × Pb) on underground Pb content and aboveground Pb content*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
Underground Pb content	35.89	< 0.01	81.23	<0.01	9.32	<0.01
Aboveground Pb content	12.33	0.78	75.81	<0.01	1.23	0.83

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Chlorophyll a, Chlorophyll b, and total Chlorophyll in *Malus royalty* leaves all reduced significantly with an increase in Pb content in the soil, with Chlorophyll a showing a more pronounced decrease than Chlorophyll, b. Research demonstrated that when the amount of Pb in the soil increased, the value of chlorophyll a/b similarly reduced. While there were various concentrations of Pb in the soil, there was no appreciable change in chlorophyll b content between *Malus royalty* leaves treated with *G. caledonium* and the control. Chlorophyll a's water content was significantly different from total chlorophyll's ($p < 0.05$), nevertheless (*Table A3*).

Table A3. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (+AMF × Pb) on chlorophyll a content, chlorophyll b content, total chlorophyll a content, chlorophyll a/b, O2•-production rate, MDA, SOD and APX*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
Chlorophyll a content	97.86	<0.01	23.88	<0.01	0.57	0.89
Chlorophyll b content	55.26	0.04	53.41	<0.01	0.12	0.55
Total chlorophyll content	20.01	<0.01	35.78	<0.01	18.56	0.70
Chlorophyll a/b	11.46	<0.01	31.23	<0.01	13.07	0.49
O2•-production rate	11.53	<0.01	65.42	<0.01	5.67	0.77
MDA	22.15	<0.01	2.86	<0.01	97.14	0.54
SOD	14.45	<0.01	55.75	<0.01	0.65	0.97
APX	55.91	<0.01	2.64	<0.01	274.69	0.23

Malus royalty's net photosynthetic rate, stomatal conductance, and transpiration rate were all significantly affected by AMF inoculation ($P < 0.01$, *Table A4*). These characteristics were significantly impacted by soil Pb as well ($P < 0.01$). However, neither of these two variables significantly affected the intercellular CO_2 content of *Malus royalty*, nor did their interaction significantly affect the parameters governing photosynthetic gas exchange.

Table A4. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (AMF × Pb) on net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
Net photosynthetic rate	13.23	<0.01	23.76	<0.01	0.49	0.72
Stomatal conductance	9.32	<0.01	19.36	<0.01	0.44	0.64
Transpiration rate	21.34	<0.01	11.73	<0.01	2.88	0.34
Intercellular CO_2 concentration	8.12	0.07	13.23	0.02	1.34	0.32

AMF inoculation had a significant effect on F_o of *Malus royalty* ($P < 0.01$, *Table A5*), but not on F_m ($P > 0.05$). Soil Pb showed significant effect on F_o and F_m ($P < 0.001$). However, there was no significant interaction effect between these two factors on F_o and F_m.

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Table A5. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (AMF × Pb) on F_o and F_m*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
F_o	25.45	<0.01	45.34	<0.01	4.59	0.21
F_m	1.42	0.53	31.43	<0.01	4.12	0.87

It is shown in *Table A6* that the effect of AMF inoculation and soil Pb on V_J and V_K of *Malus royalty* was significant ($P < 0.01$), but their interaction effect on V_J and V_K was not significant. AMF inoculation and soil Pb had significant effect on F_v/F_m, Φ_{PSII}, *ETR*, *NPQ*, $\triangle I/I_o$, PI_{ABS} of *Malus royalty* ($P < 0.01$), which is shown in *Table A6*, but they had no significant interaction effect on these parameters.

Table A6. *Two-way ANOVAs examining the effects of inoculation AMF (+AMF), Pb content (Pb) and their interaction (AMF × Pb) on V_J, V_K, Φ_{PSII}, ETR, PI_{ABS}, NPQ, F_v/F_m, $\triangle I/I_o$*

	+AMF		**Pb**		**+AMF × Pb**	
	F	***P***	***F***	***P***	***F***	***P***
V_J	86.25	<0.01	83.96	<0.01	3.44	0.04
V_K	14.81	<0.01	14.95	<0.01	0.05	0.98
Φ_{PSII}	5.16	<0.01	127.73	<0.01	141.41	0.96
ETR	300.53	<0.01	74.56	<0.01	56.32	0.74
PI_{ABS}	45.05	<0.01	8.14	<0.01	86.88	0.54
NPQ	31.07	<0.01	12.25	<0.01	68.26	0.87
F_v/F_m	58.74	<0.01	35.14	<0.01	32.47	0.98
$\triangle I/I_o$	12.51	<0.01	35.33	<0.01	12.45	0.77

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