Introduction

2,4-DNP, as a new pollutant, is highly toxic and difficult to be biodegraded [1-5]. It is often used in explosives, pesticides, and other industries [5, 6], and those industries often discharge wastewater containing 2,4-DNP into the environment. It endangers plants, animals, microorganisms, and human health [4], so it is urgent to find an effective method to purify 2,4-DNP wastewater. Physical, chemical, and bioremediation methods have been used to degrade 2,4-DNP wastewater, but these methods are often costly [1-3, 5-7]. Phytoremediation is a kind of technology with great application prospects [8]. It is widely used in the remediation of polluted water and soil because of its strong remediation ability and low cost [9]. However, phytoremediation technology also has some disadvantages, such as a long time for plant growth and toxic stress to plant from pollutants. Therefore, it is necessary to add some exogenous substances to improve the tolerance of plants to pollutants in their growth cycle [8]. Selenium is an essential micronutrient for...
many organisms [10]. The regulation of Se level in an organism is of positive significance to its physiological activities. Deng et al. found that the concentration of selenium in crops could be effectively regulated by appropriately changing the Se source and time of selenium spraying on leaf surface [11]. Hasanuzzama et al. inferred that low concentration Se has a positive effect on crop growth and stress tolerance [12]. Cao et al. found that when plants are in an adverse environment, Se addition can improve the ability of plants to produce antioxidant substances [13]. Other studies have shown that Se can protect plants from heavy metal stress, salt stress, and so on [14-17]. Therefore, it may be used to improve the tolerance of plants to 2,4-DNP stress.

*Salix babylonica* is easy to reproduce and has strong adaptability and stress resistance [18]. It has great application value in the phytoremediation of polluted soil and water [19, 20]. At present, it is mainly used for the remediation of heavy metal and cyanide pollution [21, 22]. However, there is no report on the effect of *S. babylonica* used for remediation of 2,4-DNP in polluted water and the effect of Se addition on its tolerance to 2,4-DNP. In this study, the photosynthetic physiological response and enzyme activity of *S. babylonica* under different concentrations of 2,4-DNP solution stress and the effect of exogenous Se on the physiological response of *S. babylonica* to 2,4-DNP pollutants were studied through a water culture simulation experiment. This study aims to explore the tolerance of *S. babylonica* to 2,4-DNP pollution and whether the Se addition can improve its resistant ability to 2,4-DNP stress.

### Materials and Methods

#### Experimental Materials

In early March, 2019, branches were cut from *S. babylonica* trees in East Lake Park, Tai’an, Shandong, China. The branches were cut into 25 cm in length sections and then were placed into buckets filled with tap water. Those cuttings in buckets were cultured in a plastic greenhouse in the Forestry Experimental Station of Shandong Agricultural University (117°20’E, 36°12’N), Tai’an. When they had plentiful roots, each seedling was transplanted into one 500 mL conical beaker containing 400 mL half-strength Hoagland's hydroponic nutrient solution. Those conical beakers are covered by a black plastic film to deter algal growth.

#### Experimental Design

In the experiment, the healthy and similar growth status seedlings of *S. babylonica* were treated with Hoagland nutrient solution containing 2,4-DNP and Se. Se was spiked with Na2SeO3 as 3 concentrations: 0 (Se0), 1 (Se1) and 2 (Se2) μmol·L-1. 2,4-DNP were supplied at 4 levels: 0 (2,4-DNP0), 10 (2,4-DNP10), 15 (2,4-DNP15), and 20 (2,4-DNP20) μmol·L-1. There are 10 treatments in total. Those treatments are as follows: Se0+2,4-DNP0 (control), Se1+2,4-DNP10, Se2+2,4-DNP15, Se1+2,4-DNP15, Se1+2,4-DNP15, Se2+2,4-DNP15, Se2+2,4-DNP15, Se2+2,4-DNP15, Se2+2,4-DNP15. Each treatment has four replicates. After six days of stress, the physiological indexes were measured.

#### Determination of Photosynthetic Parameters

The photosynthetic parameters were measured by Li-6800 portable photosynthesis system (Li COR, USA). The net photosynthetic rate (Pn, μmol·m-2·s-1), transpiration rate (Tr, mmol·m-2·s-1), intercellular CO2 concentration (Ci, μmol·mol-1), and stomatal conductance (Gs, mmol·m-2·s-1) of mature leaves were measured in sunny weather. Three seedlings of each treatment were randomly selected for photosynthetic parameters determination and one upper mature leaf of each seedling was measured. Each leaf was measured three times. The CO2 concentration was 400±10 μmol·mol-1, the air temperature in the leaf chamber was set 25°C, the relative humidity within the chamber was at 40-45% and photosynthetic active radiation was set at 500 μmol·m-2·s-1.

#### Determination of Chlorophyll Content

The chlorophyll content of each plant was determined by the UV-1600 ultraviolet spectrophotometer (MAPADA). Each plant was weighed with 0.2 g fresh leaves. The chlorophyll-a concentration (Chla, mg·L-1) = 12.21×OD663-2.81×OD646, chlorophyll-b concentration (Chlb, mg·L-1) = 20.13×OD646-5.03×OD663 and total chlorophyll concentration (Chla+Chlb) (mg·L-1).

#### Determination of Enzyme Activity

The enzyme activity was determined according to the determination method of Wang et al. [23]. The absorbance changes of SOD and POD were measured by UV-VIS dual-beam ultraviolet spectrophotometer (SP-756P), and their activities were calculated.

#### Data Analysis

The data were processed and graphed by IBM SPSS Statistics 20.0 and Origin 2019. The difference among treatments was analyzed by one-way analysis of variance (ANOVA) with Duncan’s multiple-range test at P<0.05.

#### Results and Discussion

**Effects 2,4-DNP and Se on Photosynthetic Parameters of *S. babylonica***

Photosynthesis is an important basis for plants to carry out normal life activities [24]. The decrease
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Fig. 1. Photosynthetic parameters of *S. babylonica* under 2,4-DNP stress and Se addition. Data are means±standard errors. Bars followed by different letters show significant differences at *P*<0.05.

...of photosynthesis indicates that plant growth is inhibited by heavy metals or other factors [25-27]. In this experiment, the 2,4-DNP alone decreased the net photosynthetic rate (\(P_n\)), transpiration rate (\(T\)), and stomatal conductance (\(G_s\)) of *S. babylonica*, whereas increased its intercellular \(CO_2\) concentration (\(C_i\)) (Fig. 1). The \(P_n\), \(C_i\), and \(T\) of *S. babylonica* under different concentrations of 2,4-DNP stress showed significant differences (*P*<0.05) (Fig. 1(a-c)). Compared with only 2,4-DNP treatment, adding Se into 2,4-DNP solution increased \(P_n\) by 0.79%, 6.84% and 6.69%, and decreased \(C_i\) by 2.77%, 9.31% and 12.04% at 2,4-DNP \(10\), 2,4-DNP \(15\) and 2,4-DNP \(20\), respectively, of *S. babylonica*. Adding Se into 2,4-DNP solution increased \(P_n\) by 6.49%, 21.06% and 19.35%, and decreased \(C_i\) by 4.89%, 10.15% and 15.94% at 2,4-DNP \(10\), 2,4-DNP \(15\) and 2,4-DNP \(20\), respectively, of *S. babylonica*. After Se treatment, the \(P_n\) and \(T\) of *S. babylonica* increased, and the promotion effect of higher Se concentration was more significant than the lower Se concentration (Fig. 1(a-c)), which is similar to the study by Zhang et al. [31]. The melioration of photosynthesis in stressed plants by addition of Se may be attributed to the decreased ROS levels, reactivation of antioxidants, restored structure of the damaged chloroplasts [32].

Some studies have shown that when plants are stressed by environmental factors, there is usually a transformation process from stomatal factors to non-stomatal factors in the decline of photosynthesis [28-30]. In this experiment, with the increase of 2,4-DNP concentration, the \(P_n\) and \(G_s\) of *S. babylonica* decreased, while the \(C_i\) increased, indicating that the decline of photosynthesis was caused by non-stomatal factors. Moreover, it can be speculated from the experimental results that, when the concentration of 2,4-DNP is 0-10 mg·L\(^{-1}\), there should be a critical 2,4-DNP concentration that changes from stomatal factor to non-stomatal factor during the decline of photosynthesis of *S. babylonica*, and its value should be further studied. Although the promotion effect of a higher concentration of Se on plants is more significant in this experiment, it is not that the higher the concentration of Se, the better the promotion effect of plants. High concentration Se is toxic to plants [33], so it is necessary to select the appropriate concentration when adding Se.

Effect of 2,4-DNP and Se on Chlorophyll Content of *S. babylonica*

Chlorophyll is an important photosynthetic pigment in plants, and its content is related to photosynthesis [34, 35]. The chlorophyll content of *S. babylonica* decreased with the increase of 2,4-DNP concentration,
indicating that the chloroplast structure of *S. babylonica* was damaged by 2,4-DNP stress (Fig. 2). In the concentration range of 2,4-DNP used in this experiment, adding Se$_1$ and Se$_2$ all improved the chlorophyll content. When the concentration of 2,4-DNP was 10 mg·L$^{-1}$, the application of Se$_1$ and Se$_2$ increased the total amount of chlorophyll-a and chlorophyll-b by 11.3% and 15.82%, respectively. When the concentration of 2,4-DNP was 15 mg·L$^{-1}$, the application of Se$_1$ and Se$_2$ increased the total amount of chlorophyll-a and chlorophyll-b by 17.69% and 33.6%, respectively. When the concentration of 2,4-DNP was 20 mg·L$^{-1}$, the application of Se$_1$ and Se$_2$ increased the total amount of chlorophyll-a and chlorophyll-b by 65.54% and 66.09%, respectively. Under the stress of medium and low concentration of 2,4-DNP, adding Se$_2$ had a more significant effect on chlorophyll content of *S. babylonica* than adding Se$_1$, but the difference was not significant at higher concentration.

In this experiment, the chlorophyll content decreased with the increase of 2,4-DNP stress. It may be that 2,4-DNP reduced the chlorophyll precursor, resulting in the inhibition of chlorophyll synthesis and photosynthesis. Under the stress of a high concentration of 2,4-DNP, the leaves of *S. babylonica* became yellow, and the growth potential was weaker than that of a low concentration of 2,4-DNP, which was consistent with the decrease of chlorophyll content and similar to the result obtained by Ibrahim et al. [36]. Their research found that under the stress of salt, the cotton chlorophyll concentration decreased. Iqbal et al. [37] and Malik et al. [38] suggest that appropriate concentrations of exogenous selenium may increase chlorophyll content and protect chloroplast structures from oxidative damage. Other studies have found that applying a certain concentration of selenium can increase the content of chlorophyll in maize, spinach, sorghum, and so on [39, 40, 48]. In this experiment, the content of chlorophyll did increase after adding Se (Fig. 2). The reason may be that the damage of chloroplast membrane structure caused by 2,4-DNP can be repaired after increasing Se, thus increasing the chlorophyll content. It is also possible that the application of selenium reduces the oxidative damage caused by hydrogen peroxide and increases chlorophyll content [41].

**Effect of 2,4-DNP and Se on the Enzyme Activity of *S. babylonica***

Pollutant stress can increase the content of reactive oxygen species (ROS) in plants, and then trigger membrane lipid peroxidation, which forms oxidative stress on plants [42]. To prevent ROS damage, plants eliminate ROS through an enzyme antioxidant defense system to avoid oxidative stress damage [43]. SOD and POD are essential enzymes for scavenging ROS. The activity of SOD and POD can be used to infer the activity of the antioxidant system of enzymes.
in plants, and then reflect the protective ability of plants [44-46]. In the absence of Se, with the increase of 2,4-DNP concentration, the activity of POD showed an increasing trend firstly, reaching the maximum value at the concentration of 15 mg·L\(^{-1}\) and then decreasing (Fig. 3a). SOD also had the same trend, but reached the maximum value at the concentration of 10 mg·L\(^{-1}\) and then decreased (Fig. 3b). After the addition of Se\(_1\), the POD and SOD activities decreased by 16.85% and 15.34% at 10 mg·L\(^{-1}\), decreased by 28.88% and 12.1% at 15 mg·L\(^{-1}\), and increased by 2.48% and 20.76% at 20 mg·L\(^{-1}\), respectively. After adding Se\(_2\), the activities of POD and SOD decreased by 24.16% and 16.47% at 10 mg·L\(^{-1}\), 31.97% and 9.18% at 15 mg·L\(^{-1}\), and increased by 20.38% and 24.19% at 20 mg·L\(^{-1}\).

In this experiment, the activities of POD and SOD increased first and then decreased with the increase of 2,4-DNP concentration (Fig. 1). POD reached the highest when 2,4-DNP concentration was 15 mg·L\(^{-1}\), while SOD was the highest when 2,4-DNP concentration was 10 mg·L\(^{-1}\), which indicated that \(S.\) babylonica could effectively activate the enzyme antioxidant defense system at a low concentration of 2,4-DNP stress and reduce the damage of oxidative stress on itself. However, this enzyme defense system is not indestructible. Excessive concentration of pollutants will produce excessive H\(_2\)O\(_2\), which may lead to the reduction of plant cell wall elongation, and eventually terminate plant growth rapidly and damage the enzyme system [46]. After Se application, the activities of the two enzymes decreased at low 2,4-DNP concentration. This may be because Se quenched the ROS (O\(_2^•\)) will be produced, and SOD activity was activated [32].

**Conclusion**

2,4-DNP could inhibit the growth of \(S.\) babylonica, and the main reason for the reduction of photosynthesis was non-stomatal factors. \(S.\) babylonica could endured well 2,4-DNP in concentration (20 mg·L\(^{-1}\) and below) used in this study. The addition of low concentration Se (2 μmol·L\(^{-1}\) or below) could promote the photosynthesis of \(S.\) babylonica to a certain extent by repairing the chloroplast and membrane system and enhance the tolerance of \(S.\) babylonica to 2,4-DNP.

**Abbreviations**

\(T_\text{r}\), transpiration rate; \(G_s\), stomatal conductance; \(C_i\), intercellular CO\(_2\) concentration; \(P_n\), net photosynthetic rate; 2,4-DNP, 2,4-dinitrophenol; Se, selenium; \(S.\) babylonica, Salix babylonica.

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**Conflict of Interest**

The authors declare no conflict of interest.
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