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RELIEF EFFECTS OF SALICYLIC ACID AGAINST THE STRESS OF HEAVY METAL IN *FRITILLARIA HUPEHENSIS* HSIAO ET K.C.HSIA

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Abstract

It is well known that salicylic acid (SA) can help plants tolerate abiotic stresses. Nevertheless, the regulatory functions of SA in plants, such as the response of *Fritillaria hupehensis* Hsiao et K.C.Hsia to exogenous SA rational application under heavy metal stress, remain unknown. This study aimed to assess the relief effects of SA on the damage of *F. hupehensis* caused by heavy metal cadmium (Cd), as measured by physiological and biochemical characteristics. The results showed that bulb germination and seedling growth of *F. hupehensis* Hsiao et k. c. Hsia decreased under different Cd toxicity treatments. The radicle length and mitotic index also significantly decreased under Cd stress ($P < 0.05$), especially under high concentrations of Cd stress. The bulb germination and seedling growth increased slightly under medium concentration treatment compared to low concentration treatment. Cd toxicity treatment significantly reduced the contents of pigment, protein, and sugar in seedlings compared with the control. The contents of total antioxidants and malondialdehyde in Cd-poisoned seedlings increased significantly. The comprehensive treatment promoted the growth of bulbs and seedlings. As a result of Cd stress, SA application significantly increased the bulb germination rate, radicle length, mitotic index, pigment, protein, and sugar content. Lipid peroxidation and total antioxidants were decreased by comprehensive treatments compared with Cd toxicity treatment. This simple study remarkably broadened our understanding of the application and protection of SA in Cd stress.

Introduction

The research on heavy metal pollution in soil has great significance. Heavy metal contamination reduces the quality and yield of crops, endangering the food chain. Moreover, it destroys the balance of the ecosystem and causes serious harm to human beings (Chen *et al.* 1999). Human activities such as wastewater irrigation, fertilization, and animal manure are the main sources of heavy metals in soil (Jiao *et al.* 2012).

Cadmium (Cd) is one of the most harmful heavy metal pollutants to plants and human beings because it is usually applied together with phosphorus fertilizer and is mobile in nature (Pinto *et al.* 2004). When vegetables are grown with ammonium nitrogen and urea nitrogen fertilizers, the soil is polluted by Cd (Fan *et al.* 2017). Cd is still the most serious pollution factor in China, with carcinogenic, mutagenic, and teratogenic effects on human health (Niu *et al.* 2013). Although Cd is an unnecessary metal element in plant physiology, the absorption of Cd by the roots of plants and its transportation to aboveground are easy to complete.

Salicylic acid (SA) is an indispensable signaling molecule in plants and plays a crucial role in enhancing plant resistance to abiotic stress (Durner *et al.* 1997). Researchers are paying increasing attention to SA because it can induce plants to take protective measures under stress factors (Sakhabutdinova *et al.* 2003). SA is known to enhance the antioxidant defense ability of plants,

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thereby improving their resistance. This is primarily due to the activation of defense genes upon the application of exogenous SA (Eltayeb *et al.* 2006).

However, the role of plants under heavy metal stress is rarely studied, especially in the interaction between Cd and SA in rapeseed oil. Therefore, this study aimed to investigate the effects of SA treatment on heavy metal stress in *Fritillaria hupehensis* seedlings. The growth, pigment, protein, sugar, and lipid peroxidation of the seedlings were measured, and total antioxidants were used to assess plant resistance to stress.

Materials and Methods

Healthy bulbs of uniform size were selected, disinfected with 0.02% mercuric chloride solution, and then rinsed with distilled water five times. The bulbs were treated with Cd at concentrations of 75 (C1), 150 (C2), and 300 mg/kg (C3). The bulbs were soaked in 0.5 mm SA or Cd. Bulb germination (BG), radicle length (RL), and cytological changes were recorded. The bulbs were planted in pots full of soil. The soil was treated as described earlier. Three replicates were used. The plants were watered, if necessary. The bulbs were cultured in an incubator with a constant temperature of 15 ± 1°C, photoperiod of 16/8 h, and light flux density of 240 $\mu mol \cdot m^{-2}s^{-1}$. The height of 15-day-old seedlings was recorded, and the first fully expanded leaf was taken for biochemical analysis.

After 48 h of planting the *Fritillaria* bulbs, the root-tip cells were observed and fixed in Carnoy solution for 24 hrs, then transferred to 75% ethanol solution. The root tips were hydrolyzed in 1 mol/l HCl for 20 min at room temperature and then stained with 2% acetyl carmine solution for 1 h (Qian *et al.* 1998). Chromosome diffusion was prepared using extrusion technology according to the method of Savaskan and Toker (1991). The mitotic index (MI) from 500 cells was studied at different times. Chlorophyll and carotenoid were determined in fresh leaf samples. Leaf samples (10 mg) were detected according to the method of Lowry *et al.* (1951), and the protein content was calculated from the standard curve of the bovine serum albumin. The total soluble sugar was quantified according to the method of Hedge *et al.* (1962). The activity of nitrate reductase was determined using modified potassium nitrate (Qiu *et al.* 2008). Lipid peroxidation (LP) was measured according to the method of Heath *et al.* (1968), in which the content of malondialdehyde (MDA) in the leaves was measured. Meanwhile, the concentration of MDA was measured using an extinction coefficient of 155 mm^{-1}. The total antioxidant capacity of plant extracts was evaluated using the method of Prieto (Prieto et al., 1999).

SPSS 20 software, one-way analysis of variance (ANOVA), and multiway ANOVA were used to evaluate the significant difference ($P < 0.05$). The mean and standard deviation were calculated from three repeated data sets.

Results and Discussion

The effects of Cd and SA treatment on BG, MI, and RL are depicted in Table 1. Cd stress significantly decreased the BG, MI, and RL, and C3 had the greatest inhibitory effect. BG and RL decreased linearly. SA increased BG and RL by 4.06 and 86.21%, respectively. SA significantly increased BG and RL compared with the Cd treatment. MI was uniform under SA treatment, but Cd decreased it at its lowest and highest concentrations, and the best concentration was C_2. The combined treatment slightly increased MI compared to the Cd treatment alone.

Cd reduced the total chlorophyll content. Seedlings treated with SA exhibited the highest chlorophyll content. The C2 treatment showed the most favorable effect on chlorophyll content. The combined treatment of SA and Cd treatment resulted in an increase in total chlorophyll content, which exceeded the levels observed in both the control and SA treatment groups.

Different carotenoid patterns were recorded. SA reduced carotenoids (44.06%) compared with the control, almost the same as C1 and C3 treatment. C2 was the best concentration, which could make the carotenoid content reach the control level. Cd + SA treatment increased the carotenoid content more than SA, C1, and C3 treatments (Table 2).

Table 1. Effects of Cd and SA on seed germination, radicle length, and mitotic index of *F. hupehensis.*

Treatment	Bulb germination (%)	Radicle length (cm)	Mitotic index
Control	82.52 ± 0.01b	2.58 ± 0.01cd	21.14 ± 0.03a
SA	86.21 ± 0.03a	4.06 ± 0.03a	21.18 ± 0.03a
C_1	68.14 ± 1.04f	2.31 ± 0.01e	11.67 ± 0.61cd
C_2	69.63 ± 1.02ef	2.42 ± 0.11de	13.42 ± 1.21b
C_3	63.52 ± 0.12g	2.23 ± 0.02f	6.83 ± 0.02e
C_1 + SA	76.76 ± 0.03c	2.64 ± 0.05b	12.64 ± 0.86bc
C_2 + SA	73.23 ± 0.02d	2.61 ± 0.03bc	16.05 ± 1.61b
C_3 + SA	71.31 ± 0.01e	2.63 ± 0.02bc	7.87 ± 1.02de

Mean ± Standard Error values followed by the same letters did not differ significantly at $P < 0.05$ (ANOVA and Duncan's multiple range test), $n = 3$. Control (untreated); SA, 0.5mM; C1, C2, and C3 were 75, 150, and 300 mg/kg concentrations of Cd, respectively.

Table 2. Effects of Cd and SA on the content of leaf pigment of *F. hupehensis.*

Treatment	Chlorophyll a (mg/g Fresh weight)	Chlorophyll b (mg/g Fresh weight)	Total Chlorophyll (mg/g Fresh weight)	Carotenoid (mg/g Fresh weight)
Control	0.295 ± 0.018ab	0.079 ± 0.003ab	0.375 ± 0.013ab	0.084 ± 0.005a
SA	0.316 ± 0.017a	0.096 ± 0.023a	0.414 ± 0.005a	0.058 ± 0.004b
C_1	0.224 ± 0.013cd	0.038 ± 0.001de	0.256 ± 0.015ef	0.061 ± 0.004b
C_2	0.249 ± 0.015bc	0.038 ± 0.002cde	0.288 ± 0.018de	0.088 ± 0.004a
C_3	0.188 ± 0.011d	0.028 ± 0.001e	0.217 ± 0.011f	0.059 ± 0.001b
C_1 + SA	0.250 ± 0.017bc	0.070 ± 0.001ab	0.321 ± 0.0193cd	0.071 ± 0.002ab
C_2 + SA	0.291 ± 0.005e	0.062 ± 0.001bc	0.354 ± 0.079bc	0.085 ± 0.004a
C_3 + SA	0.287 ± 0.001ab	0.058 ± 0.002bcd	0.347 ± 0.004bc	0.083 ± 0.004a

Mean ± Standard Error values followed by the same letters did not differ significantly at $P < 0.05$ (ANOVA and Duncan's multiple range test), $n = 3$. Control (untreated); SA, 0.5mM; C1, C2, and C3 were 75, 150, and 300 mg/kg concentrations of Cd, respectively.

The contents of protein and sugar decreased gradually with the aggravation of Cd stress. The maximum inhibition rates of C3 treatment on the protein and sugar contents were 33.70% and 24.49%, respectively. SA increased the protein and sugar contents of Cd-treated seedlings. SA and the lowest concentration of Cd did not affect the seedling height. However, a high concentration of Cd reduced the seedling height, and the maximum inhibition rate was 26.57% under the C3 treatment. The seedling height of *F. hupehensis* under the combined effect of Cd + SA increased slightly compared with that of Cd treatment alone (Table 3).

The activity of seedlings decreased significantly under Cd stress ($P < 0.05$). This reduction showed a linear relationship, with the maximum reduction rate observed in C_3 reaching 81.88%. However, treatment with SA alone and in combination with Cd showed an increase in nitrate reductase activity, lipid peroxidation and total antioxidants had similar tendencies under the same treatment. The MDA content of seedlings treated with SA decreased slightly. However, Cd gradually increased the MDA content, and the maximum value of C_3 treatment increased by 214.37%. SA decreased LP and alleviated Cd stress when applied with Cd. SA treatment did not affect the TA content, but SA treatment after Cd stress significantly increased the TA content, with the C3 treatment causing the most significant increase in the TA content. SA cut down TA when Cd was applied in higher concentrations (Table 2).

Table 3. Effects of Cd and SA on the contents of protein, sugar, lipid peroxidation, and total antioxidants in nitrate reductase from leaves of *F. hupehensis.*

Treatment	Protein (mg/g FW)	Sugar (mg/g FW)	Seedling height (cm)	Nitrate reductase (μmol NO_2 g^{-1} FW h^{-1})	LP (n mol g^{-1}FW)	TA (Abs.)
Control	104.59 ± 0.29b	29.8 ± 0.27ab	19.94 ± 0.29a	14.24 ± 0.59b	26.58 ± 0.76f	0.42 ± 0.10e
SA	107.14 ± 0.19a	31.7 ± 0.36a	20.24 ± 0.13a	17.86 ± 0.48a	22.74 ± 0.09g	0.46 ± 0.02e
C_1	93.22 ± 1.35de	25.5 ± 1.02d	18.34 ± 0.65b	11.01 ± 0.30d	57.05 ± 0.67c	2.58 ± 0.17bc
C_2	84.61 ± 0.25e	24.2 ± 0.49e	15.64 ± 0.29cd	7.83 ± 0.07f	75.30 ± 1.15b	2.68 ± 0.15b
C_3	70.89 ± 0.97f	22.5 ± 0.45f	14.64 ± 0.19d	2.57 ± 0.24g	83.85 ± 0.26a	4.41 ± 0.33a
C_1 + SA	104.14 ± 0.24c	27.3 ±0.45ab	19.24 ± 0.13ab	12.08 ± 0.16c	31.52 ± 0.93e	2.44 ± 0.48bc
C_2 + SA	102.29 ± 1.83cd	26.6 ± 0.16b	16.99 ± 0.85c	9.40 ± 0.06e	33.02 ± 2.39de	1.88 ± 0.05cd
C_3 + SA	97.24 ± 0.88e	24.7 ± 0.68c	16.44 ± 0.01c	8.88 ± 0.10e	35.96 ± 1.01d	1.49 ± 0.03d

Mean ± Standard Error values followed by the same letters did not differ significantly at $P < 0.05$ (ANOVA and Duncan's multiple range test), $n = 3$. Control (untreated); SA, 0.5mM; C1, C2, and C3 were 75, 150, and 300 mg/kg concentrations of Cd, respectively.

Our results on BG, RL and seedling height showed that Cd toxicity inhibited the germination and growth of *F. hupehensis*. The decrease in BG, MI, and RL was significantly higher in the Cd treatment regulating seedling growth. SA could induce the change in BG and promote the growth of *F. hupehensis* seedlings. Salicylic acid ameliorated RL by increasing cell division, which was evident in MI. A study reported that SA promoted RL in rice (Choudhury *et al.* 2004). SA raised the seedling height compared with the control and combined treatment because it reduced the toxic effect of Cd. The results of this study were consistent with the report of Coronado (Coronada *et al.* 1998), who suggested that spraying SA aqueous solution can promote shoot and root growth under any conditions.

The delayed BG, seedling growth, and root damage caused by Cd poisoning were the manifestations of metabolic changes related to the reduction in plant food reserves. The absorption of minerals and water by roots increased with the increase in Cd concentration, and the contents of protein and sugar reduced gradually. This might be due to the damage of the photosynthetic mechanism caused by Cd stress. SA treatment increased the contents of protein, sugar, and pigment. Our results were consistent with those of Eltayeb *et al.* (2006).

Heavy metals increase MDA, indicating lipid peroxidation and membrane damage. MDA is produced by the breakdown of unsaturated fat (Lin and Kao 2000). Several scholars have reported the good and bad effects of Cd on antioxidant compounds (Abu-Ismaileh *et al.* 1978 and Rouchard

et al. 1983). SA decreased the LP value of *F. hupehensis*, balanced the total antioxidant products to the control level, and promoted the growth of *F. hupehensis*. It alleviated the toxicity of Cd by reducing the MDA content and total antioxidant activity. The combination of SA and Cd led to the adaptation of *F. hupehensis* seedlings because TA decreased in these treatments, but the growth was promoted compared with the Cd treatment alone. The results showed that SA treatment improved the performance of *F. hupehensis* and increased the adaptability when applied in combination (SA + Cd) compared with Cd treatment alone.

SA combined with Cd and SA alone could promote the growth of *F. hupehensis* compared with the control. The resistance ability of endogenous protective enzyme systems and antioxidant substance content in the plants were enhanced by the inducement of heavy metal stress in a restricted range, but they were diminished by the exorbitant concentration of heavy metals. The optimum concentration of exogenous SA could decrease the concentration of Cd in cells, weaken the damage of Cd to the chlorophyll, improve or reduce the activities of antioxidant enzymes, reduce the content of reactive oxygen species, and slow down the oxidation of plant cell membrane by active oxygen species and the damage of cell ultrastructure by Cd.

Therefore, the role of SA in protecting *F. hupehensis* from heavy metal stress was obvious.

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