Effect of Illite on Growth Characteristic and Antioxidant Enzyme System of Rice under Cadmium Stress

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ABSTRACT: Nine combinations of illite (0,5,10g·L^{-1}) and cadmium (0,2.5,5mg·L^{-1}) were set, and the hydroponics was adopted in this experiment to explore the effect of illite on the rice growth characteristics under Cd stress, including chlorophyll content, net photosynthetic rate, plant biomass, and the antioxidant enzyme (SOD, POD and CAT) activities. The results showed that: (1) without adding illite, under severe cadmium stress (5mg·L^{-1}), the activity of antioxidant enzymes (SOD, POD, CAT) increased, chlorophyll content, net photosynthetic rate and the biomass decreased significantly ($P<0.05$), but no significant effect on rice growth and antioxidant enzyme system was observed under mild cadmium stress (2.5mg·L^{-1}). (2) without cadmium stress, leaf chlorophyll content, net photosynthetic rate and seedling biomass showed no significant difference between the treatment of adding illite of 5mg·L^{-1} and 10mg·L^{-1}, implying that illite had no obvious influence on the growth of rice; (3) Under severe cadmium stress, adding illite obviously reduced the cadmium toxicity to chlorophyll, improved the photosynthetic rate and increased the biomass.

Introduction

Heavy metal cadmium can be accumulated by organism, enters the body through the food chain, and causes chronic poisoning, threatening human health and the environment security. Rice is one of the important crops, almost half the population in the world's main food resource. Over the past 20 years in China, the problem of excessive levels of heavy metals in rice is increasingly prominent. Department of Agriculture survey found that 6.06×10^5 hm^2 farmland was polluted, accounting for about 20% of the total area of investigation; Among the total area of polluted farmland, the heavy metal polluted area accounted about more than 80%, especially Pb^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+} and their compound pollution[1]. About 10% common commercially available rice on the market in China was cadmium polluted[2]. As its high mobility and high toxic, cadmium has been widely known as one of the most threatening toxic heavy metals[3]. High concentrations of Cd^{2+} induced excessive reactive oxygen species, damaged and interfered with the normal metabolism of cells[4].Plant can resist cadmium stress through various mechanisms, for example, antioxidant systems to clear reactive oxygen species [4,5]. SOD can effectively remove plants oxygen free radicals, POD can reduce excessive peroxides in plant and CAT can eliminate excess hydrogen peroxide(H_2O_2) in the cell and protect the structure of membrane[6,7].

Currently, lots of effect of cadmium stress on rice growth has been researched, and some clay minerals have been used in the removal of heavy metals in soil and wastewater. Illite is a kind of rich potassium of layered hydrosilicate clay mineral, due to its small particle size, larger specific surface area and colloidal properties, as a low-cost adsorbent, it has been applied to industrial wastewater, cyanobacteria bloom and other environmental fields[8].The heavy metals in wastewater are removed by illite through ion exchange[9]. The research on illite application in crop growth under heavy metal stress has little reported. This study adopted hydroponics experiment to research the effect of illite on the growth and antioxidant enzyme system of rice seedling under Cd stress, to explore if illite could relieve the cadmium stress in rice seedling stage.

Materials and Methods

Experimental Materials

The illite clay used in this study was purchased from Xintai non-metallic mineral co., LTD, Hebei, the rice variety was "Jinyuan 45" supported by Institute of Crop Sciences, Tianjin Academy of Agricultural Sciences. Ceramic cylinders (13 cm in diameter, 10 cm in height, volume of 1L) with seven uniform holes lid were used in this hydroponic pot experiment.

Experimental Design

The experiment was carried out in the key laboratory of Environmental Science Department, Tianjin Agricultural University. Rice seeds were disinfected with 75% ethanol for 5 minutes and then sterilized with 0.1% sodium hypochlorite solution for 10minutes. Seeds were germinated in plastic tray in constant temperature incubator with temperature of 35℃ and humidity of 90%, seedlings were raised for 20 days in vermiculite cultures then transplanted to hydroponic pots raised with rice hydroponic formula recommended by the International Rice Research Institute, with N, P and K concentration 40, 10 and 40 $mg·L^{-1}$, respectively. Nutrient solution pH was adjusted with NaOH to about 5.5 every five days after five hours aeration.

A completely randomized design was used, with 3 replications and 9 combined treatments of Cd(0,2.5,5$mg·L^{-1}$) and illite (0,5,10$g·L^{-1}$), consistent rice seedlings were selected to make up 27 pots, 6 plants per pot. The 9 treatments are noted as T1(Cd-0,illite-0),T2(Cd-0,illite-5),T3(Cd-0,illite-10),T4(Cd-2.5,illite-0),T5(Cd-2.5,illi-te-5), T6(Cd-2.5, illite-10),T7(Cd-5,illite-0), T8(Cd-5, illite-5), T9(Cd -5,illite-10). Chlorophyll content, photosynthetic rate and the activity of SOD, POD and CAT were measured on the14th day, 21stday, 28th day, 35th day, the biomass ($g·pot^{-1}$) were measured after the harvest.

Measurement Methods

Superoxide dismutase (SOD) activity was determined according to the method of nitroblue tetrazolium (NBT) light reduction, peroxidase (POD) activity was monitored according to the method of guaiaco, catalase (CAT) activity was assayed according to the method of ultraviolet spectrophotometry[10,11]. The whole plant rinsed with deionized water, dried in an oven at 105 ℃ for 30 minutes and 70 ℃ 48h, then weighed. The chlorophyll content was determined by SPAD-502 chlorophyll meter, net photosynthetic rate (Pn) was monitored by CI-340 photosynthetic apparatus.

Statistical Analysis

All data were processed using Microsoft Excel 2013, and each value of mean and S.D.(standard deviation) in the tables represents 3 replications of measurements. The assessment of effects of Cd stress, illite application and their interaction on rice seedling growth and some physiological indices were made using ANOVA and GLM procedures of SPSS16.0 software, followed by Duncan test for multiple sample comparison.

Results and Analyses

Effect of different Cd and illite treatments on activities of SOD,POD and CAT of rice leaf

The influence of different treatments of illite and Cd^{2+}on SOD, POD, CAT activity of rice leaf was shown in Table 1. The SOD activity in T7 was 1.55 times, 1.92 times, and 4.39 times higher than T1 on the 14th day, 21st day and 35th day, respectively, but no significant difference was found between T4 and T1, indicating that only severe Cd^{2+} stress (5$mg·L^{-1}$) had significant influence on SOD activity. No significant difference existed in SOD activity among treatments of T1, T2 and T3, implying that illite application had no significant effect on SOD activity. As shown in Table 1, there existed no significant differences in POD activity between T1 and T4, T4 and T7, among different illite treatments (T1,T2 and T3; T4, T5 and T6; T7, T8 and T9), indicating that the application of illite had little effect on POD activity of rice leaf under cadmium stress. CAT activity of rice leaf showed no obvious trend under different Cd and illite treatments.

Table 1 Effects of different treatment of cadmium and illite on SOD, POD, CAT activity of rice leaf

	SOD ($U \cdot mg^{-1}$ Fw)			POD ($U \cdot mg^{-1}$ $Fw \cdot min^{-1}$)			CAT ($U \cdot mg^{-1}$ $Fw \cdot min^{-1}$)		
	14 d	21 d	35 d	14 d	21 d	35 d	14 d	21 d	35 d
T1	0.38±0.08c	0.38±0.13c	0.23±0.02e	60.48±31.54b	58.60±14.43b	69.20±42.29ab	1.38±0.53abc	0.53±0.08c	0.91±0.14b
T2	0.31±0.14c	0.55±0.11abc	0.31±0.10de	79.87±21.67ab	96.86±46.97ab	120.17±45.38a	2.69±0.64a	2.16±1.48b	1.04±0.24b
T3	0.45±0.02bc	0.41±0.09bc	0.43±0.12cde	67.34±3.15ab	79.86±9.71ab	72.97±31.74ab	1.05±0.56bc	0.61±0.72c	1.05±0.15b
T4	0.50±0.13bc	0.51±0.06abc	0.52±0.08bcde	75.65±10.81ab	65.31±28.23ab	66.12±18.35ab	0.78±0.58c	1.34±0.61bc	1.23±0.16ab
T5	0.58±0.05a	0.63±0.02ab	0.76±0.23ab	94.5±11.24a	75.89±8.36a	65.12±12.67b	1.03±0.15bc	1.44±0.33bc	1.72±0.43a
T6	0.48±0.08bc	0.54±0.08abc	0.51±0.01bcde	74.29±6.95ab	64.53±21.51ab	75.35±13.69ab	1.72±1.26abc	0.57±0.28c	1.03±0.14b
T7	0.59±0.08a	0.73±0.23a	1.01±0.27a	96.96±14.19a	92.27±13.69a	82.7±29.99ab	2.48±1.43ab	5.44±1.15a	1.25±0.38ab
T8	0.47±0.08bc	0.69±0.1a	0.55±0.14bcd	83.16±19.53ab	91.57±23.14ab	69.85±20.97ab	1.80±0.71abc	1.52±0.62bc	1.21±0.08ab
T9	0.39±0.17bc	0.45±0.07bc	0.63±0.21bc	96.97±10.27a	66.04±13.42a	83.43±10.31ab	1.02±0.21bc	4.05±0.89a	1.64±0.48a

Note: Different small letters indicate significant differences between the treatments ($p < 0.05$).

Chlorophyl content and net photosynthetic rate of rice leaf under different treatments

Table 2 Effects of different treatment of cadmium and illite on chlorophyl content (SPAD) and net photosynthetic rate (Pn) of rice leaf

	SPAD			Pn		
	14^{th} day	21stday	35^{th} day	14^{th} day	21^{st}day	35^{th} day
T1	36.36±1.17ab	39.26±3.72a	42.68±0.62a	8.24±1.34a	10.61±0.69a	8.37±1.71a
T2	38.10±3.07a	37.94±1.95ab	40.96±0.83ab	8.07±0.44ab	9.98±1.17a	7.69±1.6ab
T3	36.66±0.07ab	38.0±2.09ab	39.54±1.85bc	8.16±0.88ab	10.88±1.48a	6.03±2.9abc
T4	36.89±2.83ab	37.36±0.88ab	36.98±0.81de	6.88±0.77bc	6.59±0.62bc	5.97±1.67bcd
T5	33.69±0.69bc	33.59±1.55bc	36.30±1.43e	5.0±0.26de	6.44±0.72bc	4.3±0.78cde
T6	36.19±0.85ab	35.88±2.95ab	38.59±0.72cd	6.12±1.02cd	7.72±0.96b	5.14±1.48bcde
T7	32.0±1.56c	30.23±4.78c	29.69±0.37f	4.69±0.68e	3.21±1.07d	2.67±0.55e
T8	34.36±0.29bc	35.30±1.33ab	36.47±1.06e	7.37±0.19abc	5.13±1.28c	4.10±1.04cde
T9	36.4±1.88ab	36.08±0.3ab	35.94±1.26e	7.00±0.09abc	6.28±1.48bc	3.09±0.22de

Cadmium stress can result in biomass decreasing trough the destruction of chloroplast structure and the decrease of leaf chlorophyll content [12]. For the rice leaf chlorophyll content (SPAD), T4 was 95%, 87% of T1 and T7 was 77%, 70% of T1 in 21^{st} day and 35^{th} day, for net photosynthetic rate (Pn), T4 was 83%, 62%, 78% of T1 and T7 was 57%, 30%, 35% of T1 on 14^{th} day, 21^{st} day, 35^{th} day, respectively (seen Table 2), implying cadmium stress had obvious inhibitory effect on rice leaf chlorophyll content and net photosynthetic rate. No significant difference in chlorophyll content and net photosynthetic rate of rice leaf among T1, T2 and T3, indicated that adding illite had little effect on rice growth without Cd stress, while, chlorophyll content of T6 was significantly higher than T5 on 35^{th} day under mild cadmium stress(T4,T5, T6), chlorophyll content and net photosynthetic rate in T9 was significantly higher than those of T7 under severe cadmium stress(T7, T8,T9), proving that illite could lessen the toxicity of cadmium on rice.

Effect of different Cd and illite treatment on biomass of rice leaf

Table 3 Effects of different treatment of cadmium and illite on rice biomass（g·pot^{-1}）

Cd (mg·L^{-1})	dosage of illite（g·L^{-1}）		
	0	5	10
0	4.30±0.92ab	4.32±0.92ab	4.55±0.07ab
2.5	2.98±0.07bc	3.22±0.48abc	4.76±0.77a
5	1.80±0.44c	3.56±0.38ab	4.14±1.27ab

Mild cadmium stress had no obvious effect on biomass than the control (Cd 0), while severe cadmium stress significantly inhibited the growth of rice. The application of illite had no significant effect on rice biomass under no cadmium stress, but the rice biomass of adding 5g·L^{-1} or 10g·L^{-1} illite were both significantly higher than that without illite under mild or severe Cd^{2+} stress, which implied that illite can alleviate the toxicity of cadmium stress to rice.

Discussion

When subjected to cadmium stress, rice plant can produce $O^{2-}\cdot$, H_2O_2 and other reactive oxygen matters that damage cell macro-molecules and plasma membrane, resulting in biomass declining, unbalanced antioxidant enzyme system, and the inhibition of plant growth, even plant death[13]. SOD, POD, CAT are all major enzymes in plant antioxidant protection system to relieve the accumulation of O^{2-} and H_2O_2 and reduce damage of adversity stress[14]. It is generally believed that cadmium stress can induce the expression of plant SOD, but this promotion and duration vary with treatment concentration, plant species and plant size[15]. POD activity of plant increased with the increase of Cd^{2+} concentration and the extension of processing time[16].

In this study, SOD, POD and CAT activity all increased with the increased concentration of cadmium, chlorophyll content, net photosynthetic rate and biomass all decreased with the decreasing concentration of cadmium. Mild cadmium stress had no significant influence on rice growth and antioxidant enzyme system and biomass, while, severe cadmium stress significantly improved the activity of SOD, POD and CAT in rice leaf, inhibited leaf chlorophyll content and net photosynthetic rate, resulting in the decrease of biomass. The application of illite showed no notable effect on SOD, POD, CAT activity of rice leaf under without or mild cadmium stress, while, under severe cadmium stress, the chlorophyll content and biomass was higher in the treatments with illite than without illite, suggesting the inhibitory effect of illite on cadmium. No significant difference in rice biomass was found between T5(Cd 2.5 mg·L^{-1}, illite 5 mg·L^{-1})and T6(Cd 2.5 mg·L^{-1}, illite 10 mg·L^{-1}), T8 (Cd 5 mg·L^{-1}, illite 5 mg·L^{-1})and T9(Cd 5mg·L^{-1}, illite 10 mg·L^{-1}), which may attributed to the exchangeable adsorption of illite for cadmium[17,18].

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