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Study of the influence of Ca^{2+} content in soil on *Brassica Chinensis* L. crop yield under acid rain stress conditions

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With the rapid development of economy, the frequency and intensity of acid rains is increasing, which is a growing global environmental concern, especially in China. In recent years, techniques to protect the crop yield under acid rain stress conditions have become a focus of attention. In this study, *Brassica Chinensis* L., one of the typical crops in the northeast of China, was selected to examine crop yield under acid rain stress conditions. Based upon two series of simulation experiments, (1) growth and physiological characteristics of *Brassica Chinensis* L. were determined under different acid rain stress conditions, and (2) it was examined whether supplementation with additional Ca^{2+} to soil was able to protect *Brassica Chinensis* L. under these conditions. The results showed that additional Ca^{2+} in soil improved growth and physiological characteristics of *Brassica Chinensis* L. through the increased total and pectic acid Ca^{2+} concentrations in plants. Acid rain stress decreased the yield of *Brassica Chinensis* L, while additional Ca^{2+} administered to soil enhanced the yield. Data suggest that adding Ca^{2+} to soil was effective in preventing the actions of acid rain on *Brassica Chinensis* L. growth.

Keywords: Acid rain; Ca^{2+}; *Brassica Chinensis* L.

INTRODUCTION

Acid rain is defined as acidic precipitation with pH below 5.6, in the form of rain, snow, fog, dew and others [1, 2]. Acid rain is a global pollution concern and a continuously growing problem. In recent years, the regions influenced by acid rain have spread from urban centers to outer suburbs and further to rural areas [3, 4]. In the early eighties of the last century, acid rain only appeared in several large cities in China. However, with rapid economic development, the surface area influenced by acid rain increased. At present, China is the major acid rain distributor globally with more than 40% of the region affected by acid rain, especially in the South and East of China. It is worthwhile noting that acid rain in some areas reached even pH levels of 4.5 [5-7]. Acid deposition not only directly affects the growth conditions of plants, but also influences plant growth as a consequence of losing some nutrient elements required for plant growth including calcium (Ca) and magnesium (Mg) in the soil [8]. Previous studies showed that the ecological loss attributed to acid rain reached 45.9 billion Yuan in Jiangsu, Zhejiang and other 11 provinces during the 1990s [9]. Northeast China has relatively good resources for agricultural development and agro-ecological environment, and it is an important commodity grain region. In recent years with the development of industry in Northeast China, annually rising acid rain levels have resulted in a significant negative impact on agricultural production and threaten food security in China [10]. Therefore, the study of the influence of acid rain on typical crops constitutes an important need.

Acid rain may influence crops in different ways such as damage during the seed germination period as evidenced by diminished germination rate of rice and wheat [11]. During the plant growth period, acid rain destroys the leaves of crops such as rice and soybeans by decreasing chlorophyll concentrations in leaves, disturbing cell membrane permeability, increasing impedance stomatal factors, and inhibiting plant photosynthesis [12-14]. When comparing the response of vegetables, grains and other crops to acid rain exposure, Feng (2000) found that vegetables were more sensitive to the effects of acid rain [15].

Ca^{2+} is not only an important and essential element for plants, but it is also needed to increase resilience and act as an important messenger to enable plants to adapt to different environmental changes. In order to ensure the yields of plants, several investigators provided additional Ca^{2+} to soil and thus enhanced plant resistance under acid rain stress [16]. Qiu *et al.* (2002) examining longan found that Ca^{2+} reduced chlorophyll degradation in leaves under acid rain stress, and thus the yield was maintained [17]. Dolatabadian *et al.* (2013) showed that chlorophyll concentrations in wheat leaves were elevated by directly spraying Ca^{2+} on leaves under acid rain stress [18]. Evidence thus indicates that addition of Ca^{2+} reduces the influence of acid rain stress in some plants [19]. However, few investigators examined the effects of acid rain stress on *Brassica Chinensis* L., which is one of the main crops in northeast China. With the increasing

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intensity and frequency of acid rain occurrences in northeast China, adverse effects of acid rain on *Brassica Chinensis* L. might rise significantly. In order to maintain *Brassica Chinensis* L. yields, which is essential for food security in China, it is important to diminish the consequences of acid rain. Therefore, the aim of this study was to examine the effects of different Ca^{2+} concentrations on the physiological and nutritional quality changes of *Brassica Chinensis* L. under acid rain stress.

EXPERIMENTAL

Experimental material

The experimental site was a greenhouse in the region of Jilin University, Changchun, Jilin, which is located in the Southern district (43°49'21 "N, 125°16'47" E). Changchun, located in the hinterland of Songliao plain in the Northeast of China, has temperate continental monsoon climate. The annual mean temperature in Changchun is 4.8°C, and average annual precipitation is between 522-615 mm. The greenhouse temperature and other conditions were kept constant and maintained without natural external factors such as wind and precipitation occurring during the experiment. The crop used in this experiment was *Brassica Chinensis* L., which is an important crop sensitive to acid rain stress. The variety was Qingza *Brassica Chinensis* L. hybrids, with thick and hypertrophied leaves growing commonly in northeast China. The experimental soil was black soil which is widely distributed in northeast China [20]. The pH of the experimental soil was 6.5, organic matter content was 23.4 g/kg, available nitrogen content 108.4 mg/kg, available phosphorus 15.3 mg/kg, and potassium 160 mg/kg.

Treatments

The experiment consisted of two factors to be measured, (1) pH and (2) Ca^{2+} concentration of soil. A square wooden box with an approximate area of $0.2m^2$ in each planting area was used with plastic sheeting on the bottom to prevent water loss. A barrier of 0.3 m was placed between the plant districts to avoid interaction. The first series was run during the summer of 2014. The pH was set at two levels, 2.5 or 5.6, Ca^{2+} was added to soil. The total Ca^{2+} concentrations in soil were 240, 1260, 2520, 6300 or 12,600 mg/kg. The 240 mg/kg Ca^{2+} level was considered as soil background concentration [21]. The first series of experiments focused on the influence of different acid rain strength (pH levels) and interaction with different Ca^{2+} concentrations on *Brassica Chinensis* L. growth. The second series of experiments was conducted in the spring of 2015. The second experiment was set at pH 2.5, 4.5, or 6.89 (CK group) with Ca^{2+} added to soil at concentrations of 110, 440, 770, or 1100 mg/kg and the physiological indices of *Brassica Chinensis* L. were measured. Six indicators were measured (plant height, root length, root fresh weight, root dry weight, fresh weight of eating parts and dry weight of eating parts) which reflect the *Brassica Chinensis* L. growth characteristics.

The amount of solution required for different plots based on soil dry weight was calculated before the experiment and different concentrations of Ca^{2+} solution were added. Before seedling stage, secondary deionized water allocation simulated acid rain was added containing 0.660 mg/L K_2SO_4, 0.988 mg/L $NaSO_4$, 2.613 mg/L $CaCl_2$, 0.772 mg/L NH_4NO_3 and 1.442 mg/L $(NH_4)_2SO_4$. Acid solutions were prepared at a mole ratio of sulfuric acid and nitric acid $[SO_4^{2-}]/[NO_3^-]$ of 5:1, mixed and adjusted to pH 1. Different amounts of acid solution were added to the simulated acid rain, and desired acidity of the latter was determined with a pH meter. The simulated natural rainfall with no acid solution served as a background. During the seedling stage considered as growth of 6 true leaves, 500 ml of simulated acid rain was sprayed at different concentrations on the leaves of *Brassica Chinensis* L. until water dripped from the leaves at 16:00-17:00 every two days. The spraying was maintained uniform and the intensity of the spray was consistent.

Plant harvesting

Samples were collected at 9:00 on the 7^{th}, 14^{th}, 21^{st} day, by randomly selecting three leaves of *Brassica Chinensis* L. from each group. Different forms of Ca^{2+} concentrations in *Brassica Chinensis* L. were measured according to Ohat *et al.* [22]. The content of soluble sugar was measured by the anthrone method [23]. The chlorophyll meter (SPAD-502 PLUS) was used to measure the chlorophyll content in the leaves [24]. The dry plant weight was obtained after drying in an oven at 105°C. Plant height, petiole length and root length were measured directly by a ruler, 5-10 points with different length were selected for each measurement and the mean values were recorded. In order to more accurately analyze the effect of acid rain and Ca^{2+} on the growth of *Brassica Chinensis* L., three different measuring dates were selected at various growth stages, the results reflect the characteristics during the whole growing process.

Statistical analysis

In this study, Ca^{2+} concentrations in the soil and degree of acid rain stress (pH) were regarded as two factors. All data were analyzed by a two-way analysis of variance (two-way ANOVA: different soil Ca^{2+} concentrations and degree of acid rain stress were regarded as two factors) using SPSS 20.0.

RESULTS AND DISCUSSION

Ca^{2+} concentrations

With the increasing soil Ca^{2+} concentration, the contents of different kinds of Ca^{2+} in *Brassica Chinensis* L. obviously changed, and the results are shown in Figure 1. In the control group (pH=5.6), the contents of different kinds of Ca^{2+} in *Brassica Chinensis* L. increased under low soil Ca^{2+} concentration, then decreased, and finally became stable. When the concentration of Ca^{2+} in the soil was 1260 mg /kg, the contents of most kinds of Ca^{2+} in the plants were higher than those growing with other soil Ca^{2+} concentrations. Especially the concentration of total Ca^{2+} was 49.1mg/kg, which is the highest Ca^{2+} concentration in this experimental group. The concentration of Ca^{2+} in the plants did not gradually increase with the increasing soil Ca^{2+} concentration when the pH of acid rain was 5.6. *Brassica Chinensis* L. absorbed most Ca^{2+} at soil Ca^{2+} concentration of 1260 mg /kg. When the soil Ca^{2+} concentration was higher than 1260 mg/kg, the ability to absorb the Ca^{2+} from the soil may be suppressed, which caused a decrease in the concentration of Ca^{2+} when the soil Ca^{2+} concentration was higher than 1260 mg /kg.

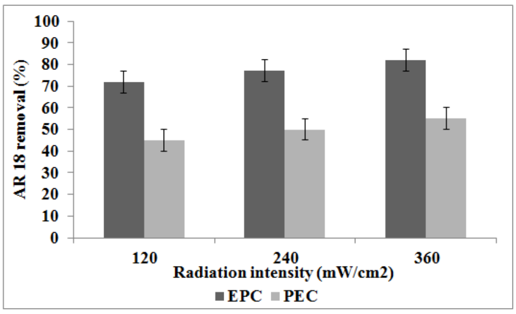

Fig. 1. Influence of Ca^{2+}concentrations treatment on Ca^{2+} concentrations in the *Brassica Chinensis* L. under acid rain stress

Soluble sugar and chlorophyll

Chlorophyll and soluble sugar of *Brassica Chinensis* L. under different pH and soil Ca^{2+} concentrations are illustrated in Figure 2. Compared to the plant grown under normal rain (pH = 5.6), the soluble sugar concentration was considerably higher than that in plants grown under acid rain stress (pH=2.5). With an increase in soil Ca^{2+}concentration, the levels of soluble sugar rose. When the soil Ca^{2+} concentration was 6300 mg/kg, soluble sugar content was 3.75 mg/kg, the highest value attained. Under different degrees of acid rain stress, chlorophyll concentration increased with increase in soil soluble calcium, and did not maintain a stable concentration in the range of soil soluble Ca^{2+} concentration range designed in this study. In the control group, the chlorophyll concentration of the plant which was not influenced by acid rain increased and tended to stabilize when the soil soluble calcium increased. The chlorophyll concentration of *Brassica Chinensis* L. influenced by light acid rain stress (pH=4.5) was higher than that treated with heavy acid rain stress (pH=2.5).

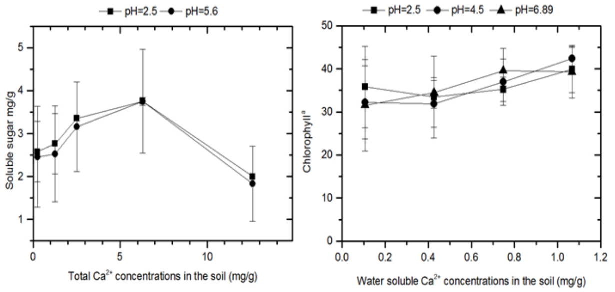

Fig. 2. Influence of Ca^{2+} concentrations treatment on soluble sugar and chlorophyll of *Brassica Chinensis* L. under acid rain stress

Growth characteristics of Brassica Chinensis L.

Six growth characteristics were determined and the results are presented in Figure 3. Data show that high concentrations of soil Ca^{2+} significantly increased the height, root fresh weight and root dry weight in a concentration-dependent manner. With the increasing soil Ca^{2+} concentration, the trends of fresh weight and dry weight of root were similar. When *Brassica Chinensis* L. grew without acid rain stress, root dry weight and fresh weight increased with increasing soil Ca^{2+} concentration (from 0.44 to 1.1 g/kg). The fresh weight and dry weight of roots in the control group were highest under the same soil Ca^{2+} concentration. Acid rain stress did not markedly affect plant height. In contrast to plant height, the root lengths were predominantly influenced by pH. In the control group without acid rain, the root lengths increased under low soil Ca^{2+} conditions. However, with the soil Ca^{2+} concentration increasing from 0.44 to 1.1 g/kg, the root lengths manifested a clear decreasing trend from 8.93±0.55 cm to 7.71±0.56 cm and increased to 8.44±0.39 cm when the soil Ca^{2+} concentration was higher than 0.75 g/kg. When the *Brassica Chinensis* L. grew under acid rain stress, the root length gradually increased after the first decrease (from 0.11 to 0.44 g/kg) with the concentration of soil Ca^{2+} increasing from 0.44 to 1.1 g/kg. With the soil Ca^{2+} concentration increasing, the root length change was not significant and the influence of Ca^{2+} concentration was lower than other degrees (pH = 4.5, 6.89) of acid rain stress.

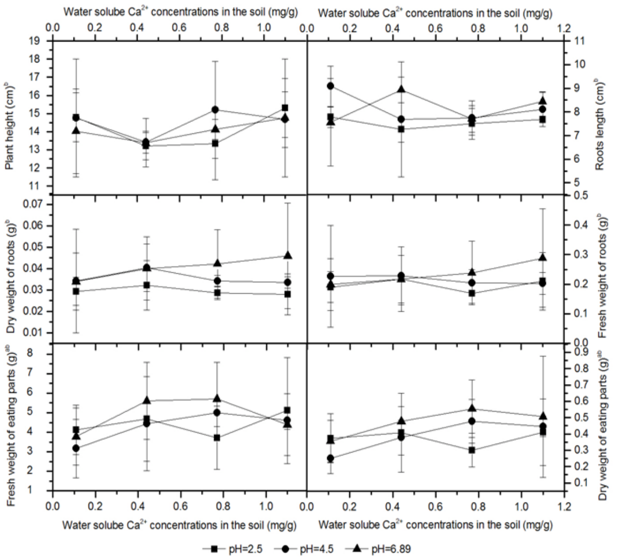

Fig. 3. Influence of Ca^{2+} concentrations treatment on the growth character of *Brassica Chinensis* L. under acid rain stress

Effects of different kinds of Ca^{2+} concentrations

When *Brassica Chinensis* L. grew under acid rain stress, the trend of different kinds of Ca^{2+} concentrations in the plant was different from those growing in a normal (pH=5.6) environment. When the pH of acid rain was 2.5, with the increasing soil Ca^{2+} concentration, the trend of the Ca^{2+} concentrations in the plants could be grouped in two kinds of trends. The trend of inorganic Ca^{2+} and soluble Ca^{2+} concentrations increased with the increasing soil Ca^{2+} concentration while the concentration of other kinds of Ca^{2+} increased after a decreasing trend and finally stabilized. For example, the total Ca^{2+} concentration in the plants was 30200 ± 5100 mg/kg when the soil Ca^{2+} concentration was 240 mg/kg, while, when the concentration of Ca^{2+} in the soil as 1260 mg/kg, the concentration of Ca^{2+} in the plant was the lowest at a value of 26500 ± 2020 mg/kg. When *Brassica Chinensis* L. grew under acid rain stress, the concentration of total Ca^{2+} in the plants was not always increasing with the increasing concentration of Ca^{2+} in the soil. After a short decreasing trend, it could continuously increase when the soil Ca^{2+} concentration was higher than 1260 mg/kg. The trends of pectin acid calcium, tricalcium phosphate and residual calcium concentrations in the plants were similar. Based on the results of the concentrations of different kinds of Ca^{2+}, we found that the acid rain stress could influence the process of plant absorbing Ca^{2+} from the soil, and it is more obvious for pectin acid calcium and tricalcium phosphate and residual calcium when the soil Ca^{2+} concentration was low. The decrease in these three kinds of Ca^{2+} concentrations leads the total Ca^{2+} concentration in the plants to decrease.

Comparing the contents of different kinds of Ca^{2+}in these two groups, the same soil Ca^{2+}concentrations, the content of Ca^{2+} in the plants was higher than in those growing under serious acid rain stress. When the soil Ca^{2+} concentration was low, the decreasing trend of Ca^{2+} concentrations in the plant was more obvious, which means that the ability of the plant to absorb Ca^{2+} from the soil could be inhibited by the acid rain. When the acid rain stress was strong, the ability to absorb Ca^{2+} might be weakened while with the increase in soil Ca^{2+}concentration, the degree of influence by acid rain on the Ca^{2+} absorption ability of *Brassica Chinensis* L. could be decreased, and the the plant could absorb more Ca^{2+}. When the Ca^{2+} concentration in the soil was high enough, the Ca^{2+} in the plant could be similar to those growing without acid rain.

Effects of soluble sugar and chlorophyll

Chlorophyll and soluble sugar are two important indicators for researchers to know the plant growth condition and are widely used in previous studies [25]. According to Fig. 2, the soluble sugar concentrations in the plant changed significantly when soil Ca^{2+} is different and the pH of acid rain is similar, it is not obvious in turn, so the soluble sugar concentrations in the plant were mainly controlled by soil Ca^{2+} concentration. Some previous studies have focused on the relationship of soluble sugar concentrations of *Brassica Chinensis* L. and acid rain without changing the soil Ca^{2+} concentration [26]. The results showed that the weak acid rain could increase the synthesis rates of soluble sugar of *Brassica Chinensis* L.

Soluble sugar synthesis rate at pH 2.5 was lower than at pH 4.5, and soluble sugar synthesis rates at pH 4.5 was lower than CK. Based on our experimental results, when the soil Ca^{2+} concentration and acid rain were regarded as two control factors, the influence of the acid rain stress on soluble sugar concentration of *Brassica Chinensis* L. was not obvious, and the soluble sugar concentration of the plant was mainly influenced by the soil Ca^{2+}concentration.

Comparing chlorophyll concentration of plants with different degrees of acid rain stress and soil soluble Ca^{2+} concentrations, high concentrations of soil Ca^{2+} benefit the chlorophyll synthesis of the plant. And with the soil Ca^{2+} concentration increasing, chlorophyll concentration could reach a stable concentration at different degrees of acid rain stress. When *Brassica Chinensis* L. grew under acid rain stress, increased concentration of soil Ca^{2+} could promote the synthesis of chlorophyll and keep the chlorophyll concentration in the plant stable and normal. Although no stable chlorophyll concentration of the plants was reached in this experiment, it was found that high concentration of Ca^{2+} treatment could increase chlorophyll concentrations of *Brassica Chinensis* L. to levels higher than those not influenced by acid rain. These results might mean that the light acid rain stress could increase the chlorophyll concentration of *Brassica Chinensis* L., while with increasing acid rain stress the chlorophyll concentration of the plant could be suppressed. Acid rain could decrease the chlorophyll synthesis rate of *Brassica Chinensis* L. and decrease photosynthetic capacity, which is similar to previous studies through other kinds of plants. High concentrations of Ca^{2+} in the soil could promote the synthesis of chlorophyll, and make the chlorophyll concentrations of plants influenced by acid rain similar with those growing in a normal environment. In previous studies, high concentrations of Ca^{2+} could increase the seeds germination rates of *Medicago sativa* under acid rain stress, and decrease the effect of acid rain stress on the photosynthesis of *Zephyranthes candida* and longan [27]. In our study, high Ca^{2+} concentration also could decrease the effect of acid rain on *Brassica Chinensis* L. and protect the photosynthesis by keeping the chlorophyll concentration, which is similar to previous studies on other kinds of plants.

Effects of growth characteristics

In order to understand the influence of acid rain and soil Ca^{2+} concentration on the growth characteristics, six kinds of indicators (plant height, root length, root fresh weight, root dry weight, fresh weight of eating parts and dry weight of eating parts) were determined, shown in Fig. 3.

With the acid rain stress increasing, the dry weight and fresh weight of roots were both decreasing. When *Brassica Chinensis* L. grew under acid rain stress, with the soil Ca^{2+} concentrations increasing, the dry weight of roots decreased after a short increase trend. High concentration of soil Ca^{2+} could promote the growth of roots when the plant grew without acid rain stress. High concentration of soil Ca^{2+} and strong acid rain stress could inhibit the growth of the roots and reduce the root dry weight. When *Brassica Chinensis* L. grew without influence by acid rain, the food fresh weight and dry weight decreased after a short peak with soil Ca^{2+} concentration increasing. It can be inferred that the fresh weight could grow well under the suitable soil Ca^{2+} concentration. When it grew under light acid rain stress, this suitable soil Ca^{2+}concentration did not change significantly.

Interaction of acid rain and Ca^{2+} concentration

Based on the two-way ANOVA analysis, the Ca^{2+} concentration in the soil and the degree of acid rain stress were regarded as two factors in the *Brassica Chinensis* L. change while there was no significant influence on the physiological characteristics. Except the height, acid rain stress caused significant differences on the root length, eat fresh weight, dry weight of edible part, root fresh weight and root dry weight of the plant. The analysis results showed that the influence of the interaction between different soil Ca^{2+} concentrations and acid rain stress on the *Brassica Chinensis* L. was not significant. So we can infer that the impacts of Ca^{2+} concentration and acid rain stress on this plant are independent.

For example, when the degree of acid rain increased, the weight of the edible parts of *Brassica Chinensis* L. decreased, while increasing soil Ca^{2+} concentrations could eliminate this influence gradually, and make the yield of *Brassica Chinensis* L. not decrease significantly. So, this reason could explain why the interaction of Ca^{2+} concentration and acid rain on *Brassica Chinensis* L. is not significant.

Two-way ANOVA for growth character, soluble sugar and chlorophyll of Brassica Chinensis L. under acid rain stress and Ca^{2+} stress are shown in Table 1.

CONCLUSIONS

Based on all experimental results, it can be inferred that pH and soil Ca^{2+} concentrations influence the physiological characteristics of *Brassica Chinensis* L. Acid rain could influence the key indicators like edible parts weight, root length of plants while, when the soil Ca^{2+} concentration increased, it could reduce the influence caused by acid rain, and keep the yields of the plant, so we can adjust *Brassica Chinensis* L. cultivation methods in the acid rain region according to this study results. For example, an appropriate increase in soil Ca^{2+} concentration could reduce the influence of acid rain stress on the yield in the regions significantly influenced by acid rain.

Table. 1. Two-way ANOVA for growth character, soluble sugar and chlorophyll of *Brassica Chinensis L.* under acid rain stress and Ca^{2+} stress

	Calcium concentration		pH		Calcium concentration & pH	
	F	P	F	P	F	P
Total calcium	11.861	.000	16.452	.001	23.214	.000
Total calcium	6.433	.002	36.361	.000	16.948	.000
Inorganic calcium	7.390	.001	61.842	.000	4.494	.009
Water soluble calcium	4.983	.006	108.329	.000	4.459	.010
Pectic acid calcium	1.699	.190	16.428	.001	3.662	.021
Tricalcium phosphate	1.836	.162	5.435	.030	2.226	.103
Calcium residue	11.861	.000	16.452	.001	23.214	.000
Plant height	.621	.609	3.185	.062	.219	.966
Roots length	.864	.475	3.507	.049	.686	.663
Fresh weight of eating parts	9.906	.000	14.162	.000	.687	.662
Dry weight of eating parts	3.911	.023	3.669	.043	.473	.820

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