Effects of Pb Pollution on Seed Vigor of Three Rice Cultivars

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Abstract: An experiment was conducted to study the effects of different Pb concentrations on rice seed germination and seedling growth in three cultivars (Jiayu 293, Jiayu 948 and Xieyou 963). The method of qualitative traits combining with quantitative traits by orthogonal polynomials was applied to analyze rice seed vigor by regression model. The seeds from different rice cultivars had different sensitivity to Pb poison. In the Pb concentration of 100-700 mg/L, Pb significantly decreased root length and shoot height, and the degree of the restraint would aggravate with the increasing of Pb concentration. However, Pb did not reduce root dry weight and shoot dry weight, which indicated that different physiological characteristics had different sensitive reactions to Pb. The predicting equations of regression were developed, which could be used to predict the effects of different Pb concentrations on rice seed vigor.

Key words: rice; lead; seed vigor; pollution; regression analysis

With the improvement of industrialization and the development of science and technology, heavy metals from different sources are accumulated in the soil gradually, resulting in environment contamination, and serious damage to seed germination and seedling growth of various crops. It is reported that industrial waste gas, waste water and solid waste polluted nearly 6.7 million ha of farmland, and the area of polluted farmland at various degrees in China had already reached 10 million ha in 1992 [1]. At present, nearly 20 million ha of arable area (accounting for 1/5 of total arable areas) in China is contaminated by the heavy metals, causing a loss of 12 billion kg grain every year. Heavy metals such as cadmium, mercury, lead, arsenic, chromium, etc., are highly noxious to human, animals and plants, also have already become one of the most serious pollutants of farmland in some areas, of which Pb is becoming a main pollutant of environment, and has a serious threat to human, especially to children.

The research about the influence of heavy metals on plant growth has been reported[2, 3], however less information is available on the effects of Pb pollution on rice seed germination and seedling growth. The objective of this study is to investigate the influence of Pb on rice seed germination and seedling growth by simulating Pb contamination. Similar research in the past mostly adopted general test or factorial design method [4, 5] which can not be used to predict the effect. But regression analysis method can achieve this purpose [6] and be used to estimate the dynamic changes of the influence of the independent variable on dependent variable [7]. The independent variables used in regression analysis usually are quantitative variable; sometimes, qualitative variable such as crop cultivar can be used in regression analysis [8]. However, qualitative variable needs to be transformed to indicator variable (namely 0, 1 or -1) before analysis[9]. In this paper, qualitative trait combined with quantitative trait was used to analyze the relation between Pb and rice seed vigor by regression model. The example of regression analysis by combining qualitative trait and quantitative trait was supplied in this paper.

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MATERIALS AND METHODS

Materials

Seeds from three rice cultivars, Jiayu 293 (indica), Jiayu 948 (indica) and Xieyou 963 (indica/hybrid rice) were used.

Methods

Rice seeds were soaked in water at a constant temperature of 25℃ for 24 h, and then sown in a plastic germination box with sands containing 0 (control), 100, 300, 500 and 700 mg/L of Pb, respectively. Fifty seeds were sown in each box and were incubated for 14 days at 30℃ with a 12-h light period. The test was ran with three replications. Seven days after germination, the covers of germination boxes were removed, then 15 mL solution with corresponding Pb concentration was added to each germination box every day. The number of germinated seeds (visible radicle protrusion) was recorded daily. Root length, shoot length, dry weight of root and shoot (weighed after drying at 80℃ for 72 h) were measured after growing for 14 days. Germination percentage after growth for seven days was counted. Germination index [GI=$\Sigma(Gt/Tt)$, where Gt is the number of the germinated seeds in the t day, Tt is the time corresponding to Gt in days] and vigor index (VI= GI × seedling height) were calculated [10,11].

Statistical analysis

Data analysis was made with SAS. Data of percentage were transformed according to y= arcsin [sqr (x/100)]. Two-way factorial design analysis was conducted, and the predicted formula of the relation between rice seed vigor and Pb concentration was constructed by the orthogonal polynomials regression analysis based on the combination of qualitative traits and quantitative traits.

RESULTS

Effects of different Pb concentrations on rice seed germination

The results of two-way factorial design analysis showed that when the Pb concentration was lower than 500 mg/L, Pb pollution had no significant effect on seed germination percentage and germination index of the three rice cultivars (Jiayu 293, Jiayu 948 and Xieyou 963). Pb (700 mg/L) significantly restrained germination percentage and germination index in Jiayu 293 and Jiayu 948.

The harmful degree of root length and shoot length of rice seedling would increase with the increasing of Pb concentration. The most serious damage was observed at the concentration of 700 mg/L (Table 1). The harmful degrees in root length and shoot length in Jiayu 293 and Jiayu 948 were severer than those of Xieyou 963. There was no significant difference in root dry weight and shoot dry weight of the three rice cultivars at 100–700 mg/L of Pb concentrations, and no significant difference between all the treatments and the control except at 700 mg/L of Pb concentration in Jiayu 293.

Table 1. Effects of different Pb concentration on rice seed germination and growth.

Cultivar	Pb^{2+} (mg/L)	Germination percentage (%)	Germination index	Root length (cm)	Root dry weight (mg)	Shoot length (cm)	Shoot dry weight (mg)
Jiayu 293	CK	100.0 a	16.6 a	13.1 a	2.6 a	12.8 a	5.7 ab
	100	98.7 ab	15.8 ab	11.6 b	2.6 a	12.0 a	5.9 ab
	300	98.7 ab	16.2 ab	11.1 bc	2.3 a	11.8 ab	5.5 a
	500	99.3 a	16.3 a	11.3 b	2.5 a	11.0 b	5.7 ab
	700	96.0 b	15.5 b	10.2 c	2.6 a	9.6 c	5.3 b
Jiayu 948	CK	98.0 a	16.2 a	11.3 a	2.4 a	12.1 a	5.1 a
	100	96.7 ab	15.9 a	10.3 b	2.3 a	10.9 b	5.2 a
	300	96.0 ab	15.7 a	10.8 ab	2.3 a	10.7 b	5.0 a
	500	96.7 ab	15.3 a	11.4 a	2.2 a	10.6 b	5.1 a
	700	93.3 b	14.0 b	10.1 c	2.4 a	9.6 c	5.1 a
Xieyou 963	CK	92.7 a	15.1 a	11.8 a	2.6 a	13.1 a	5.6 a
	100	94.7 a	15.5 a	11.1 ab	2.8 a	13.1 a	6.0 a
	300	95.3 a	15.4 a	11.3 a	2.5 ab	12.6 a	5.8 a
	500	95.3 a	15.3 a	10.6 b	2.8 a	11.1 b	5.7 a
	700	93.3 a	15.4 a	10.5 b	2.8 a	11.1 b	5.9 a

Within a column, data followed by different letters for the same cultivar mean significant difference at 0.05 level (LSD).

Effects of different Pb concentrations on seed vigor based on qualitative traits combining with quantitative traits

Orthogonal polynomial regression analysis based on the qualitative traits combining quantitative traits

Using the method with combination of qualitative traits and quantitative traits, the regression relation between the seed vigor of the three rice cultivars and Pb concentrations was showed by orthogonal polynomial regression analysis.

Because rice cultivar was qualitative trait in the experiment, two variables X_1 and X_2 could be achieved by transferring the rice cultivar to indicator variable (Table 2). The Pb concentration in this experiment had four levels and each with an interval of 200 mg/L, so it was used as quantitative trait for orthogonal polynomials regression analysis [12, 13]. According to orthogonal polynomial coefficient for quantitative trait within four levels (Table 3), the factors and levels of orthogonal polynomials were established (Table 4).

The whole regression model was achieved according to the above conditions and was indicated as follows:

$$Y=b_0+b_1X_1+b_2X_2+\ldots+b_vX_v+\varepsilon,$$

where b_0, b_1, b_2,…, b_v is the parameters of regression model; X_1, X_2,…, X_v is the independent variables of regression model; ε is residual effect.

Stepwise regression method [14] was used to analyze the vigor indices (germination percentage, germination index and vigor index) according to the whole regression model.

Table 2. Qualitative trait (cultivar, A) transferred to quantitative trait.

Contrast (df = 2)	A_1^a (Jiayu 293)	A_2 (Jiayu 948)	A_3 (Xieyou 963)
X_1	1	0	-1
X_2	0	1	-1

$^a\sum A_i=0$, i = 1, 2, 3.

Table 3. Establishment of orthogonal polynomials coefficient for quantitative trait within four levels.

Level	k=4		
	P_1 (Linear)	P_2 (Quadratic)	P_3 (Cubic)
1	-3	1	-1
2	-1	-1	3
3	1	-1	-3
4	3	1	1
C	20	4	20
λ	2	1	10/3

k, Number of levels; λ, Constants; P_j, Contrast vector for jth degree orthogonal polynomial; C, Squared length for P_j.

Table 4. Factors and levels of orthogonal polynomials for quantitative trait (Pb concentration, B).

Contrast (df = 3)	B_1	B_2	B_3	B_4
X_3 (Linear)	$-3/\sqrt{20}$	$-1/\sqrt{20}$	$1/\sqrt{20}$	$3/\sqrt{20}$
X_4 (Quadratic)	$1/\sqrt{4}$	$-1/\sqrt{4}$	$-1/\sqrt{4}$	$1/\sqrt{4}$
X_5 (Cubic)	$-1/\sqrt{20}$	$3/\sqrt{20}$	$-3/\sqrt{20}$	$1/\sqrt{20}$

Regression prediction model between vigor and Pb concentration

Germination percentage ($\hat{Y}_1$), germination index ($\hat{Y}_2$) and vigor index ($\hat{Y}_3$) were used as dependent variables for linear regression analysis with repeated measurement data. The equations of regression prediction model were obtained as shown in Table 5.

All of the three regression equations provided the regression relation between the vigor indices and the rice cultivars, and their relations with Pb concentration. All of the vigor indices had regression relation with the cultivars and Pb concentration. Germination percentage only had quadratic relation with Pb concentration; germination index not only had the quadratic relation with Pb concentration, but also had linear relation with Pb concentration and its interaction with cultivar; vigor index had linear and cubic relation with Pb concentration, and also had linear and cubic relation with Pb concentration and their interaction with cultivar. All regression parameters showed in the parentheses of Table 5 had reached significant or highly significant levels, indicating that the three regression equations could effectively predict the relation among vigor, cultivar, Pb concentration and their interaction.

Table 5. Predicting equation of regression model for rice vigor, cultivar and Pb concentration.

Vigor parameter	Predicted model of regression[a]
Germination percentage(%)	$\hat{Y}_1 = 79.50106 - 2.77622X_1 - 4.12973X_4$ ($b_0<0.0001$, $b_1=0.0045$, $b_4=0.0089$) [b]
Germination index	$\hat{Y}_2 = 15.52851 + 0.418122X_1 - 0.30073X_2 - 0.54939X_3 - 0.37262X_4 - 0.63357X_2X_3$ ($b_0<0.0001$, $b_1=0.0017$, $b_2=0.019$, $b_3=0.0032$, $b_4=0.0379$, $b_{23}=0.0052$)
Vigor index	$\hat{Y}_3 = 124.107 + 4.52X_1 - 11.79X_2 - 5.96X_3 - 3.64X_5 - 4.04X_2X_3 - 4.45X_2X_5$ ($b_0<0.0001$, $b_1<0.0001$, $b_2<0.00001$, $b_3= 0.0002$, $b_5=0.0131$, $b_{23}=0.0232$, $b_{45}=0.0132$)

[a] $X_3 = c_{(1)}/\sqrt{C_{(1)}} = \lambda_1(\frac{x-m}{\Delta})/\sqrt{C_{(1)}}$; $X_4 = c_{(2)}/\sqrt{C_{(2)}} = \lambda_2[(\frac{x-m}{\Delta})^2 - (\frac{k^2-1}{2})]/\sqrt{C_{(2)}}$; $X_5 = c_{(3)}/\sqrt{C_{(3)}} = \lambda_3[(\frac{x-m}{\Delta})^3 - (\frac{x-m}{\Delta})(\frac{3k^2-7}{20})]/\sqrt{C_{(3)}}$;

[b] The value in parentheses is *P* value of each regression parameter.

Table 6. Comparison of vigor index between real and predicted values by regression model.

Rice cultivar	Pb concentration (mg/L)	Observed value VI	Predicted value $\hat{V}I$	$(VI - \hat{V}I)/VI$
Jiayu 293	100	134.1477	133.4390	0.0053
	300	126.0533	127.5180	-0.0116
	500	133.2565	129.7360	0.0264
	700	121.0501	123.8150	-0.0228
Jiayu 948	100	117.7206	120.8340	-0.0265
	300	114.7723	109.1260	0.0492
	500	112.2115	115.5080	-0.0294
	700	104.5808	103.8000	0.0075
Xieyou 963	100	135.9592	132.4840	0.0256
	300	127.0925	132.3500	-0.0414
	500	129.0945	130.4040	-0.0101
	700	133.3427	130.2700	0.0230

DISCUSSION

Pb pollution can be harmful to crops, and cause many health problems in human being. Rice is one of the main cereal crops, so it is important to verify the critical poison concentration of Pb to rice seedling growth during rice production. The present paper did primary investigation on this aspect. The results showed that the emergence of rice seeds in different cultivars had different sensitivities to Pb concentration. There was no significant influence on emergence of rice seeds when Pb concentration was lower than 500 mg/L; when the Pb concenrtration reached 700 mg/L, it restrained germination percentage and germination index of rice. Between 100 and 700 mg/L concentrations, Pb significantly reduced the root length and shoot length of rice seedling, and their inhibited level increased with the increase of Pb concentration; however, Pb had no significant inhibition effect on root and shoot dry weight. It indicated that different physiological characteristics of rice seedling had different sensitivities to Pb.

Present results showed, by combining qualitative traits with quantitative traits, the regression of orthogonal polynomials could be used, after transferring the qualitative traits to quantitative traits, to obtain regression equations effectively predicting the relation among rice seed vigor, cultivar and Pb concentration. As an example of vigor index, the predicted value (Table 6) was calculated according to its equation, the results showed the difference between observed values and predicted values was very small. Predicted results indicated that the regression equation of qualitative traits combining with quantitative traits by orthogonal polynomials could be used to effectively predict the influence of Pb concentration on rice seed vigor. The dynamic changes of Pb pollution influence on rice seed vigor could be showed according to prediction equation. As an example of Jiayu 293, the experimental responses of rice seed vigor was only for four levels of their corresponding Pb concentrations, but when the Pb concentrations were not the established values during the experiment, such as 150, 250, 350, 450, 550 and 650 mg/L, the levels of rice seed vigor could be predicted through regression equation, the corresponding germination index was 16.20, 16.26, 16.23, 16.11, 14.96 and 14.51 respectively; the corresponding vigor index was 130.31, 127.60, 127.95, 129.31, 115.70 and 110.40, respectively.

Multiple-way factorial designs with qualitative trait and quantitative trait are commonly used in research, however their analysis results were only given the responses corresponding to the established values, and was unable to know responses between the established values. The method of qualitative traits combining with quantitative traits by orthogonal polynomial regression analysis used in the present paper could effectively analyze the relations between rice seed vigor and different Pb concentrations. Comparing to the multiple-way factorial analysis, the regression method we designed was more effective, suggesting that this method could be used in other experiments with multiple levels and multiple factors including qualitative traits and quantitative traits.

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