Effects of simulated acid rain on Growth of Lolium perenne

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Abstract. Lolium perenne is an important forage in China. In order to reduce the effect of acid rain on the quality and yield of Lolium perenne, Lolium perenne was used as material to study the changes of seedling growth and physiological characteristics under different pH conditions. The results showed that under the influence of simulated acid rain, the biomass and chlorophyll content of ryegrass changed greatly. The aboveground biomass and total biomass increased at pH 4.0 ~ 7.0, and the aboveground biomass and total biomass were the largest at pH 5.0, indicating that moderate acidity promoted the growth of Lolium perenne leaves. The chlorophyll content showed a double peak curve with the change of acid rain acidity, and reached the peak value at pH = 7 and pH = 3.5, respectively, indicating that acid environment was also conducive to the synthesis of chlorophyll in Lolium perenne leaves. Malondialdehyde content and relative conductivity increased significantly with the aggravation of acid rain stress, which was also a kind of adaptation and response mechanism of plants to environmental stress.

1. Introduction

Acid rain, also known as acid rain, consists mainly of the deposition of certain acid gases and particles, resulting in the pH of precipitation is less than 5.6. Acid rain pollution has become a common problem in China. At present, about 40% of soil pH is less than 5.6 in China, ranking as the third largest acid rain area after Europe and North America. Based on the data of 10 acid rain observation stations in Sichuan Province, it is found that the acid rain pollution in Sichuan is serious [1]. Acid rain has covered 80% of the province, including 21 cities, most of the state areas [2]. Acid rain enters the soil and changes soil properties, which indirectly affects plant growth. At the same time, it also directly affects plants, destroys plant morphological structure, damages plant cell membrane and inhibits plant metabolic function [3-6]. However, some studies have shown that acid rain can promote plant growth in a certain pH range. Only when the acidity of acid rain reaches a certain value, it will destroy the ultrastructure of plants, cause and intensify the membrane lipid peroxidation of cells, reduce chlorophyll content and photosynthetic efficiency, and lead to crop yield reduction [4].

Lolium perenne is a good feed for livestock and poultry. It is mainly used as fine forage in China. The research of Lolium perenne is mainly focused on improving yield and quality, while its physiological and biochemical characteristics under stress, especially in acid soil, are less studied. In this experiment, Lolium perenne was used as material to study the effects of acid rain on biomass,
chlorophyll content, relative electrical conductivity and malondialdehyde content, to provide theoretical basis for the growth of Lolium perenne in acid rain polluted areas.

2. Study methods

2.1. Study materials
Lolium perenne L. (Dannon seed company, Denmark)

2.2. Preparation of simulated acid rain
According to the ratio of $\text{SO}_4^{2-} : \text{NO}_3^- = 8:1$ (V/V), the acid rain mother liquor with pH 1.0 was prepared. Adjust the pH of the solution to 7.0, 6.0, 5.0, 4.0, 3.5, and 3.0 by pH meter.

2.3. Research design
The seeds of Lolium perenne were sterilized with 0.1% $\text{HgCl}_2$ solution for 8 min, and then soaked in deionized water for 24 h. Then 100 seeds were placed in a Petri dish with two layers of filter paper and cultured in an incubator at 25 °C / 18 °C (day / night). After 25 days, the seedlings with the same growth trend were selected and transplanted into the plastic pot containing Hoagland solution (Hogland nutrient solution) for stress culture in greenhouse. The stress gradient of Hoagland solution was pH 7.0, 6.0, 5.0, 4.0, 3.5 and 3.0. Each treatment was repeated three times. One week later, the biomass, chlorophyll content, relative electrical conductivity and malondialdehyde content (MDA) were taken for determination.

2.4. Determination method
The weight of aboveground ($W_1$) and underground parts ($W_2$) was determined by drying method. The root shoot ratio and biomass were calculated using the following formula [4].

$$\text{Root shoot ratio} = \frac{W_1}{W_2}; \text{Biomass} = W_1 + W_2$$

Chlorophyll was extracted with 80% acetone and determined by spectrophotometer [7]. The content of malondialdehyde was determined by TBA method [7]. The relative conductivity was measured and calculated by conductivity meter [7].

3. Results and analysis

3.1. Effects of simulated acid rain on biomass
The aboveground and underground biomass and root shoot ratio directly reflect the influence of acid rain on the growth of Lolium perenne. It can be seen from table 1 that under the influence of simulated acid rain, the biomass changes in a single peak curve, and increases with the decrease of acidity, reaching the maximum value at pH 5.0. The aboveground biomass, underground biomass, and total biomass is 14.536g, 4.544g, and 19.080g respectively, which is extremely significant compared with the control. When pH was less than 5.0, the growth began to decrease. It indicated that weak acid rain stimulated the growth of Lolium perenne seedlings to a certain extent. It may be because the cation activity in weak acid rain is higher, which supplements mineral nutrients to a certain extent, and promotes the growth of Lolium perenne. The strong acid rain destroyed the leaves of Lolium perenne, affected the photosynthesis of leaves and inhibited the growth of seedlings. The variation of root shoot ratio was similar to that of biomass. When pH = 5, the root shoot ratio reached 0.313, which was much higher than that at pH=3 and pH=7.
Table 1. Effects of simulated acid rain on biomass.

| pH | aboveground biomass/g | underground biomass/g | root shoot ratio | biomass/g |
|----|------------------------|------------------------|------------------|-----------|
| 3  | 9.024±0.941            | 1.135±0.531            | 0.126±0.031      | 10.159±1.439 |
| 3.5| 8.554±0.941            | 1.971±0.531            | 0.230±0.031 *    | 10.525±1.439 |
| 4  | 10.849±0.941           | 2.138±0.531            | 0.197±0.031      | 12.987±1.439 |
| 5  | 14.536±0.941 *         | 4.544±0.531 **         | 0.313±0.031 **   | 19.080±1.439 * |
| 6  | 10.842±0.941           | 3.321±0.531 *          | 0.306±0.031 **   | 14.163±1.439 |
| 7 (ck) | 11.263±0.941        | 1.585 ± 0.531           | 0.141±0.031      | 12.848±1.439 |

Note: "*" and "**" in the table indicate that they are significant at 0.05 and 0.01 levels respectively.

3.2. Effects of simulated acid rain on chlorophyll content

It can be seen from Fig.1 that the chlorophyll content does not show a regular change trend with the change of acidity, but presents a multi peak curve change. The maximum chlorophyll content was 12.953 mg / g at pH = 3.5. When the pH value was 5, the chlorophyll content was the lowest, which was 10.0637 mg / g, which reached a very significant level compared with the control, with a reduction of 22.23%. When pH = 3, the chlorophyll content was also low, which was 11.244 mg / g, which was significantly lower than that of the control.

![Fig 1. Effects of simulated acid rain on chlorophyll content.](image)

3.3. Effect of simulated acid rain on malondialdehyde content

It can be seen from Fig. 2 that with the increase of acidity, the content of malondialdehyde in leaves of Lolium perenne increased regularly. When pH = 3, the content of malondialdehyde reached 0.0157 umol / g FW. When pH = 7, the content of malondialdehyde was the lowest, which was 0.0136 umol / g FW, which was significantly lower than that of pH = 3 ~ 4.
Fig 2. Effect of simulated acid rain on malondialdehyde content.

3.4. Effect of simulated acid rain on relative conductivity

It can be seen from Fig. 3 that the relative conductivity of leaves increases with the increase of acidity. When pH = 3, the relative conductivity is 26.5, which is 43.3% higher than that of the control. The results of statistical analysis showed that the relative conductivity of the control was significantly lower than that of the acid rain treatment. When the pH is between 3.5 and 4, the relative conductivity is basically stable at about 23.

Fig 3. Effect of simulated acid rain on relative conductivity.

4. Discussion

Consistent with previous studies [8-10], both biomass and root shoot ratio increased slightly under weak acid, but decreased significantly under strong acid in this study. It indicated that weak acid rain
stimulated the growth of Lolium perenne seedlings to a certain extent. It may be because the cation activity in weak acid rain is higher, which supplements mineral nutrients to a certain extent, and promotes the growth of Lolium perenne. The strong acid rain destroyed the leaves of Lolium perenne, affected the photosynthesis of leaves and inhibited the growth of seedlings.

Leaf is the organ of photosynthesis. Acid rain affects the structure of plant leaves and normal physiological and biochemical processes, and then indirectly affects the growth and development of plants [4]. The results showed that chlorophyll content indicated the photosynthesis of plant leaves. Acid rain would damage the lamellar structure and thylakoid membrane of chloroplast, and reduce the photoelectric conversion efficiency. High acidity and long-term stress would lead to serious damage to Lolium perenne [5]. In this study, the chlorophyll content was 11.2439 mg / g when pH = 3.0, which was significantly lower than that of the control. This may be due to the acid rain when the acidity is too high to elute the cations from the aboveground parts of plants, resulting in the inhibition of chlorophyll formation. A bimodal curve was found in this study, which was also found in previous reports. Huang Hui et al. pointed out that the hyperbola may be due to the rapid renewal cycle of chlorophyll in the plant body [11], and the influence of acid rain on soil, which promoted the change of chlorophyll. The factors leading to the change of chlorophyll content include not only the influence of acid rain concentration, but also the change of soil properties and the growth cycle and stress resistance of plants [12].

MDA is an important product of membrane lipid peroxidation, which can aggravate membrane damage. The content of MDA can reflect the damage degree of plants under stress. Overall, the MDA content of Lolium perenne after acid rain was higher than that of the control. The content of MDA with acidity lower than 4 was significantly higher than that of the control, which was basically consistent with the conclusion of Zhang Pei et al [6].

Relative conductivity is an important index to measure the permeability of cell membrane. The membrane system is damaged and the permeability of cell membrane is increased, which leads to the increase of electrolyte extravasation capacity. The higher the electrical conductivity is, the more electrolyte permeates, the more serious the damage of cell membrane is. The conclusion of this study is also consistent with most previous reports [4, 6]. However, when pH = 3.5-5, the relative conductivity of ryegrass leaves showed a relatively stable change trend, which also showed that ryegrass had a certain adaptability to acid rain, and the antioxidant system also actively responded to resist external damage, eliminate free radicals and prevent membrane system damage [5].

5. Conclusion
The results showed that under the influence of simulated acid rain, the biomass and chlorophyll content of ryegrass changed greatly. The aboveground biomass and total biomass increased at pH 4.0 ~7.0, and the aboveground biomass and total biomass were the largest at pH 5.0, indicating that moderate acidity promoted the growth of Lolium perenne leaves. The chlorophyll content showed a double peak curve with the change of acid rain acidity, and reached the peak value at pH = 7 and pH = 3.5, respectively, indicating that acid environment was also conducive to the synthesis of chlorophyll in Lolium perenne leaves. Malondialdehyde content and relative conductivity increased significantly with the aggravation of acid rain stress, which was also a kind of adaptation and response mechanism of plants to environmental stress.

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