

Effects of Alkali Stress on the Growth and Plant Acid Secretion of Different Energy Grasses

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Abstract: Alkali stress can seriously affect plant growth, but plants can cope with stress damage through measures such as secreting organic acids. The purpose of this paper is to investigate the response of energy grasses to alkali stress and the effect of alkali stress on organic acid secretion. In this experiment, different alkali-tolerant energy grasses were used as materials; by simulating high alkali stress environment with red mud residue, the growth of plants, the chemical characteristics of soil samples and the organic acid secretion in root systems were studied. The results showed that high alkali stress could seriously inhibit the growth of energy grasses, but when organic matters were added, energy grasses could grow vigorously. Among them, *Pennisetum hydridum* and *Pennisetum purpureum* Schumab cv. could even obtain enormous biomass, effectively improve the alkaline soil and reduce pH values. After alkali stress occurs, energy plants secrete organic acids rapidly. The organic acid contents of energy herbs, as well as the speed and duration of organic acid secretion are closely related to the adaptability of plants to alkali stress. This study provides useful information for the preliminary response of different energy grasses to alkali stress such as organic acid secretion, and provides a direction for further research on the adaptation mechanism of different energy grasses to alkali stress.

1. Introduction

Today, soil salinization is becoming more and more serious worldwide. The main damage to plants can be divided into osmotic stress and ion toxicity. For plants, damage caused by alkali stress is much greater than that caused by salt stress. Red mud is a kind of strong alkaline waste discharged from the aluminum industry with the pH value from 10 to 12. Its utilization and treatment are worldwide problems due to its strong alkalinity and low organic composition. There are relatively few reports on its phytoremediation.

In recent years, the research on remediation of saline-alkali soil through dominant plant cultivation has attracted more and more attention. Liang and his colleagues proposed that saline-alkali-tolerant forage and rice varieties could be effectively used to restore the saline-alkali ecosystem. Dagar's team found that licorice could adapt to alkaline soil and significantly reduce soil pH value. Ma and coworkers reported that sweet sorghum could also be used to effectively improve high alkali red mud residue. However, *Pennisetum hydridum*, a more tolerant energy plant, can grow well in saline-alkali wastelands and beach lands, showing great potential for the comprehensive treatment of saline-alkali soil.

Organic acids are a kind of low molecular weight compounds with carboxyl group as buffer. Organic acids secreted by plant roots can help plants to withstand the damage caused by adversity. However, although there are many reports about organic acids and stress, there are few reports about organic acids and salt-alkali stress, especially alkali stress. It is speculated that the organic acids secreted by roots may be involved in soil formation and reduce the damage of toxic metals to plants, thus regulating the resistance of plants to adverse environment (alkali stress).

In this study, different alkali-tolerant energy grasses were used as materials to study the changes and differences of plant growth, soil chemical characteristics and root organic acid secretion under

the condition that red mud residue serves as the high alkali stress cultivation medium. The primary response of different energy grasses to alkali stress and the physiological role of organic acids in the process were discussed in order to provide a theoretical basis for the adaptation mechanism of different energy grasses to alkali stress.

2. Research Materials and Research Methods

2.1 Research materials

The materials are *Pennisetum hybridum* (H), *Pennisetum purpureum* Schumab cv. (R) and *Miscanthus floridulus* (M) grown in the campus of Shaoguan University.

2.2 Material culture and stress treatment

Single segments of *Pennisetum hybridum*, *Pennisetum purpureum* Schumab cv. and *Miscanthus floridulus* were cultured in red soil in a plastic greenhouse; the plants were used for alkali stress test when the lateral buds of segments germinated and grew to 3-4 leaves.

Alkali stress test started in May 2016. The cultivation tank was 2 m long, 2 m wide and 30 cm deep. The tank was placed in a plastic greenhouse; its bottom was covered with waterproof black plastic film and filled with ordinary red soil (regular soil treatment, RS), red mud (high alkali stress treatment, RM) and 10% volume of farm manure organic matter mixed with red mud (high alkali mixed organic matter treatment, MS). The pH values were 5.17, 8.52 and 8.38 respectively.

Three energy grasses and three alkali stress tests were set up. 36 plants of energy grasses were planted at intervals of 30 cm * 30 cm in each experiment. After energy grass seedlings with 3-4 leaves were planted, tap water was irrigated every 3 days; soil moisture was 60%.

2.3 Sampling and observation of growth characteristics

According to different treatment time (10 days, 20 days, 60 days, 120 days and 180 days), three energy grass plants were randomly rooted in each test area. The tiller numbers and height growth characteristics were observed. At the same time, about 1 kg of mixed soil samples from 5-10 cm soil layer around the root system of the plant were taken out and put into sealed bags for treatment.

2.4 Sample processing and index measurement

Soil chemical properties were determined after samples were placed in the shade to dry. The pHS-25 acidity meter was used to determine PH value; DDS-11C digital display conductivity meter produced by Shanghai INESA was used to determine EC value; Kjeldahl method was used to determine total N content in soil; potassium dichromate-external heating method was used to determine total C content in soil. The roots were washed with pure water, then placed in the oven for 10 minutes at 105 degree to kill enzymes, and then dried at 80 degree to achieve constant weight. The organic acids in plant samples were determined by acid-base titration.

2.5 Data Processing

The data were processed and plotted by Excel 2007, and analyzed by SPSS 16.0 software.

3. Results and Analysis

3.1 Effects of different alkali stresses on the growth of energy grasses

Table 1 shows that, the average plant height of *Pennisetum hybridum* was the highest; the fresh weights of fibrous roots and aerial parts, as well as the amount of plant growth were the largest. *Miscanthus floridulus* was the shortest and smallest; *Pennisetum purpureum* Schumab cv. was in the middle. Under alkali stress, the growth of energy grass was greatly affected. The growth index of each plant decreased significantly; the growth of each plant was seriously inhibited, and the plants became short and yellowish. Among them, the growth amount of *Pennisetum hybridum* decreased by nearly 70%. *Miscanthus floridulus* showed strong adaptability and tolerance; the decline was

slightly smaller than that of the other two plants. In the group of mixed organic matter treatment, the growth of each plant was significantly improved; the fresh weight of fibrous roots and plant growth were significantly increased. The growth of *Pennisetum hydridum* was the best. It can be seen that after adding organic matter, the plants under high alkali stress can still good well.

Table 1. Different growth characteristics of energy grasses under different alkali stress conditions

treatment	height (cm)	tiller number	fresh weight of root (g)	fresh weight of aerial part (g)	growth amount (g)
HRS	220.15±21.38	4.23±0.21	10.43±1.45	523.31±46.87	365.44±32.17
HRM	155.75±21.8	2.32±0.01	7.85±0.88	281.45±22.18	112.25±8.96
HMS	276.24±11.53	5.61±0.08	14.38±2.26	872.48±81.45	631.45±24.73
RRS	196.42±15.62	4.72±0.35	5.36±4.27	434.39±38.94	274.56±27.11
RRM	152.34±10.08	3.63±0.57	3.92±0.15	302.22±18.34	152.34±9.58
RMS	268.52±19.23	6.14±2.51	6.81±1.21	624.57±78.36	483.26±35.81
MRS	53.16±3.24	6.49±0.52	0.25±0.02	15.27±1.32	10.93±1.58
MRM	43.68±3.54	5.62±1.23	0.18±0.01	11.62±1.03	6.32±0.54
MMS	68.76±9.87	7.63±1.52	0.33±0.01	23.46±1.94	18.28±1.95

3.2 Effects of energy grass planting on chemical properties of alkaline substrate soil

After energy grasses were planted, the chemical indexes of alkaline soils changed in varying degrees (see Table 2). Among them, the planting experiment of *Pennisetum hydridum* changed the most, while that of *Miscanthus floridulus* was relatively small. In the alkali stress treatment experiment, except for the slight increase of the pH value of the control group, the pH values of all other groups decreased after planting different plants. The EC values of all treatments decreased in varying degrees, but the lowest reduction was achieved under high alkali condition. Among them, the experiment of adding organic matter decreased the most, and EC decreased for more than 20%. The decreases of N were very significant in all treatments, especially in the soil of the experimental area of planting *Pennisetum hydridum* with the control treatment, the decrease of N content was more than 90%. Relatively speaking, under the condition of high alkali test with pH of 8.5, especially when organic matter is not mixed, the available N and C nutrients are seriously inadequate.

Table 2. Effects of energy grass planting on soil under alkali stress

Treatment	pH		EC (ms/cm)		N (g/Kg)		C (g/Kg)	
	begin	end	begin	end	begin	end	begin	end
HRS	5.17±0.33	5.69±0.25	0.18±0.01	0.13±0.02	3.28±0.24	0.21±0.01	1.48±0.12	4.12±0.24
HRM	8.52±0.45	8.19±0.66	2.85±0.12	2.74±0.15	0.45±0.02	0.32±0.01	9.64±1.07	6.45±0.61
HMS	8.38±0.42	7.72±0.54	3.55±0.35	2.51±0.13	15.51±1.13	7.67±0.53	57.46±2.73	44.08±3.27
RRS	5.17±0.33	5.34±0.38	0.18±0.01	0.14±0.01	3.28±0.24	0.94±0.05	1.48±0.12	2.23±0.12
RRM	8.52±0.45	8.31±0.74	2.85±0.12	2.61±0.16	0.45±0.03	0.22±0.01	9.64±1.07	5.47±0.37
RMS	8.38±0.42	7.98±0.61	3.55±0.35	2.83±0.18	15.51±1.13	8.47±0.52	57.46±2.73	45.93±3.51
MRS	5.17±0.33	5.23±0.34	0.18±0.01	0.13±0.01	3.28±0.24	2.21±0.01	1.48±0.12	1.85±0.42
MRM	8.52±0.45	8.38±1.18	2.85±0.12	2.82±0.15	0.45±0.05	0.01±0.00	9.64±1.07	3.12±0.26
MMS	8.38±0.42	8.05±0.76	3.55±0.35	3.27±0.24	15.51±0.13	12.84±1.07	57.46±2.73	45.54±3.88

3.3 Effects of different alkali stresses on organic acids secreted by energy grass

Different alkali stresses significantly increased the content of root exudates of *Pennisetum hydridum*, and the total organic acid secretion increased more than two times after 60 days of stress, while the organic acid secretion of the control group decreased slightly (see Figure 1). Ten days after high stress, the organic acid secreted by the roots of *Pennisetum hydridum* reached 1.8 times as much as that before the beginning of stress, and then the rate of increase slowed down. The relative highest level was reached at the 60th day. Under mixed organic matter treatment, organic acid secretion was relatively late, but continued to reach 2.4 times at the 120th day, and then

maintained at a relatively high level.

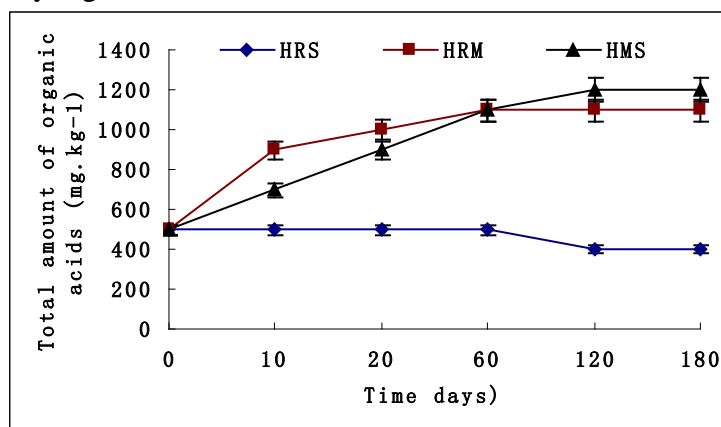


Figure 1. Organic acid secretion in root system of *Pennisetum hybridum* under alkali stress

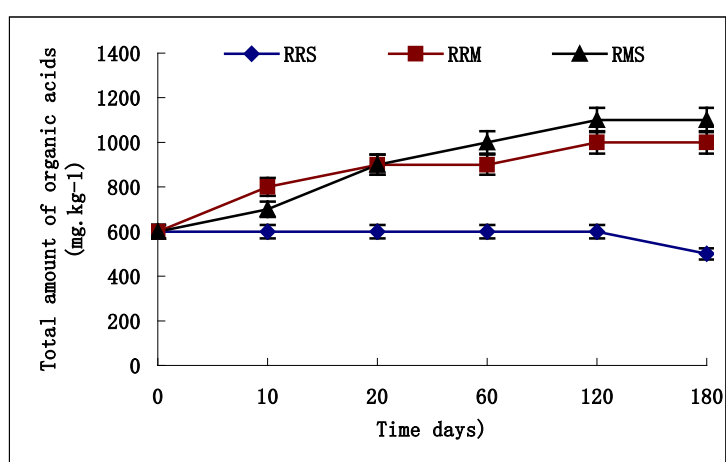


Figure 2. Organic acid secretion in root system of *Pennisetum purpureum* Schumab cv. under alkali stress

From Figure 2, we can see that the amount of organic acid secreted by the root system of *Pennisetum purpureum* Schumab cv. increased slowly after alkali stress. In the early stage of high alkali stress, the increased rate was relatively fast. The amount increased slowly to the highest level at the 120th day under the mixed organic matter treatment.

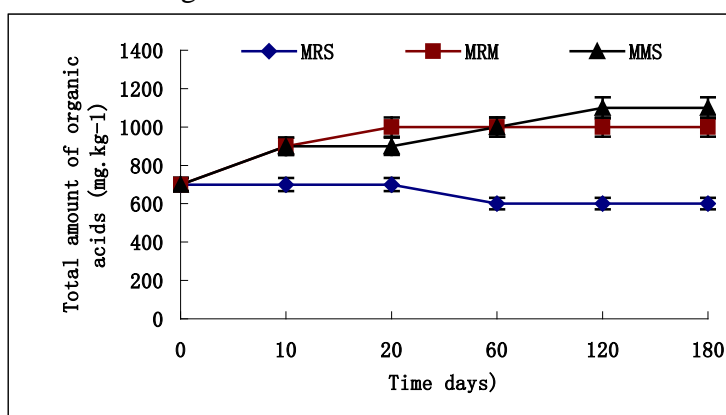


Figure 3. Organic acid secretion in root system of *Miscanthus floridulus* under alkali stress

Under alkali stress, the rapid organic acid secretion from the roots of *Miscanthus floridulus* increased by nearly 30% in the early stage, while it reached the highest level after the 20th day and remained at that level (see Figure 3). In the mixed organic matter treatment group, the organic acid secreted by roots increased slowly to nearly 1.4 times at the 120th day and remained stable. In the

control group, the organic acid secreted by roots decreased after 20 days.

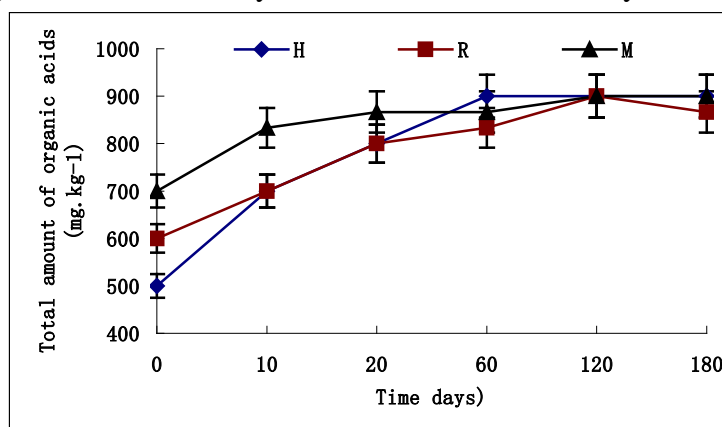


Figure 4. Differences of organic acid secretion in roots of three energy grasses under alkali stress

Overall, the organic acid secretion of *Miscanthus floridulus* in the early stage was higher than that of *Pennisetum hydridum* and *Pennisetum purpureum* Schumab cv. After alkali stress, the organic acid secretion of *Pennisetum hydridum* and *Pennisetum purpureum* Schumab cv. reached the same level as that of *Miscanthus floridulus* (see figure). Especially, the amount of organic acid secreted by the root system of *Pennisetum hydridum* increased the fastest and lasted the longest, and nearly doubled the amount.

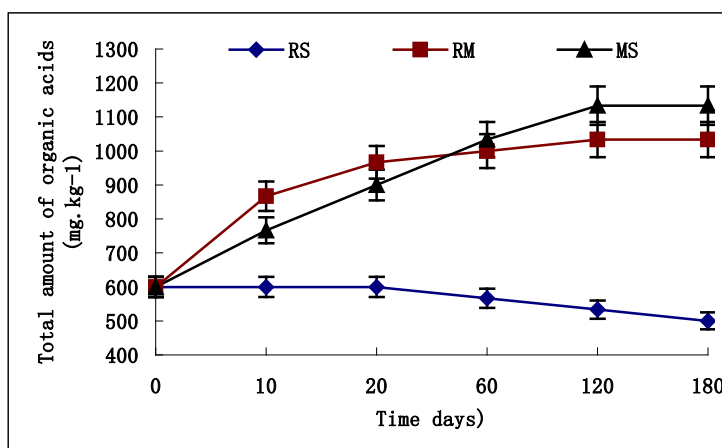


Figure 5. Effects of alkali stress on organic acids secretion in roots of three energy grasses

Comparing three different alkali stress conditions, it was found that in the control group of regular soil treatment, the amount of organic acid secreted by plant roots was always at a low level (see Figure 5). After alkali stress, the root system of each plant secreted organic acids rapidly. The rates increased rapidly in the first 20 days after alkali stress, and then slowed down. In the treatment of mixed organic matter, the root system of each plant continued to secrete organic acids, which eventually increased by nearly 90% compared with that before the stress.

4. Conclusion

Under different alkali stress levels, the growth characteristics of different energy grasses vary greatly. Even under higher alkali stress conditions, *Pennisetum hydridum* and *Pennisetum purpureum* Schumab cv. can still grow vigorously in fertile soil. The well-developed roots and huge biomass of *Pennisetum hydridum* are much better than those of *Miscanthus floridulus*. It has great application potential in desert restoration and biomass energy. Under the condition of strong alkali stress and poor fertility, *Miscanthus floridulus* showed stronger adaptability than *Pennisetum hydridum* and *Pennisetum purpureum* Schumab cv. It can be used as a pioneer plant in desert lands with harsh conditions.

Plants can effectively improve the physical structure of soil through the interpenetration of their roots. At the same time, the decay of their branches, leaves and roots can increase the contents of organic matters in soil, thus improving the nutrient status and chemical properties of earth. The results showed that the planting of energy plants could effectively improve the physical and chemical properties of alkaline soils. The planting of *Pennisetum hydridum* which had developed roots had the most obvious effect, which again verified the conclusions of our previous experiments. When the soil fertility is high, the utilization of nutrients can be greatly improved; plants can grow vigorously. *Pennisetum hydridum* has more vigorous growing power. It can be seen that energy grasses such as *Pennisetum hydridum* have better prospects for the restoration of desert lands with lower fertility.

The synthesis and secretion of organic acids in plants are the response to environmental stress. After alkali stress occurs, the root system of energy grass can secrete a large amount of organic acids. The stronger the stress, the faster the secretion rate will be. It is speculated that the organic acids can be used to quickly adapt to the damage caused by strong alkali stress. The improvement of soil fertility under alkali stress is helpful to the continuous secretion of organic acids, which reflects stronger and more effective stress adaptability, thus ensuring the normal growth of plants. It is worth noting that under the condition of strong alkali and fertility, the *Miscanthus floridulus*, which can still grow tenaciously, can secrete more background organic acid. It is concluded that plants' secretion amount and speed of organic acids can be used as an important indicator of plants' resistance to alkali stress. In the future, the response mechanism between organic acids and alkali stress needs to be further studied.

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