Effects of Different Soil Environments on Root Structure of Cynanchum Chinense

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Abstract. Cross-cutting sectioning of the roots of Cynanchum chinense grown in different habitats showed that the root structure of Cynanchum chinense in different habitats was significantly different, but it did not affect its normal growth. Under the salty environment, the number of layers of Cynanchum chinense root cortex cells is relatively small, and the cells are arranged more closely, which is beneficial to the plants to absorb water and nutrients in the soil. The soil physical and chemical properties of different habitats of Cynanchum chinense and the concentration of cultivable microorganisms in rhizosphere soil were compared and found that the obvious difference in root structure of Cynanchum chinense may be caused by the difference of soil soluble salt content and soil culturable microorganism concentration. The research results of this subject provide a theoretical reference for the introduction, breeding and cultivation of Cynanchum chinense.

1. Introduction

Salt stress is a kind of abiotic stress that is ubiquitous in the world, and it is also one of the main environmental factors that limit plant growth and development. The area of saline-alkali land in the world has increased year by year. Due to the presence of a large amount of salt, it has a negative impact on the normal growth, high yield and quality of plants. Studying the salt tolerance of plants, screening salt-tolerant plants, and improving the salinization of soils has become a hot topic in the world [1, 2, 3]. Roots are the main organs of plants that absorb water and nutrients, directly affecting the survival and development of the entire plan. The most direct damage to the plant in the soil is the root of the plant. Its distribution characteristics under adverse conditions are the most direct adaptation characteristics of plants to effectively absorb and utilize soil nutrients [4]. Therefore, it is of great significance for the biological study of salt-tolerant plant roots.
Yuncheng is located in the southwestern part of Shanxi Province, at 110°15′—112°04′E, 34°35′—35°49′N. Yuncheng Salt Lake is a typical inland saltwater lake. A large number of minerals containing salt are concentrated here. After long-term precipitation and evaporation, a natural salt lake is formed. Yuncheng City is affected by monsoon activities throughout the year and is a warm temperate continental monsoon climate. The winter is cold, the summer is hot, the lake water evaporation is strong, some of the topsoil is rich in salt, and the soil has a certain salinization phenomenon. *Cynanchum chinense* belongs to the genus *Cynanchum* [5], which is a common entangled herb around the salt lake.

This project aims to investigate the effects of different soil environments on the root structure of *Cynanchum chinense*, and carry out hand-slicing on the roots of *Cynanchum chinense* in different habitats [6]. From the cross-cutting and slitting of roots, make temporary loadings for both [7, 8], observation and comparison under an electron microscope. The research results of this subject provide a theoretical reference for the wider breeding, introduction and cultivation of *Cynanchum chinense*.

2. Materials and methods

2.1. Study area and soil sampling

The sampling points around the salt lake were selected as the experimental group. The sampling points about 10 km away from the salt lake were used as the control group. Some of the similar *Cynanchum chinense* plants were excavated, and placed in the tissue culture flask and quickly brought back to the laboratory for pretreatment. Cut the slice by hand. In addition, the soil was taken close to the sampling point with a soil drill, and a part of the mixture was placed in a previously sterilized EP tube to determine the concentration of soil culturable microorganisms. A part of it was put into a ziplock bag and brought back to the laboratory for natural air drying to determine the physical and chemical properties of the soil.

2.2. Research methods

Bare-handed sections for temporary loading and observation

2 cm down at the junction of the rhizome, cut the roots into 2-3 cm long roots with a blade, and cut the roots into a water-dish culture dish to cut several pieces continuously, then place the blade in the culture dish. As soon as it sways, the slice floats in the water. The sectioned material was placed in 30% alcohol and transferred to clear water after 5 minutes. After washing, stain with 1% aqueous solution of saffron for 1 h, pour off the aqueous solution of saffron, and rinse with water several times, and then soak for 5 min in 50% alcohol. After 5 min, transfer the material from 50% alcohol to 70% alcohol. Continue to decolorize until the lignified cell wall is red, and the other parts are pink or close to colorless. Wash the excess dye solution with 95% alcohol and dehydrate it with absolute alcohol for 5 min. A drop of water was added to the slide with a dropper, and the well-stained slice was placed on the slide with a brush. When the cover slip was added, bubbles should be avoided at a 45° angle, affecting the observation effect [9]. After the tablet is completed, it is placed under a microscope and photographed.

Analysis of soil physical and chemical properties

The soil water content was determined by the drying method, and the pH was measured using the potentiometric method [10, 11], the soil soluble salt content was determined by the gravimetric method as well as [12].

Soil culturable microbial colony count

In addition, the cultureable microbial colony count was measured by plate scribing [13-14].

2.3. Statistical analysis

Variance analysis was conducted to test the difference between the test group and control group using the SPSS 20.0 for windows. Axio Observer was used to shooting the cross-sectional structure of the root system.
3. Results and analysis

3.1. Comparison of horizontal cuts of plant root structure

The root section of *Cynanchum chinense* is a nearly circular structure (Fig 1 A, D). The root section of the experimental group has a narrower xylem and the xylem tube diameter is relatively small (Fig 1 A); thin, cells are small, cells are densely arranged; the number of layers of cortical cells is relatively small, the cells are densely arranged, and the volume is small (Fig 1 B). The fewer the number of layers of cortical cells, the distance of water and nutrients transported by roots. The shorter the transport speed, the faster the transport of water and nutrients from the soil to the xylem of the roots; the vascular column is the main component of the cross-section of the root system, accounting for about 3/4 of the cross-section of the entire root system. Left and right, the pith is large in volume and contains a lot of calcium oxalate clusters (Fig 1 A, B, C), which is an important structure for plants to adapt to salt stress. The roots of the control group had larger xylem in the transverse section and more large vessels (Fig 1 D). The root epidermis was thicker and the cell volume was larger (Fig 1 D, E); the cortical cells were larger and arranged. It is looser, the number of cell layers is increased, the cortex is larger, the cell gap is larger, and the myeloid is significantly smaller (Fig 1 E, F). These structural features are significantly different from the experimental group, see Table 1.

![Figure 1. Cross-cutting sectioning of *Cynanchum chinense* vine roots (A, B, C: experimental group; D, E, F: control group)](image)

| Root structure  | Test group                                      | Control group(CK)                                      |
|-----------------|-------------------------------------------------|--------------------------------------------------------|
| epidermis       | Thinner, smaller cells, dense cells              | Thicker, larger cell size                               |
| Cortex          | The number of cell layers is relatively small,   | The cell is large in size and loosely arranged,         |
|                 | the cells are densely arranged and the volume is small. | the number of cell layers is increased, the cortical    |
|                 |                                                 | volume is large, and the cell gap is large.             |
| Vascular        | The pith is large, the xylem is narrow, and the   | The marrow is obviously smaller, the xylem is larger,   |
| column          | diameter of the catheter is relatively small.    | and there are many large catheters.                     |

3.2. Soil physical and chemical properties

The water content of the surface soil sample of the experimental group was about 23%, and the water content of the soil sample of the control group was about 20%. Because the soil samples collected by the experimental group are close to the salt lake, about 10 km away from the salt lake, the soil water content is higher, while the soil samples of the control group are located in the grass near the road, the
The relative water content is lower, 10-20 cm, 20-30 cm. The water content trend is consistent with the topsoil sample (Fig 2 c). The pH value of the surface soil sample of the experimental group was about 8.5, and the pH value of the soil sample of the control group was about 8.3. The analysis of variance was not significant (P>0.05), the pH of 10-20 cm, 20-30 cm and the surface soil sample. The value trend is consistent (Fig 2 b). The content of soluble salt in the surface soil of the experimental group and the control group was significantly different, about 1.8% in the experimental group and about 0.2% in the control group. The results of variance analysis showed that there were significant differences in soil salinity in different habitats (P<0.05). The 10-20 cm, 20-30 cm and surface of soil samples have the same trend of soluble salt content (Fig 2 a).

**Figure 2.** Comparison of physical and chemical properties of different habitats of *Cynanchum chinense* (a, soluble salt content; b, pH; c, soil water content). The histogram and error represent the mean ± standard deviation, n= 3.

### 3.3. Comparison of the number of cultureable microbial colony

The colony forming unit (CFU) per ml of the soil sample of the experimental group was about 1.90×10⁴, while the colony forming unit (CFU) per ml of the soil sample of the control group was about 2.80×10⁵ (Fig 3). The content of culturable microorganisms in the rhizosphere soil of the experimental group was relatively small at a dilution of 10⁻³.
Figure 3. Colony distribution of the rhizosphere soil samples of *Cynanchum chinense* under the gradient of $10^{-3}$ (A experimental group, B control group)

4. Discussion

For the first time, this experiment systematically studied the root structure of *Cynanchum chinense* around Yuncheng Salt Lake and 10 km from Salt Lake by hand-slicing technique. By comparing the transverse and longitudinal sections of the roots of *Cynanchum chinense*, it was found that the root structure of *Cynanchum chinense* was different in different soil environments, but it did not affect the normal growth and development of the plants. In the experimental group, the number of cortical cells in the root section was small, and the cells were densely arranged because the fewer the number of layers of cortical cells, the shorter the distance between the water and nutrients transported by the roots, and the faster the lateral transport of water and nutrients. A structural feature contributes to the lateral transport of moisture and nutrients from the soil to the root xylem [15]. The number of conduits in the xylem is relatively large, and the diameter of the conduit is relatively small. Some scholars [16, 17, 18] believe that: the diameter of the xylem conduit is small, increasing the resistance to water flow, thereby preserving a part of the water for the critical period such as the flowering period of the plant. The characteristics are important for the rational use of water in the soil by plants. The pith is large in volume, and the pith is composed of a large number of parenchyma cells. The plant dilutes the absorbed salt by increasing the number of parenchyma cells, absorbing and storing a large amount of water, that is, diluting the intracellular salt concentration by a large amount of water absorption to make the plant body The salt concentration is maintained at a low level [19], which is beneficial for plants to adapt to the salt stress environment. The characteristics of the cell layer are relatively small, the cells are densely arranged, and the volume is small. The characteristics of the *Cynanchum chinense* are suitable for adapting to the salt stress environment. These structural features are beneficial to the plant to absorb water and nutrients in the soil.

The soil physical and chemical properties such as water content, pH value and soluble salt content were also analyzed. The difference of soil soluble salt content was found to be the most significant. The reason why the soluble salt content may be one of the causes of the difference in root section of the two plants. By analyzing and comparing the content of cultivable microorganisms in the rhizosphere soil of plants, it was found that the content of cultivable microorganisms in the rhizosphere soil of the experimental group was lower than that of the cultivating microorganisms in the rhizosphere soil of the same dilution concentration, indicating that the salt content was higher. The number of microorganisms that can survive in the environment is small. The soil around the rhizosphere and the rhizosphere of the plant constitute a unique micro-ecological environment [20]. The microbes in the soil are closely related to the plants, which indicates that the difference in
microbial content may also be an important reason for the root structure of plants. There are still some shortcomings in this experiment, and the future work will start with more about the soil soluble salt content and the mechanism of how the soil culturable microbial concentration affects the plant root structure.

5. Conclusion
The root structure of *Cynanchum chinense* was significantly different in different soil environments. The number of layers of *Cynanchum chinense* root cortex cells in the salty environment was relatively small, and the cells were arranged more closely, which was more conducive to the absorption of water and nutrients in the soil. In addition, the study also found that the number of cultureable microbial colony in the salt-producing environment is relatively low, which may be one of the reasons for the structural differences in the root system of *Cynanchum chinense*.

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