Comparison of Antioxidant Enzyme Activity of Pioneer Plants in Karst Areas of the Northwest Guangxi

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Abstract. The selection of pioneer species is one of the key technologies for ecological restoration in Karst Rocky desertification areas. The purpose of this study is to explore the relationship between antioxidant enzyme activities of different pioneer plants in Karst Rocky desertification areas and their living conditions, environmental adaptability and other ecological factors, so as to screen out excellent species with good ecological and economic benefits and provide a theoretical basis for ecological restoration in Karst Rocky desertification areas. In this study, the pioneer plant samples were collected from the karst area of the Northwest Guangxi and their SOD, POD, CAT, GSH, APX and other antioxidant enzymes activities were determined and compared. Our results showed that the antioxidant enzyme activities of different plants were significantly various due to the influence of themselves and various external factors. The plant species with high antioxidant enzyme activities often had better soil ecological restoration effect. The activity of antioxidant enzymes in pioneer plants was closely related to soil organic matter, soil enzyme activity and other physical and chemical indexes. Antioxidant enzyme activity of plants in Karst regions may be a critical indicator for species selection for ecological restoration of Karst Rocky desertification.

1. Introduction

Guangxi is one of the provinces with the largest karst area in China. The karst areas in Guangxi cover $9.87 \times 10^4 \text{ km}^2$, accounting for 41.57% of the land area. Among them, the bare rock area covers $7.47 \times 10^4 \text{ km}^2$, accounting for 75.7% of the karst landform area and 31.5% of the land area [1]. For a long time, the contradiction between people and land is extremely prominent due to the rapid growth of population. Furthermore, the unreasonable interference of human activities worsens the karst environment, which is already very fragile. Finally, ecological geological disasters, like rocky desertification, show up.

Karst Rocky desertification refers to the process of land degradation caused by unreasonable social and economic activities of human beings in a fragile Karst environment of subtropical region, resulting in serious soil erosion, large area of bedrock exposure, serious decline in land productivity, and land degradation processes similar to desertification landscape appearing on the surface [2]. Since the 1980s, people in Guangxi has been committed to the research of the management of Karst Rocky desertification in the area and the related problems. They put forward many valuable suggestions and solutions, as a result, some effective governance models with regional characteristics have been explored, such as PingGuo pattern (Zenia insignis pattern), TianYang pattern (Dendrocalamus minor pattern), ecological
agriculture pattern (GongCheng pattern) and Longla pattern, etc [3]. Vegetation restoration is an important measure for the control of Karst Rocky desertification, and how to select excellent pioneer species is the key. Therefore, the selection of pioneer species plays a very significant role during this process.

Based on various considerations, scholars at home and abroad have done some related researches on the selection of pioneer tree species in the restoration process of damaged ecosystems with different geological backgrounds. But most of the research focuses on structural biology, water physiology, photosynthetic characteristics, cultivation physiology, introduction and domestication of superior species, drought resistance exercise, artificial simulation of water stress, salt stress, heavy metal stress, and ecological characteristics of plant community in extreme areas, including desertification, loess plateau of Northwest China and mining wasteland. So far, there are few systematic studies on the screening of pioneer species for the ecological restoration of Karst Rocky desertification. Therefore, taking the karst area in the northwest Guangxi as the main research object, the author explores the feasible method of selecting pioneer species from the perspective of plant antioxidant enzyme activity and its interaction with habitat, providing necessary reference for the control of rocky desertification in Guangxi and surrounding areas.

All the metabolisms of living organisms work through enzyme catalysis, and controlled and regulated by enzymes. Antioxidases mainly include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), etc. SOD, POD and CAT are important protective enzymes in plant enzymatic defence against membrane lipid peroxidation [4]. Relevant researches shows that when these three enzymes coordinates with each other, free radicals in cells could be maintained at a low level, preventing oxidative damage of biological oxygen [5]. On the other hand, APX, dehydroascorbic acid reductase (DHAR) and glutathione reductase (GR) are vital enzyme components in the oxidation-reduction pathway of AsA-GSH in plants, having an important role in promoting the AsA and GSH regeneration and maintaining the cycle operation of AsA-GSH [6]. Some scholars have done some relevant studies on the above enzymes [7-10]. In recent years, there have also been some reports on the effects of stress on antioxidant enzyme activities in plants [11-13]. However, theses researches are mostly implemented in artificial simulation conditions, the backgrounds are usually not karst area, and the index is relatively few. At the same time, there are few systematic comparative studies focusing on the activities of antioxidant enzymes such as CAT, POD, SOD, APX and GSH in karst areas. Thus, this study tried to select the excellent pioneer tree species suitable for the ecological restoration of the northwest Karst Rocky desertification of Guangxi through the comparative study on the antioxidant enzyme activity of common tree species in karst area, so as to provide theoretical support for the governance and ecological restoration of Karst Rocky desertification.

2. Materials and methods

2.1. Overview of the research area
This research was performed mainly in Hechi City, a typical karst area in the northwest Guangxi, which is located in 106°34'-109°09' E, 23°41'-25°37' N. This area is 228 km long, 260 km wide, the total area is 3.35×10^4 km² (Karst area accounts for 66%), the highest altitude is 1693 m, the average annual sunshine is 1447-1600 h, the average annual temperature is 16.9-21.5℃, and the average annual rainfall is 1200-1600 mm. This place has a mild climate, summer heat and winter cold would not been seen here. Four seasons are distinct, belonging to subtropical monsoon climate zone, which makes this place conducive to plants growth.

2.2. Sample collection and treatment
The samples of this study were the leaves of the pioneer species for the restoration of rock hill plants in karst area of the northwest Guangxi. Fresh leaves of Pteroceltis tatarinowii, Cyclobalanopsis glauca, Zenia insignis, Bauhinia variegata var.candida, Cinnamomum bunaniae, Thuja cccidentalis and other species common in karst areas of the northwest Guangxi were collected in Xiajian, Liumei village, white
dragon park, as well as LuoCheng County and Huanjiang County and other places of Yizhou District, Hechi City. According to the basic requirements of ecological field investigation, 5 plants of similar size were randomly selected from each tree species in the selected plot and sampled from the sunny side of each plant as far as possible. Samples were put into plastic sealing bags, followed by the corresponding record, number and label were made, and then were put in a curling pot for temporary preservation. After this, samples were brought back to the laboratory as soon as possible, the dust on the leaf surface was cleaned with distilled water. The water on the leaf surface was dried with clean absorbent paper. At last, it was stored in a refrigerator at 4℃ for the following experiments.

2.3. Instruments and reagents

**Instruments** UV-1800 ultraviolet-visible spectrophotometer (Shimadzu International Trading (Shanghai) Co., Ltd); 722N visible spectrophotometer (Shanghai Precision and Scientific Instrument Corporation); MP200A electronic analytical balance (Shanghai Jingke Balance); FCD-217SEN refrigerator (Haier Group); TGL-16R refrigerated centrifuge (Heima Medical Instrument Co., Ltd), PSH-3C pH meter (Shanghai San-Xin Instrumentation Inc.), HH-6 thermostatic water bath with digital display (Changzhou Guohua Electric Appliance Co., Ltd.), Aike DZG-303A barnstead (Taiwan Aike Chengdu Kangning Experimental Pure water equipment Factory).

**Reagents** sodium hydrogen phosphate, mono potassium phosphate (Guangdong Shantou Xining Chemical Plant), 30% hydrogen peroxide (Guangzhou New fine chemical Plant); Methionine (MET), NBT, riboflavin, polyvinylpyrrolidone (PVP), tris (hydroxymethyl) aminomethane sulphate (Sinopharm Chemical Reagent Co., Ltd.); EDTA-Na2 (Tianjin Kemiou Chemical Reagent Co., Ltd.); guaiacol (Tianjin Guangfu Fine Chemical Research Institute), hydrochloric acid (36-38%), sodium hydroxide, acetylsalicylic acid, 5,5’-Dithio bis-(2-nitrobenzoic acid), reduced glutathione hormone (Sinopharm Chemical Reagent Co., Ltd.). All the above drugs and reagents are analytical reagent (AR).

2.4. Methods

SOD, POD, CAT and AXP activities were determined by NBT photochemical reduction method, Guaiacol process, deoxidation of Hydrogen peroxide [14] and ultraviolet spectrophotometry [15], respectively. GSH content was assessed by TDNB color method [16].

2.5. Statistical analysis

Five replicates were set for each sample, and the results were averaged. The experimental data were processed and analyzed by Excel 2003 and SPSS18.0.

3. Results and analysis

In this study, a total of 19 common pioneer species for rock hill plants restoration were collected in the karst area of the northwest Guangxi. The antioxidant enzyme activity of various plant samples were shown in Table 1, and physiological indexes and soil physical and chemical characteristics of corresponding samples were shown in Table 2.

| Name of plants         | SOD (u.g⁻¹FW.h⁻¹) | POD (u.g⁻¹FW.min⁻¹) | CAT (u.g⁻¹FW.min⁻¹) | GSH (µg.g⁻¹FW⁻¹) | APX (u.g⁻¹.min⁻¹) |
|------------------------|-------------------|---------------------|---------------------|------------------|------------------|
| *Pteroceltis tatarinowii* | 1068.93±18.78     | 315.87±26.69        | 42.15±13.06         | 712.07±146.96    | 136.96±12.01     |
| *Zenia insignis*       | 5915.88±25.23     | 841.43±67.92        | 199.54±0.13         | 483.28±69.83     | 211.70±16.32     |
| *Cyclobalanopsis glauca* | 1084.37±26.83     | 108.99±15.5         | 61.96±11.92         | 417.49±31.34     | 389.07±81.35     |
| Name of plants                  | Malonaldehyde (µ mol·g⁻¹) | Proline (µg·mL⁻¹) | Cell membrane permeability (%) | Soil organic matter content (g·kg⁻¹) | Soil water content (%) | Soil protease activity (µg·g⁻¹) |
|--------------------------------|---------------------------|-------------------|-------------------------------|---------------------------------------|------------------------|-------------------------------|
| Pinus sylvestris               | 3784.90±506.92            | 41.99±28.11       | 409.79±23.43                  | 140.65±53.20                       | 67.59±18.18            |                               |
| Cinnamomum bumani               | 1059.04±12.24             | 712.29±15.65      | 28.96±11.65                   | 548.33±86.77                        | 271.49±49.12           |                               |
| Liquidambar formosana           | 644.39±0.09               | 6.75±0.469        | 72.19±41.95                   | 269.53±42.62                        | 294.82±35.83           |                               |
| Miscanthus floridulus           | 1463.13±643.05            | 8865.12±314.19    | 162.20±22.69                  | 210.18±45.93                        | 138.51±26.23           |                               |
| Dendrocalamus minor             | 2362.65±337.58            | 5300.98±570.79    | 304.02±12.50                  | 497.97±27.19                        | 139.36±32.83           |                               |
| Neyraudia reynaudiana           | 988.77±176.35             | 1334.75±265.63    | 217.06±25.76                  | 216.40±31.91                        | 126.70±26.31           |                               |
| Thuja occidentalis              | 6131.22±272.60            | 286.53±196.23     | 303.21±41.08                  | 384.31±62.49                        | 175.60±6.39            |                               |
| Eucalyptus globulus             | 6684.48±173.47            | 17.63±5.58        | 1599.46±42.85                 | 425.92±52.75                        | 901.61±80.58           |                               |
| Cynodon dactylon                | 2123.16±218.76            | 4075.68±112.26    | 147.02±39.02                  | 393.71±59.74                        | 133.31±5.12            |                               |
| Vitex negundo                   | 5565.53±174.39            | 15.99±8.99        | 527.85±296.12                 | 585.75±60.75                        | 446.93±11.34           |                               |
| Bauhinia variegata var. candida | 492.80±47.90              | 736.14±121.97     | 301.78±69.07                  | 601.99±93.48                        | 193.99±19.01           |                               |
| Ulmus parvifolia                | 4761.90±575.38            | 217.41±4.39       | 277.78±13.97                  | 1272.71±211.98                      | 620.71±22.51           |                               |
| Handeliodendron bodinieri       | 6809.52±67.34             | 584.24±142.47     | 17.31±8.97                    | 199.64±23.46                        | 89.66±1.75             |                               |
| Koelreuteia minor               | 4428.57±508.43            | 597.91±37.56      | 234.50±61.40                  | 405.41±25.62                        | 612.56±52.33           |                               |
| Pistacia weinmannifolia         | 4619.05±67.34             | 3.33±1.09         | 538.88±132.42                 | 534.25±9.82                         | 679.91±35.35           |                               |
| Platycarya longipes             | 4520.58±238.38            | 2.44±0.31         | 320.75±152.53                 | 1019.94±75.17                      | 648.31±46.23           |                               |

Table 2. Physiological indexes of pioneer plants and soil physical and chemical properties
3.1. Comparison of antioxidant enzyme activities of different pioneer species

3.1.1. Comparison of SOD activity. Studies have shown that SOD is the first antioxidant enzyme to play a role in the process of reactive oxygen scavenging reaction, and its level can reflect the strength of plants’ resistance to oxidation, that is, resistance to inversion [17]. As can be seen from Table 1, SOD activity of different pioneer species differs (Figure. 1). The SOD activity is relatively high in Handeliodendron bodinieri, Eucalyptus globulus, Thuja ccidentalis, Zenia insignis and Vitex negundo, while low in Liquidambar formosana, Neyraudi reynaudiana and Bauhinia variegata var.candida. The highest SOD activity is as much as 10 times than the lowest. The distinction of SOD activity among species may be related to its biological characteristics. In addition, the diverse soil physical and chemical properties in different sampling sites may also have a certain impact on the activity of SOD in plants, while the specific reasons need to be further analyzed.

3.1.2. Comparison of POD activity. POD is very sensitive to various adverse environments. When plants are subjected to stress, POD activity will change. Therefore, POD can be used as an indicator to judge plant resistance. As shown in Table 1, POD activity varies greatly among different plant species. Among them, the POD activity of Miscanthus floridulus was the highest, followed by that of Dendrocalamus minor and Cynodon dactylon, while in Liquidambar formosana, Neyraudi reynaudiana and Bauhinia variegata var.candida. The highest SOD activity is as much as 10 times than the lowest. The distinction of SOD activity among species may be related to its biological characteristics. In addition, the diverse soil physical and chemical properties in different sampling sites may also have a certain impact on the activity of SOD in plants, while the specific reasons need to be further analyzed.

3.1.3. Comparison of CAT activity. The main function of CAT is to clear H2O2 from peroxisomes and mitochondria in plant’s body [18]. The CAT activity of plants in this study was ordered as follows: Eucalyptus globulus > Pistacia weinmannifolia > Vitex negundo > Pinus sylvestris > Platycarya longipes > Dendrocalamus minor > Thuja ccidentalis > Bauhinia variegata var.candida > Ulmus parvifolia > Koelreuteta minor > Neyraudi reynaudiana > Zenia insignis > Miscanthus floridulus > Cynodon dactylon > Liquidambar formosana > Cyclobalanopsis glauca > Pteroceltis tatarinowii > Cinnamomum humanii > Handeliodendron bodinieri (Figure 1). According to the experimental results, the difference between the highest and lowest CAT activity was more than 100 times, indicating that the scavenging ability of hydrogen peroxide varied greatly among different species. As an important scavenging agent of hydrogen peroxide in plants, the enhancement of CAT activity was conducive to the regulation of H2O2 level in vivo, delaying or preventing the occurrence of cell apoptosis [19].

3.1.4. Comparison of GSH content. Glutathione reductase (GR) is a key enzyme in the antioxidant enzyme system of plant cells, catalyzing the reduction of glutathione disulfide (GSGS) to reduced glutathione hormone (GSH), maintaining a high GSH/GSGS ratio [20]. Therefore, the activity of GR enzyme can be determined by the content of GSH. As shown in Fig. 4, plant order according to the
GSH content is as follows: *Ulmus parvifolia* > *Platycarya longipes* > *Pteroceltis tatarinowii* > *Bauhinia variegata var. candida* > *Vitex negundo* > *Cinnamomum humanii* > *Pistacia weinmannifolia* > *Dendrocalamus minor* > *Zenia insignis* > *Eucalyptus globulus* > *Cyclobalanopsis glauca* > *Koelreuteia minor* > *Cynodon dactylon* > *Thuja occidentalis* > *Liquidambar formosana* > *Neyraudi reynaudiana* > *Miscanthus floridulus* > *Handeliodendron bodinieri* > *Pinus sylvestris*. Among the plants studied, there was a nine-fold difference between the highest and lowest GSH levels.

### 3.1.5. Comparison of APX activity

Among the plants studied, *Eucalyptus globulus* had the highest APX activity (1055.40 u.g⁻¹FW.min⁻¹), followed by *Pistacia weinmannifolia*, *Platycarya longipes*, *Ulmus parvifolia* and *Koelreuteia minor*, whose APX activity exceeded 600 u.g⁻¹FW.min⁻¹. *Vitex negundo* and *Cyclobalanopsis glauca* ranked third, with values ranging from 380 to 450 u.g⁻¹FW.min⁻¹. *Liquidambar formosana*, *Cinnamomum humanii*, *Bauhinia variegata var. candida* and *Thuja occidentalis* were the fourth, with values between 175 and 300 u.g⁻¹FW.min⁻¹. The difference between *Dendrocalamus minor*, *Miscanthus floridulus*, *Pteroceltis tatarinowii*, *Cynodon dactylon* and *Neyraudi reynaudiana* was not significant, and the value ranged from 125 to 140 u.g⁻¹FW.min⁻¹. *Handeliodendron bodinieri* and *Pinus sylvestris* were the smallest, with values less than 100 u.g⁻¹FW.min⁻¹ (Figure 1). *Eucalyptus globulus* was an alien species, and its APX was higher than all native plants.

Comparing the antioxidant enzyme (SOD, POD, CAT, GSH, APX) activity data of all kinds of above plants would lead to more intuitive results (Figure 1). It can be seen that the antioxidant enzyme activity of different tree species varies greatly, and its value was not only related to the factors of tree species themselves, but also may depend on the living conditions, environmental adaptability and other ecological factors. Through comparison, the variation trend of antioxidant enzyme activity in different tree species was also disparate.

Plants growth was affected by a variety of adverse environmental factors. Many conditions can increase reactive oxygen, including superoxide ion (O²⁻), hydroxyl radicals (HO⁻), singlet oxygen (¹O₂) and hydrogen peroxide (H₂O₂) levels, destroying the balance of antioxidant enzymes (SOD, POD, CAT, APX, GR) activity in plant body. Enzyme activity of *Dendrocalamus minor*, *Eucalyptus globulus*, *Miscanthus floridulus*, *Pinus sylvestris*, *Zenia insignis*, *Thuja occidentalis*, *Cynodon dactylon*, *Vitex negundo*, *Koelreuteia minor*, *Ulmus parvifolia*, *Handeliodendron bodinieri*, *Platycarya longipes* and *Pistacia weinmannifolia* was relatively high, indicating that their ability to withstand a hostile environment are stronger. Studies have shown that when plants are under various stress conditions, the activity of SOD in the body will be significantly increased. SOD catalyzes the disproportionation reaction of oxygen free radicals to produce hydrogen peroxide, which can be converted by CAT into harmless molecular oxygen and water. Peroxidase is one of the key enzymes in plant enzymatic defense system under adverse conditions. It coordinates with superoxide dismutase and catalase to eliminate excess free radicals and maintain free radicals at a normal dynamic level to improve plant stress resistance [22].
Figure 1. Comparison of antioxidant enzyme activities of different pioneer plants

3.2. Correlation analysis
Malonaldehyde (MDA), proline (Pro) and cytoplasmic membrane permeability (TSC) are important indicators of plant resistance physiology. And soil organic matter (SOM), soil water content and soil enzyme activities are key indexes of soil quality. In order to figure out the correlation between antioxidant enzyme activities and stress resistance of plants, the correlation between antioxidant enzyme activities (SOD, POD, CAT, GSH, APX) and MDA, proline, cytoplasmic membrane permeability, soil organic matter, soil moisture content and soil protease (SP) was analyzed. The results are shown in Table 3.

Table 3. Correlation between antioxidant enzyme activity and physiological indexes of plant resistance

|       | SOD  | POD  | CAT  | GSH  | APX  | MDA  | Pro  | TSC  | SOM  | H2O  | SP  |
|-------|------|------|------|------|------|------|------|------|------|------|-----|
| SOD   | 1    |      |      |      |      |      |      |      |      |      |     |
| POD   | -0.347 | 1    |      |      |      |      |      |      |      |      |     |
| CAT   | 0.492* | -0.194 | 1    |      |      |      |      |      |      |      |     |
| GSH   | 0.121 | -0.274 | 0.036 | 1    |      |      |      |      |      |      |     |
| APX   | 0.430 | -0.373 | 0.717** | 0.441 | 1    |      |      |      |      |      |     |
| MDA   | -0.204 | 0.328 | -0.196 | -0.111 | -0.135 | 1    |      |      |      |      |     |
| Pro   | -0.149 | -0.101 | -0.219 | 0.021 | -0.331 | 0.057 | 1    |      |      |      |     |
| TSC   | 0.172 | -0.016 | -0.216 | -0.410 | -0.134 | 0.242 | -0.105 | 1    |      |      |     |
| SOM   | 0.363 |      |      |      |      |      |      |      |      |      |     |
| H2O   | -0.009 | -0.181 | -0.370 | 0.310 | -0.128 | -0.338 | 0.301 | -0.089 | 0.381 | 1    |     |
| SP    | -0.318 | -0.157 | -0.265 | 0.061 | -0.358 | 0.064 | 0.545* | -0.285 | 0.205 | 0.489* | 1  |

* There was a significant correlation at the 0.05 level (bilateral)
** There was a significant correlation at the 0.01 level (bilateral)

As can be seen from Table 3, there were significant correlations between SOD and CAT, GSH and SOM, soil moisture and soil protease. POD was significantly negatively correlated with SOM. What’s more, positive correlations could be seen between CAT and APX, Pro and soil protease. Other indexes were not significantly correlated. These indicated that the factors affecting the activity of plant antioxidant enzymes were quite complex. The difference of soil background and human factors may
affect the growth status of plants, leading to the difference of enzyme activity. At the same time, the various plant growth environment and soil physical and chemical properties were bound to make a change in the activity of antioxidant enzymes in plants. The changes of each enzyme did not eliminate the relationship between each other. In fact, they influenced each other. When plants were subject to adverse environmental factors, enzymes worked together to make plants better able to resist and survive. However, which enzyme is more important remains to be further studied.

3.3. Cluster analysis
Antioxidant enzyme is one of the critical physiological indexes of plant resistance and its activity can reflect plants’ adaptation to environment or response to adverse environmental stress. According to the determination results of antioxidant enzyme system in the leaves of 19 plants in karst areas, the clustering figure was shown in Figure 2. Within the Euclidean range of 5, 19 species commonly found in karst areas can be divided into four categories: the first category included six species, including Cinnamomum humanii, Bauhinia variegata var.candida, Cyclobalanopsis glauca, Neyraudi reynaudiana, Liquidambar formosana and Pteroceltis tatarinowii. The second category contained Dendrocalamus minor and Cynodon dactylon. Ulmus parvifolia, Platycarya longipes, Pistacia weinmannifolia, Koelreutetia minor, Pinus sylvestris, Zenia insignis, Thuja occidentalis, Vitex negundo, Handeliodendron bodinieri and Eucalyptus globulus formed the third category. And the fourth category only contained Miscanthus floridulus. In the third category, Ulmus parvifolia, Platycarya longipes, Pistacia weinmannifolia, Koelreutetia minor and Pinus sylvestris clustered first, and Zenia insignis, Thuja occidentalis, Vitex negundo and Handeliodendron bodinieri also clustered, finally with Eucalyptus globulus. Within the Euclidean range of 5-8, 19 kinds of rock hill plants can be divided into three categories, the first and second plants can be classified into one category, and the third and fourth plants remained unchanged. Within the range of 10 Euclidean distances, these rock hill plants can be divided into two groups. The plants clustered in the same group had higher similarity with each other. On the contrary, difference was great.

The above clustering analysis only considered the growth of the plants themselves. If the influence of plants on the corresponding soil was also taken into consideration, the clustering results will change. Figure 3, showed the results of the cluster analysis of the antioxidant enzyme system of 19 common karst plants and the corresponding soil physiological indexes. As shown in Figure 3, the 19 species commonly found in karst areas can be divided into four categories in the range of Euclidean distance 5. The first category included Ulmus parvifolia, Platycarya longipes, Pistacia weinmannifolia, Koelreutetia minor, Vitex negundo, Pinus sylvestris, Zenia insignis, Thuja occidentalis, Handeliodendron bodinieri, Eucalyptus globulus. The second category contained Cinnamomum humanii, Cyclobalanopsis glauca, Pteroceltis tatarinowii, Bauhinia variegata var.candida, Liquidambar formosana and Neyraudi reynaudiana. The third category contained two species, Dendrocalamus minor and Cynodon dactylon.
Miscanthus floridulus was the only one included in the last category. In the first category, Ulmus parvifolia, Platycarya longipes, Pistacia weinmannifolia, Koelreuteia minor, Vitex negundo and Pinus sylvestris clustered first, and Zenia insignis, Thuja occidentalis and Handeliodendron bodinieri clustered first too, and then clustered with Eucalyptus globulus into a large group. Within the range of Euclidean distance 5-8, 19 species of rock hill plants can be divided into three categories, the second and third categories can be grouped into one category, and the other two categories remained unchanged.

By comparing these two graphs, it can been seen that the clustering order and classification of tree species had not changed much, while the clustering position of Vitex negundo varied a little. Vitex negundo clustered with Zenia insignis, Thuja occidentalis, Handeliodendron bodinieri into one group, according to the antioxidant enzymes system. After considering the impact on the soil, Vitex negundo clustered with Ulmus parvifolia, Platycarya longipes, Pistacia weinmannifolia, Koelreuteia minor and Pinus sylvestris, but in the end, they all went to the first category. This indicated that after considering the soil properties, Vitex negundo was close with Ulmus parvifolia and other species. It can also be seen from the figure that the influence of plants on soil was corresponding, basically consistent with the correlation analysis results in Table 3.

4. Discussion
The activity of antioxidant enzymes in various plants is different, which can be employed as a key evaluation index of plant stress resistance. Plants growth is affected by drought, barren, high temperature, cold, acid rain, saline, heavy metal pollution and other adverse environmental factors. In these case, plants will produce a large number of superoxide anions, hydrogen peroxide, singlet oxygen, hydroxyl radicals and other toxic substances, while the increase of activities of the antioxidant enzymes SOD, APX, GR and CAT in plants can enhance the resistance of plants to various oxidative stresses [17]. A large number of studies have found that increasing the activity of antioxidant enzymes and the level of antioxidant metabolism in plants are effective ways to improve its stress resistance [23,24]. In this research, the SOD activity of Handeliodendron bodinieri, Eucalyptus globulus, Thuja occidentalis, Zenia insignis and Vitex negundo were all high. And the POD activity of Miscanthus floridulus, Dendrocalamus minor and Cynodon dactylon were also high. This may indicate that they were good pioneer plants and can be widely used for ecological restoration. However, the problem should not be absolute. Other factors should be taken into consideration, such as the plant’s ability to repair soil, its adverse effects on the environment, economic benefits, whether the plant cultivation technology is relatively simple, maintenance consumption, economic feasibility, etc.

It was found that Eucalyptus globulus, Pinus sylvestris and other plants had excellent tolerance to environmental stress and could survive in some harsh habitats, even with some short-term economic benefits. However, due to its many adverse effects on the soil microenvironment, like a sharp decrease in soil moisture content, soil acidification, reduction of biodiversity, and unsuitability for the survival of other biological species, whether it is worthwhile to promote large-scale cultivation should be carefully considered, so as to avoid the deterioration of the ecological environment. Especially in karst areas, excessive reliance on a few exotic tree species, like Eucalyptus, for forestry development and excessive consideration of immediate interests makes it easy to ignore the protection of forest ecological security and green ecological barrier, or even cause serious ecological disasters.

5. Conclusion
Based on the above results, analysis and discussion, the following conclusions can be drawn preliminarily:

(1) If the antioxidant enzyme activity (physiological characteristics) is the only one factor taken into account, Dendrocalamus minor, Eucalyptus globulus, Miscanthus floridulus, Pinus sylvestris, Zenia insignis, Thuja occidentalis, Cynodon dactylon, Vitex negundo, Koelreuteia minor, Ulmus parvifolia, Handeliodendron bodinieri, Platycarya longipes and Pistacia weinmannifolia have stronger ability to adapt the environment and can be seen as candidates for the first batch of pioneer plants karst vegetation restoration in karst region.
(2) Considering the impact of plants on the soil environment and the ecological and economic benefits, *Eucalyptus globulus* should not be planted as a pioneer species in a large area for its adverse impact on the soil ecological environment.

(3) The selection of excellent pioneer species must comprehensively consider the plants’ physiological and ecological characteristics, as well as the ecological and economic benefits. Besides, the soil geological background of different regions should also be taken into account. Native species should be paid attention to minimize and avoid the blind introduction of foreign species. Excessive pursuit of immediate economic benefits might lead to disastrous consequences for karst ecological environment.

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