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The Migration Characteristics of *cfp* Fluorescent Labeled Rhizobium in *Dolichos lablab L.* Plants and Rocky Desert Surface Soil

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Abstract

In this study, cyan fluorescent protein (*cfp*) labeled endophytic rhizobia of *Dolichos lablab* L. was used as the test strains which had strong nitrogen-fixing ability, low fluorescence loss rate, and good genetic stability. Through methods such as reinoculation and spot application of bacterial suspension in rocky desert mountain areas, we investigated the quantity and distribution of fluorescently labeled rhizobia in *Dolichos lablab* L. plants at different growth stages, as well as the migration characteristics of these bacteria on the surface of rocky desertified soil in the absence of hosts. The results indicate that in terms of spatial distribution, the fluorescently labeled rhizobia concentrated in the roots primarily. Specifically, during the vegetative stage, they are mainly present in the taproot, while from the budding stage to the pod-setting stage, they are predominantly found in the lateral roots. In terms of temporal dynamics, the highest count during the vegetative stage and the lowest during the budding stage. The number of labeled bacteria in the aboveground part of the plant was only 39.93% of that in the root, mainly distributed in the stem tip, flower bud and pod. During the development from flower buds to pods, the quantity of labeled bacteria shows a trend of increase-decrease-increase, with the lowest count observed inside the flowers. The surface soil of rocky desertification provides a suitable microenvironment for the survival of fluorescently labeled rhizobia. These labeled rhizobia can colonize the surface soil of rocky desertification and migrate across the soil surface over time. They can migrate from the central point O in vertical, oblique, and horizontal directions, but their distribution at various sampling points is discontinuous and unstable.

Introduction

Dolichos lablab L. is a high-quality leguminous green manure crop. It can establish a symbiotic relationship with soil bacteria-rhizobia-allowing the plant to grow in nitrogen-deficient soil conditions (Zhang et al. 2020). Rocky desertification is a significant driver of ecological degradation. Studies have demonstrated that rhizobia associated with leguminous crops can enhance the physical and chemical properties of rocky desertified soils, reduce soil pH, and significantly increase the levels of organic matter and essential nutrients, such as nitrogen, phosphorus, and potassium. These findings highlight the potential of rhizobia

to improve soil quality and contribute to ecological restoration (Du et al., 2025). Therefore, introducing rhizobia into rocky desertification soils, even in the absence of leguminous crops, can improve soil properties, provide a source of nitrogen, and enhance overall soil health.

In this experiment, fluorescently labeled rhizobia of *Dolichos lablab* L. were introduced into *Dolichos lablab* L. plants and surface soils of rocky desertification areas. The study investigated the quantity and distribution of the labeled bacteria in different plant parts and at various growth stages of *Dolichos lablab* L., analyzed their movement within the plants, and examined their colonization ability and distribution characteristics in rocky desertified soils without a host plant. This research provides a theoretical foundation for the production of seeds pre-inoculated with rhizobia and offers valuable insights into improving soil conditions in the absence of leguminous crops.

Methods

Test Seeds: The experimental seeds used were *Dolichos lablab* L. of the "Rungao" variety, with a purity of over 90%.

Test Strain: The experimental strain was a genetically stable rhizobium strain (Y-1) capable of producing cyan fluorescence.

Preparation of Bacterial Suspension: After activation, strain Y-1 was inoculated into YMA liquid medium and cultured at 28°C with shaking at 120 r/min until the optical density (OD_{600nm}) reached 0.5-0.8. The culture was then centrifuged at 4000 r/min for 10 minutes to remove the supernatant, leaving the bacterial cells. The cells were resuspended in an equal volume of sterile water and dispersed to prepare the bacterial suspension.

Seed Treatment and Sowing: *Dolichos lablab* L. seeds were placed in a sterilized Erlenmeyer flask and soaked in 5% povidone-iodine solution for 5 minutes, followed by rinsing with sterile water 5-6 times. The seeds were then soaked in the bacterial suspension for 2 hours. Using the hole-sowing method, seeds were planted in plots with a spacing of 40 cm between holes and 45 cm between rows, with one seed per hole.

Bacterial Suspension Application: Every 30 days, 30 ml of the bacterial suspension was applied to each plant. Watering was adjusted as needed to maintain adequate soil moisture.

Plant Sampling: At various growth stages of *Dolichos lablab* L. (130 days [vegetative stage], 216 days [squaring stage], 226 days [Florescence], and 235 days [pod stage]), three plants were randomly selected at each stage. The selected plants, including their roots, were carefully uprooted, washed, and air-dried to remove surface moisture. Using sterile scissors, the plants were divided into different parts: root, stem, leaves, stem tip, and stage-specific parts, such as flower buds (squaring stage), flowers (Florescence), and pods (pod stage). Each plant tissue sample was surface-sterilized, ground, diluted, and plated following Zhang (2012). Colony counts were recorded for each sample, with each part tested in triplicate.

Soil Sampling in Rocky Desertification Areas: A rocky desertification site with no vegetation was selected for soil sampling. The site was located in Wudang District, Guiyang City, Guizhou Procince. Sampling points were established in three directions: horizontal (H), vertical (V), and diagonal (D), with five points along each direction. Adjacent points in each direction were spaced 30 cm apart (Fig. 1). The central point (O) was treated with 100 mL of bacterial suspension. Soil samples were collected from each sampling point on the 7th, 14th, and 21st days following bacterial application. For each sample, 1 g of soil was placed in a

sterile Erlenmeyer flask, mixed with 100 mL of sterile water to prepare a soil suspension. The suspension was diluted, plated, cultured, and colony counts were recorded following Zhang (2012).

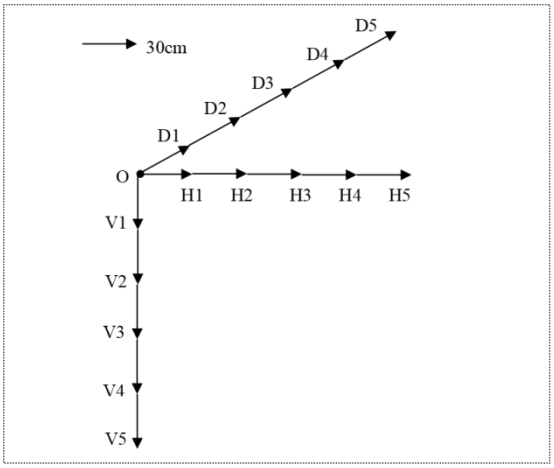

Fig.1 The distribution map of rocky deseritification mountain bacteria solution.

Results

[Quantity and distribution of labeled bacteria in different parts of Dolichos lablab L. plant at different growth stages]

Table 1 highlights the variation in the distribution of labeled bacteria across different parts of the root system and growth stages. During the vegetative stage, the quantity of labeled bacteria in the tap root was 14.58 times, 42.98 times, and 39.68 times higher than that in the squaring stage, florescence, and pod stages, respectively. Lateral roots exhibited the highest bacterial counts during the squaring stage, while the labeled bacteria in hair roots showed a gradual increase as the plant grew. In terms of timing, the root had the highest bacterial counts during the vegetative stage, with the lowest counts observed during the squaring stage. Within the vegetative stage, labeled bacteria were detected in the lower stem, lower leaves, and stem tip, with the highest concentration in the stem tip - 201.17% and 534.29% higher than in the lower stem and lower leaves, respectively. However, the distribution of bacteria in stems and leaves was discontinuous across different growth stages. During the reproductive growth phase, labeled bacteria were detected in all reproductive organs of *Dolichos lablab* L., with the highest counts observed in flower buds. The bacterial counts in flower buds were 5.59 times, 10.71 times, and 1.25 times higher than those in floral primordia, flowers, and pods, respectively.

Table 1 The number of labeled bacteria in each site of *Dolichos lablab* L. plants during different growth stage

Plant parts	The number of labeled bacteria (cfu·g^{-1}·FW)			
	Vegetative stage	Squaring stage	Florescence	Pod stage
Tap roots	71297	4577	1621	1752
Lateral roots	3563	4860	1101	701
Hair roots	6667	10230	18994	24276
Down Stem	7407	91	-	10886
Down leaves	3517	-	-	-
Stem tip	22308	-	-	-
Floral primordia	1341	-	-	-
Flower bud	-	7500	-	-
Flower	-	-	700	-
Pod	-	-	-	6000

Note: "-" means no labeled rhizobia. The same as below.

The quantity and distribution of labeled bacteria at different sampling points in surface soil of the karst mountainous area over time

Table 2 The number of labeled rhizobia at each point at different sampling times

Sampling point	The number of labeled bacteria at different sampling time (cfu·g^{-1}·FW)		
	Day 7	Day 14	Day 21
O	6971	1967	-
H1	-	31527	-
H2	-	-	-
H3	-	-	1196
H4	-	-	50
H5	-	-	3162
D1	-	-	+
D2	-	19927	149
D3	-	75	325
D4	-	+	5645
D5	-	667	1166
V1	2379	575	+
V2	-	-	27634
V3	-	-	9481
V4	-	7409	535
V5	-	-	50

Table 2 reveals that the quantity of labeled bacteria at the central point (O) gradually decreases as the inoculation time increases. Horizontal direction: On the 7th day, no labeled bacteria were detected at any sampling points. By the 14th day, labeled bacteria were detected only at H1, while on the 21st day, they were detected only at H3. This indicates that labeled bacteria migrate horizontally over time and distance. Vertical direction: On the 7th day, labeled bacteria were detected only at V1. By the 14th day, they were detected at V1 and V4. By the 21st day, labeled bacteria were detected at all points except V1. Diagonal direction: On the 7th day, no labeled bacteria were detected at any point. By the 14th day, labeled bacteria were detected at D2, X3, and D5. By the 21st day, labeled bacteria were detected at all points except D1.

In conclusion, labeled bacteria can effectively colonize surface soils and demonstrate tracer effects, providing valuable insights into their distribution characteristics in rocky desertification environments.

Discussion

Most labeled bacteria were primarily distributed in the plant's root, consistent with previous studies, which have shown that while endophytic bacteria can move within plants, they exhibit a preference for specific parts (Zhang 2012; Zhang et al. 2020). Labeled bacteria within the root can migrate to the above-ground parts of the plant, but their distribution is discontinuous. For instance, bacteria have been observed moving from roots to stems and leaves in tobacco, rice, and clover plants. The variation in microenvironments across different plant parts results in endophytic bacteria occupying distinct ecological niches within various organs and tissues (Chi 2006; Gyaneshwar et al. 2001; Zhang et al. 2020). During the development from flower buds to blooms, the number of labeled bacteria in flowers decreased, likely because the nutritional environment during development failed to induce bacterial chemotaxis. Endophytic bacteria can move and adhere to root surface through chemotaxis or by chance, facilitated by root exudates. After mutual recognition and penetration of the root surface, they can colonize the host plant (Artur et al. 2019). When inoculated onto rocky desertified surfaces, the distribution of labeled bacteria in different directions was discontinuous. However, their widespread presence at greater distances from the inoculation point demonstrates that bacteria can migrate to deeper soil layers and spread through capillary water. Additionally, even in the absence of a host, labeled bacteria can colonize rocky desertified soils as independent entities. This suggests that rocky desertified soil provides a microenvironment conducive to bacterial survival and colonization.

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