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RESEARCH

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Analysis of exogenous lactic acid bacteria on growth and development of different herbaceous peony varieties and rhizosphere soil nutrients

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Abstract

There are replanting problems in the production of herbaceous peony. If ramet seedlings are replanted in the original planting hole, they weaken year-by-year until their death, which reduces the land utilisation rate and increases the production costs. In this study, exogenous lactic acid bacteria (LAB, the main component is *Lactobacillus plantarum*) were applied to improve the planting soil of herbaceous peony for the first time to alleviate the replanting problems, to reduce the production costs, and to provide a new way to promote the of the herbaceous peony industry. In this study, herbaceous peony main cultivars varieties 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' were selected, and experiments were conducted using exogenous LAB. Morphological, rhizosphere soil, and root physiology indexes were measured by sampling at the end of the high-growth periods of herbaceous peony. The results showed that after LAB treatment, the plant height, flowering rate, other morphological indexes and root vitality of 'Hongxiuqiu' were increased, with a better promoting effect than that of 'Dafugui' and 'Zifengyu'. The 'Dafugui' rhizosphere soil nutrient content and enzyme activity were improved, followed by 'Hongxiuqiu' and 'Zifengyu'. The rhizosphere soil free salicylic acid content and root abscisic acid content decreased, whereas the soil bacterial abundance, root antioxidant enzyme activity, proline and paeoniflorin content increased in all varieties. This study found that LAB application can improve soil fertility and enzyme activity, promote the growth and development of herbaceous peony, increase the flowering rate and improve the ornamental value. However, the influence of LAB on different herbaceous peony varieties varied. Therefore, it is necessary to further expand the number of varieties, optimise the application concentration and frequency of LAB application, alleviate the replanting problems in herbaceous peony production, improve the utilisation rate of land, promote excellent varieties of herbaceous peony, and provide a new methods and references.

Keywords *Lactobacillus plantarum*, Herbaceous peony, Soil environment, Replanting problems

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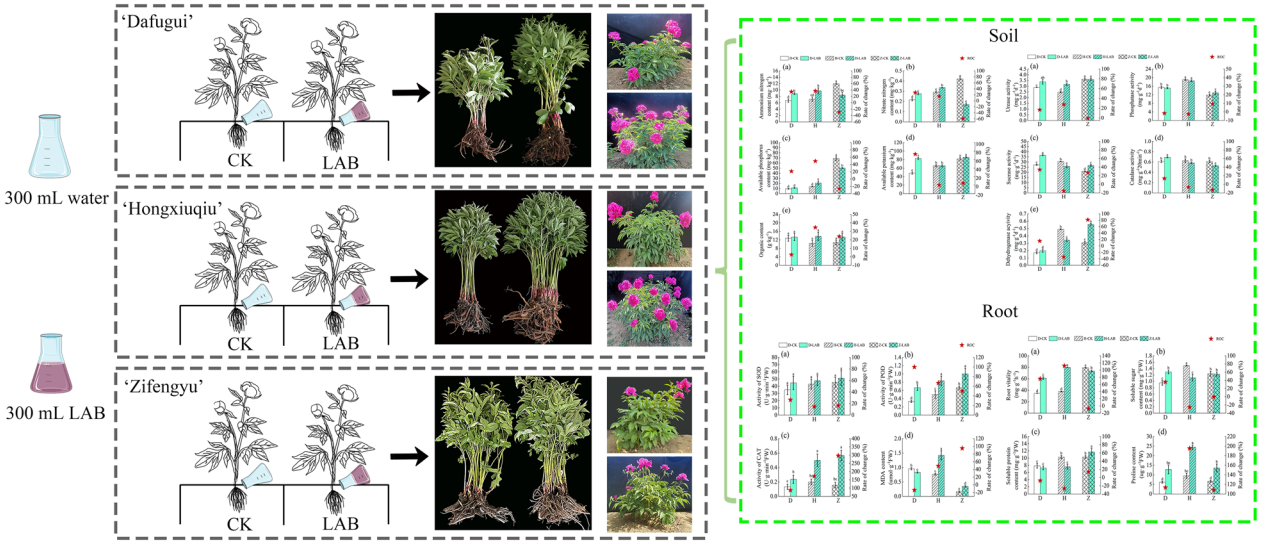
Graphical abstract



Introduction

Herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional Chinese flower that has long history of cultivation and rich cultural deposits. Herbaceous peony has a wide variety of species, including large, colourful, light, and elegant flowers with high ornamental value. Research on herbaceous peony has focused on fresh-cut flowers and medicinal uses, and the cultivation of high-efficiency, high-quality and high-yield is also the focus of current research [1, 2]. To maintain the excellent characteristics of ornamental herbaceous peony, propagation with root ramets is used in production; however, replanting ramet seedlings back to the original planting hole is prone to replanting problems, leading to an increase in the content of secondary metabolites in the soil, also root vitality and soil nutrient is decreased, and there is an imbalance in the soil microbial community structure. This is accompanied by a gradually weakening of herbaceous peony growth, eventually leading to plant death [3]. As the number of years herbaceous peony replantation increased, the phenolic acid content of the soil gradually increased. Exceeding a certain concentration will aggravates the occurrence of soilborne diseases, reduces the ability of the root system to absorb nutrients, affects the growth and development of the plant [4], and limits the development of herbaceous peony production and the promotion of elite varieties. However, how to improving the soil is conducive to the growth of herbaceous peony, which is an urgent problem that needs to be solved for current production.

Rhizosphere soil contains bacteria, fungi, actinomycetes, and other microorganisms, and the interactions between these microorganisms and plants plays a beneficial role in plant growth and development, adaptation, and biodiversity [5]. Furthermore, variations in plant cultivars and soil conditions lead to differences in the abundance of rhizosphere microorganisms. Therefore, the application of microbial agents to the plant rhizosphere microbial communities has attracted considerable attention. Some studies have shown that microbial agents contain a large number of active microorganisms, and are plant growth promoters and soil conditioners [6]. Common microbial agents include PGPR, *Bacillus subtilis*, *Trichoderma harzianum*, and lactic acid bacteria, which are beneficial microbial agents that can improve the physical and chemical properties of the soil and thus enrich its microbial population [2]. The application of lactic acid bacteria compound fertiliser on saline land accelerates the decomposition of organic and insoluble substances, increases the nutrient elements in the soil, improves the growth indices of strawberry plants, such as height and stem thickness, and promotes the growth and development of plants [7, 8]. The PGPR treatment increased the number of flowers per plant and improved chrysanthemum biomass [9]. PGPR (YDSY1-13) was isolated from the rhizosphere soil of herbaceous peony, enhanced the photosynthetic capacity, nutrient accumulation, and

antioxidant enzyme activity of herbaceous peony, and it also promoted the growth of herbaceous peony roots [10].

LAB is a general term for a class of microorganisms that produce large amounts of lactic acid [11]. Among the various beneficial LAB strains, *Lactobacillus plantarum* is of great significance in promoting plant growth and development, enhancing plant resistance to adversity, and improving crop nutritional value [12–15]. However, *Lactobacillus plantarum* is mostly used in wheat, cucumber, and other crops or vegetables, there are few studies on ornamental plants, and there are no reports on herbaceous peony [13, 16]. Therefore, this study is the first to analysed the effects of exogenous LAB on the physicochemical properties, enzyme activity of rhizosphere soil, and root physiology of herbaceous peony, attempted to scientifically improve and regulate the soil microbial environment using safer and low-cost exogenous LAB, and lay a foundation for solving the replanting problems of herbaceous peony, improving the soil for planting herbaceous peony at a low cost, and promoting its growth and development. Furthermore, this study provides a theoretical reference for the cultivation of high-efficiency, high-quality, and high-yield of herbaceous peony, as well as the application and popularisation of exogenous LAB.

Materials and methods

Material selection and processing

This study was conducted from April 2021 to October 2022 at the Herbaceous Peony Resource Nursery and Horticultural Experiment Centre in the Horticultural Experiment Station of Shandong Agricultural University. The experimental park has a temperate continental monsoon climate, four distinct seasons, rain and heat at the same time, an average annual temperature of 13.1 ℃, an average annual precipitation of 675.3 mm, soil pH of 7.35, soil organic matter content of 8.86 $g{\cdot}kg^{-1}$, an available nitrogen content of 6.56 $mg{\cdot}kg^{-1}$, an available phosphorus content of 20.79 $mg{\cdot}kg^{-1}$, and an available potassium content of 49.25 $mg{\cdot}kg^{-1}$. The main cultivars 'Dafugui', 'Hongxiuqiu', and 'Zifengyu' in production were used in the experiments; these plants grown for 3 years in a 3-year plantation and those that were in good health, i.e., free of diseases and pests after division were selected. The main component of exogenous LAB is *Lactobacillus plantarum* (effective viable count of *Lactobacillus plantarum* $>1\times10^{10}$ CFU/g, mixed bacteria rate $\leq$ 1%, water content $\leq$ 13%, Carrier: dextrin), Provided by Henan Hebi Xingwang Biotechnology Co., Ltd. The LAB product was diluted 100 times and used as the mother liquor (effective viable count of *Lactobacillus plantarum* $\geq1\times10^{8}$ CFU/g), the mother liquor was diluted with water at 1:50, and 300 mL of diluent was used for root irrigation of herbaceous peony.

The test herbaceous peony varieties were set up in control groups (CK) and treatment groups (LAB), labelled as: 'Dafugui' control group (D-CK), 'Dafugui' treatment group (D-LAB), 'Hongxiuqiu' control group (H-CK), 'Hongxiuqiu' treatment group (H-LAB), 'Zifengyu' control group (Z-CK), and 'Zifengyu' treatment group (Z-LAB). Three replicate plots were established for each treatment group, with 20 plants per plot. Isolation plates (resin-based composite fibre-reinforced plastic) were buried 60 cm deep between adjacent treatments and arranged into randomised groups [17]. Four times of root irrigation treatment (April 8, July 22, October 27, 2021 and March 15, 2022) with 300 mL of dilution were applied each time to the control group; 300 mL of water was applied simultaneously to the control group, with regular cultivation and maintenance management.

The rhizosphere soil (the soil was collected 1–2 mm from the surface of the root) and root systems of herbaceous peony were sampled at the end of the high growth period (April 21, 2022, when the soil was in the microbial activity period) for simultaneous determination of morphological indicators, and three plants were randomly selected from each treatment. The entire plant was pull out, the rhizosphere soil was collected using a sterile brush, and the soil was divided into two parts. One part was used for microbial count determination. Another part was stored in a cool, ventilated place and dried with a 1 mm sieve for the determination of soil physical and chemical properties. The fine roots removed and washed with distilled water, cut into 2 cm segments, placed in sample tubes, and transported to the laboratory in liquid nitrogen. Samples were stored at − 80℃ for determination of physiological indices.

Determination of plant morphological indexes

On April 21, 2022, three plants were randomly selected to determine their plant morphological indexes. The plant height was measured using a tape measure. Stem thickness at 3 cm from the soil surface was measured using a Vernier calliper. The third functional leaf of the plant facing south was selected to determine leaf length, width, and area using a YMJ-B leaf area-measuring instrument. Fresh weight (FW) is the weight of the aboveground part of each plant, dry weight (DW) is the weight of aboveground part dried at 85 ℃ for 24 h when the weight of the sample does not change. The plant water content (WC) was determined using the following formulae:

$WC=(FW-DW)/FW\times100\%$. The flowering rate and root to shoot ratio were calculated using the following formulae:

Flowering rate = number of flowers/number of stems (lateral buds were excluded from the flowering rate calculation).

Root to shoot ratio = dry weight of roots/dry weight of above ground parts.

Determination of physical and chemical properties of rhizosphere soil

Determination of pH and conductivity of rhizosphere soil

The rhizosphere soil pH and conductivity were determined using a 5:1 water-to-soil ratio suspension, with a PHS-3C acidity meter, and a Raycom DDB-303A portable conductivity meter [18].

Determination of organic matter and nutrient content of rhizosphere soil

The nitrate and ammonium nitrogen contents were determined using the KCl leaching indophenol blue colorimetric method, the fast-acting phosphorus content was determined using the molybdenum antimony ant colorimetric method, fast acting potassium content was determined using the ammonium acetate flame photometer method, and the organic matter content was determined using the potassium dichromate sulfuric acid dilution heat method [18]. The detailed methods are described in the Additional file 1.

Determination of rhizosphere soil enzyme activity

The sucrase activity was determined using the 3,5-dinitrosalicylic acid colorimetric method, phosphatase activity was determined using the sodium phosphate colorimetric method, urease activity was determined using the sodium phenol sodium hypochlorite colorimetric method, catalase activity was determined using the potassium permanganate titration method, and dehydrogenase activity was determined using the TTC method [19]. The detailed methods are described in the Supporting Information.

Determination of rhizosphere soil microbial population

Bacteria were cultured observed and recorded after 1 day of incubation in LB medium at 38 °C. Fungi were cultured observed and recorded after 2 days of incubation in PDA medium at 28 ℃. Actinomycetes were cultured observed and recorded after 3–4 days of incubation in Gauze's No.1 medium at 28 ℃ [20].

Determination of the secondary metabolite content in rhizosphere soil

The salicylic acid content of the rhizosphere soils was determined using a high-performance liquid chromatography. Extraction method: about 0.50 g of herbaceous peony rhizosphere soil was weighed, then 1 mL of precooled 90% methanol aqueous solution was added, and extracted at 4 ℃ overnight. Centrifugation was then performed at 8500 r/min for 10 min. The residue was extracted with 90% methanol aqueous solution (0.5 mL) for 2 h, and the supernatant was removed after centrifugation. The two supernatants were combined, evaporated until no organic phase was present at 40 ℃. 20 μL 1 mg/mL TCA Solution was added, mixed well and then shaken for 1 min. A mixture of 1 mL of ethyl acetate (1 mL) and cyclohexane (1:1v/v) was added and extracted twice. The upper organic phase was transferred to a new EP tube, dried with nitrogen, dissolved by 0.5 mL of methanol, filtered and tested.

HPLC liquid phase conditions: Baiso C18 reversed phase chromatographic column (250 mm*4.6 mm, 5 μm) was used for analysis. The column temperature was maintained at 35 ℃, and the detection wavelength of the UV detector was set at 294 nm. 1% acetic acid water (mobile phase A) and methanol (mobile phase B) were used as mobile phases (where A:B = 4:6). The flow rate was set at 0.8 mL/min and the retention time was 30 min. The chromatographic column was washed with the mobile phase, and the sample was detected after the baseline was stable.

Determination of plant root physiological indicators

Determination of root antioxidant enzyme activity and malondialdehyde content

Root superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) activities were determined using the method established by Cang Jing and Zhao Huijie [21, 22]. The detailed methods are described in the Supporting Information.

Determination of root vitality and osmotic adjustment substances

The root is an important organ for plants in order for the plant to fix themselves to the ground and for absorbing water and nutrients. The strength of root vitality indicates the growth and development of roots. The root vitality was determined using the triphenyl tetrazolium chloride method [23], soluble sugar content was determined using the anthrone colorimetric method, soluble protein content was determined using the Coomassie Brilliant Blue method, and proline content was determined using the sulfosalicylic acid method [22]. The detailed methods are described in the Supporting Information.

Determination of the phytohormone content in the root system

Indole acetic acid (IAA), abscisic acid (ABA), and spermine contents in the root system were determined using high-performance liquid chromatography. About 0.20 g of herbaceous peony root sample was weighed,

and 1 mL precooled reagent 1(methanol:water:acetic acid = 80:20:1) was added, centrifuged at 8500 r/min 4 ℃ for 10 min was carried out. The residue was extracted with 0.5 mL reagent for 2 h. After centrifugation, the supernatant was removed, and they were merged twice. The organic phase was dried using nitrogen at a temperature of 40 ℃. Then, 0.5 mL of petroleum ether was added to the extract and decolorize three times, and the upper ether phase was discarded. The pH was adjusted to 2.8 using a saturated citric acid solution. The organic phase was combined after three times of extractions with ethyl acetate and dried under nitrogen atmosphere. Finally, methanol (0.5 mL) was added to dissolve the vortex, and the test was performed after filtration.

HPLC liquid phase conditions: a Compass-C18 chromatographic column (4.6 mm*250 mm, 5 μm) was used for analysis. The column temperature was maintained at 35℃, and the detection wavelength of the UV detector was set at 254 nm. 1% acetic acid water (mobile phase A) and methanol (mobile phase B) were used as mobile phases (where A:B = 5:5). The flow rate was set at 0.8 mL/min and the retention time was 40 min. The chromatographic column was washed with mobile phase, and the sample was detected after the baseline was stable.

The preparation method for spermidine samples refers to the method of referring ABA and IAA. HPLC liquid phase conditions: Sepax BIO-C18 reversed phase chromatographic column (250 mm*4.6 mm,5 μm) was used for analysis. The column temperature was maintained at 40 ℃, and the detection wavelength of the UV detector was set at 340 nm. Methanol (mobile phase A) and water (mobile phase B) were used as mobile phases. Table 1 lists the details of the elution procedures.

Determination of the paeoniflorin content in the root system

The paeoniflorin content of the root system was determined using high-performance liquid chromatography. Extraction method: about 0.20 g of herbaceous peony root sample was weighed, and with 1 mL of precooled 50% ethanol water was added out. It was then grounded into a slurry, transferred into EP tube, and heated at 60 ℃ for 30 min. Centrifugation at 14000 r/min 4 ℃ for 10 min was carried out, then the supernatant was taken and tested after passing through a membrane.

HPLC liquid phase conditions: Sepax BIO-C18 reversed phase chromatographic column (250 mm*4.6 mm,5 μm) was used for analysis. The column temperature was maintained at 30 ℃, and the detection wavelength of the UV detector was set at 230 nm.

Acetonitrile (mobile phase A) and 0.015% phosphoric acid in water (mobile phase B) were used as mobile phases. The elution procedures are listed in Table 2.

Data analysis

A one-factor analysis of variance, as implemented using IBM SPSS Statistics 23.0 (SPSS, Inc., Chicago, IL, USA), was conducted to analyze the plant morphology, soil and other indicators data among the treatments. Duncan's multiple range test was then performed using the least significant differences test or Tamhane's T2 post hoc test. The threshold for determining the significant differences was $P < 0.05$. Using Origin 2021 software was used for mapping.

Results

Morphological changes in herbaceous peony under LAB treatment

LAB treatment promoted the growth of herbaceous peony. Compared with the control group, the plant height and flowering rate of 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' increased significantly (Fig. 1, Table 3). There was no significant difference in thick stems between the treatment groups of 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' and the control group. After LAB treatment, the leaf length, leaf width and leaf area of 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' were not significantly

Table 1 Gradient elution programs of spermidine

Time(min)	Flow rate(mL min^{-1})	Percentage of mobile phase (%)	
		Methanol (Mobile phase A)	Water (Mobile phase B)
0. 00	0.8	80	20
3.00	0.8	80	20
14.00	0.8	100	0
16.00	0.8	100	0
27.00	0.8	80	20
40.00	0.8	80	20

Table 2 Gradient elution programs of paeoniflorin

Time (min)	Flow rate (mL min^{-1})	Percentage of mobile phase (%)	
		Acetonitrile (Mobile phase A)	0.015% Phosphoric acid water (Mobile phase B)
0. 00	0.8	8	92
5.00	0.8	12	88
17.50	0.8	13	87
20.00	0.8	14	86
38.00	0.8	20	80
48.00	0.8	30	70
63.00	0.8	60	40
73.00	0.8	8	92

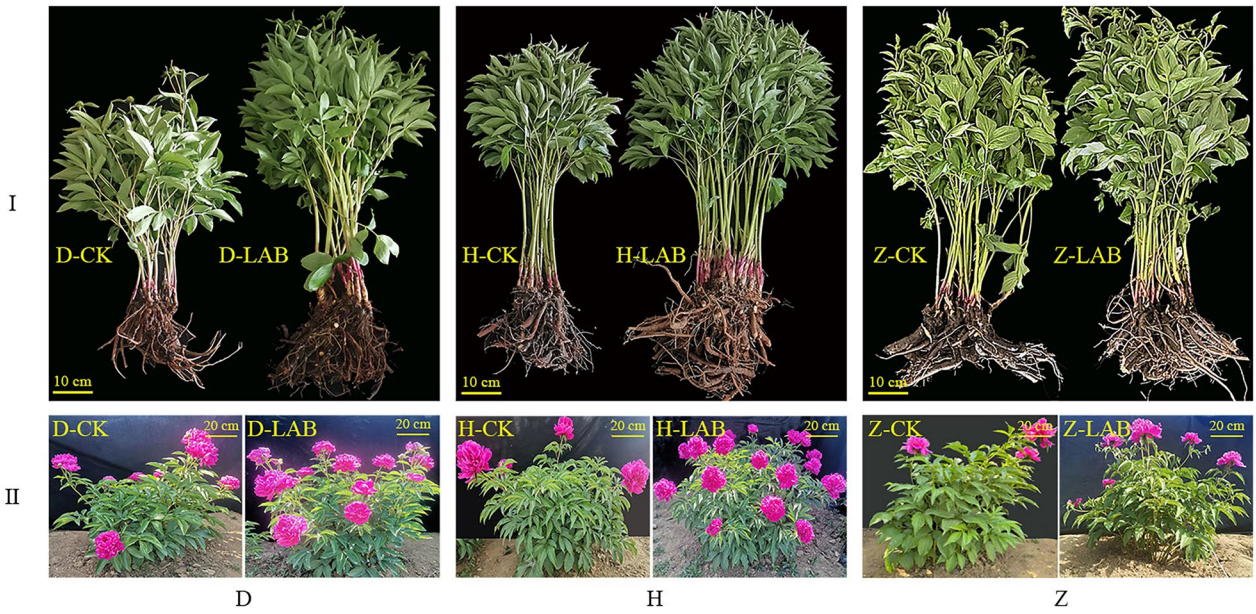


Fig.1 Morphology of herbaceous peony varieties 'Dafugui', 'Hongxiuqiu', and 'Zifengyu' after different treatments**.** I: Whole plant state of herbaceous peony, II: the herbaceous peony flowering state. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB*: 'Zifengyu' treatment group

Table 3 Effects of LAB on growth index growth rate of different varieties of Herbaceous peony

Variety	Plant height	Flowering rate (%)	Thick stems (mm)	Leaf length (mm)	Leaf width (cm)	Leaf area (cm^2)	Root–shoot ratio	Plant water content
D-CK	63.20 ± 0.53 e	65.86 ± 1.33 b	7.49 ± 0.13 b	7.68 ± 0.13 bc	4.26 ± 0.17 ab	24.19 ± 1.28 b	1.69 ± 0.01 a	68.74 ± 0.80 b
D-LAB	69.48± 0.68 d	73.53 ± 1.78 a	8.25 ± 0.14 ab	7.83 ± 0.03 bc	4.58 ± 0.12 a	27.57 ± 0.57 b	1.23 ± 0.03 bc	42.67 ± 2.03 d
H-CK	70.00 ± 1.97 d	62.58 ± 0.98 b	6.18 ± 0.18 c	5.18 ± 0.32 d	2.90 ± 0.18 c	11.42 ± 0.87 c	1.48 ± 0.11 ab	63.67 ± 2.40±
H-LAB	76.50 ± 1.70 c	74.00 ± 0.58 a	7.32 ± 0.34 bc	6.22 ± 0.57 cd	3.29 ± 0.24 bc	15.48 ± 0.80 c	1.52 ± 0.03 a	77.00 ± 1.00 a
Z-CK	89.95 ± 1.68 b	56.28 ± 1.11 c	9.18 ± 0.33 a	10.69 ± 0.23 a	5.04 ± 0.07 a	37.68 ± 1.21 a	1.08 ± 0.05 c	69.00 ± 1.15 b
Z-LAB	98.14 ± 1.02 a	66.57 ± 1.72 b	8.13 ± 0.27 ab	9.25 ± 0.81 ab	4.71 ± 0.45 a	34.55 ± 0.02 c	1.09 ± 0.02 c	61.00 ± 0.58 c

All values were represented as the mean ± SE of three replicates ($n = 3$). The same letter in a column indicates that the difference did not reach a significant level ($P > 0.05$) and different letters indicate significant differences ($P < 0.05$).

D-CK 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group

different from those of the control group. After applying LAB, the root–shoot ratio of 'Dafugui' decreased, and the difference between 'Hongxiuqiu' and 'Zifengyu' was not significant difference. The plant water content of 'Dafugui' and 'Zifengyu' decreased, the plant water content of 'Hongxiuqiu' increased (Table 3).

Changes in the rhizosphere soil of herbaceous peony under the treatment of LAB

Changes in rhizosphere soil pH and conductivity

Rhizosphere soil pH increased for all varieties following LAB treatment, by 5.75%, 2.87%, and 1.33% for 'Dafugui', 'Zifengyu', and 'Hongxiuqiu' (Fig. 2a). Compared with the control group, the conductivity of 'Hongxiuqiu' decreased significantly by 3.7%, 'Zifengyu' showed no significant difference, 'Dafugui' increased by 30.37% (Fig. 2b).

Changes in rhizosphere soil nutrient and organic matter content

The effects of rhizosphere soil ammonium and nitrate nitrogen content on different herbaceous peony varieties were inconsistent. The contents of ammonium nitrogen and nitrate nitrogen in rhizosphere soil of 'Dafugui' and 'Hongxiuqiu' were significantly increased compared with the control group, 'Dafugui' increased by 34.5% and 27.59%, respectively. 'Hongxiuqiu' increased by 36.99% and 14.97%, respectively, the content of 'Zifengyu'

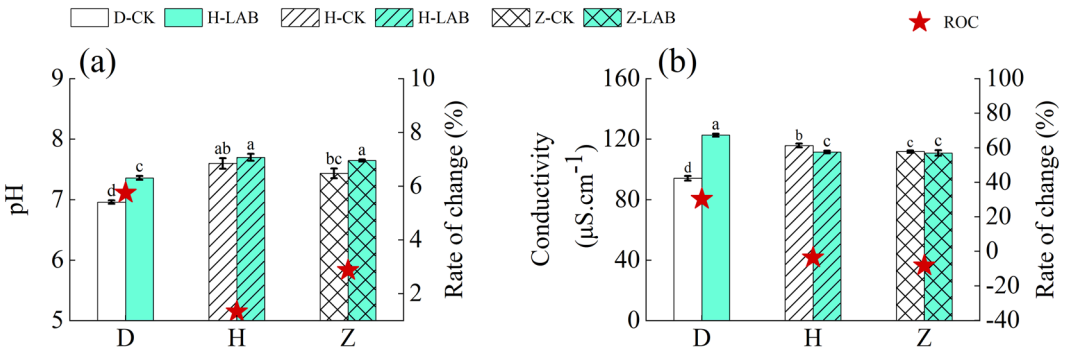


Fig. 2 Effects of LAB on rhizosphere soil pH and conductivity of herbaceous peony and the rates of change**. a** pH; **b** Conductivity. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates ($n = 3$). The same letter in a column indicates that the difference did not reach a significant level ($P > 0.05$), while different letters indicate significant differences ($P < 0.05$)

rhizosphere soil decreased by 29.48% and 59.1%, respectively (Fig. 3a, b); after treatment with LAB, the content of available phosphorus in the rhizosphere soil of 'Hongxiuqiu' was significantly different from that of the control group, which increased by 49.22%, 'Dafugui' showed no significant difference, 'Zifengyu' decreased by 26.6% (Fig. 3c); the content of available potassium in the rhizosphere soil of 'Dafugui' increased significantly by 72.93% when compared with the control, 'Hongxiuqiu' and 'Zifengyu' were no significant difference (Fig. 3d); the content of organic matter in rhizosphere soil of three varieties increased after LAB treatment, the change of rate was 'Hongxiuqiu' (44.38%) > 'Zifengyu' (33.79%) > 'Dafugui' (2%) (Fig. 3e).

Changes in rhizosphere soil enzyme activity

After LAB treatment, the urease activity in the rhizosphere soil of 'Dafugui' and 'Hongxiuqiu' increased by 16.89% and 27.76%, respectively, and was significantly higher than that of the control group, 'Zifengyu' was no significant difference (Fig. 4a). After LAB treatment, there was no significant difference in phosphatase activity in the rhizosphere soil of 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' compared with the control group (Fig. 4b). Compared to the control group, the rhizosphere soil sucrase activity of 'Dafugui' and 'Zifengyu' increased by 34.16% and 26.92%, respectively, while that of 'Hongxiuqiu' decreased by 15.59% (Fig. 4c). The rhizosphere soil catalase activity of 'Dafugui' increased by 13.66%, while that of 'Hongxiuqiu' and 'Zifengyu' decreased by 6.84% and 12.98%, respectively (Fig. 4d). LAB treatment had a significant effect on the activity of dehydrogenase in three variety rhizosphere soil, the dehydrogenase activity in the rhizosphere soil of 'Zifengyu' was significantly increased by 81.42%, 'Dafugui' is no significant difference, 'Hongxiuqiu' was reduced by 34.1% (Fig. 4e).

Changes in rhizosphere soil microorganism quantity

After the application of LAB, there was no significant difference in the number of bacteria in the rhizosphere soil of 'Dafugui' 'Hongxiuqiu' and 'Zifengyu' (Fig. 5a). After treatment with LAB, the rhizosphere soil fungi quantity for 'Hongxiuqiu' and 'Zifengyu' decreased by 38.89% and 15.31%, respectively, while that of 'Dafugui' increased by 55.99% (Fig. 5b). The rhizosphere soil actinomyces quantity in the three varieties increased by 52.39% for 'Hongxiuqiu', 27.36% for 'Zifengyu', and 16.90% for 'Dafugui' (Fig. 5c).

Changes in rhizosphere soil free salicylic acid content

Following the LAB treatment, the soil salicylic acid content decreased by 37.83% in 'Dafugui', 23.90% in 'Hongxiuqiu', and 19.75% in 'Zifengyu'. Among them, the degradation effect of LAB on rhizosphere soil salicylic acid content for 'Dafugui' was better than that of 'Hongxiuqiu' and 'Zifengyu' (Fig. 6).

Physiological changes of herbaceous peony root systems treated with LAB

Changes in antioxidant oxidase activity and root MDA content

The SOD activity of herbaceous peony roots increased following LAB treatment, with the largest increase in 'Dafugui' (26.54%), followed by 'Zifengyu' (16.54%) and 'Hongxiuqiu' (14.72%) (Fig. 7a). The POD activity increased in the root systems of all three varieties, there is a significant difference compared with the control group, with the largest increase in 'Dafugui'

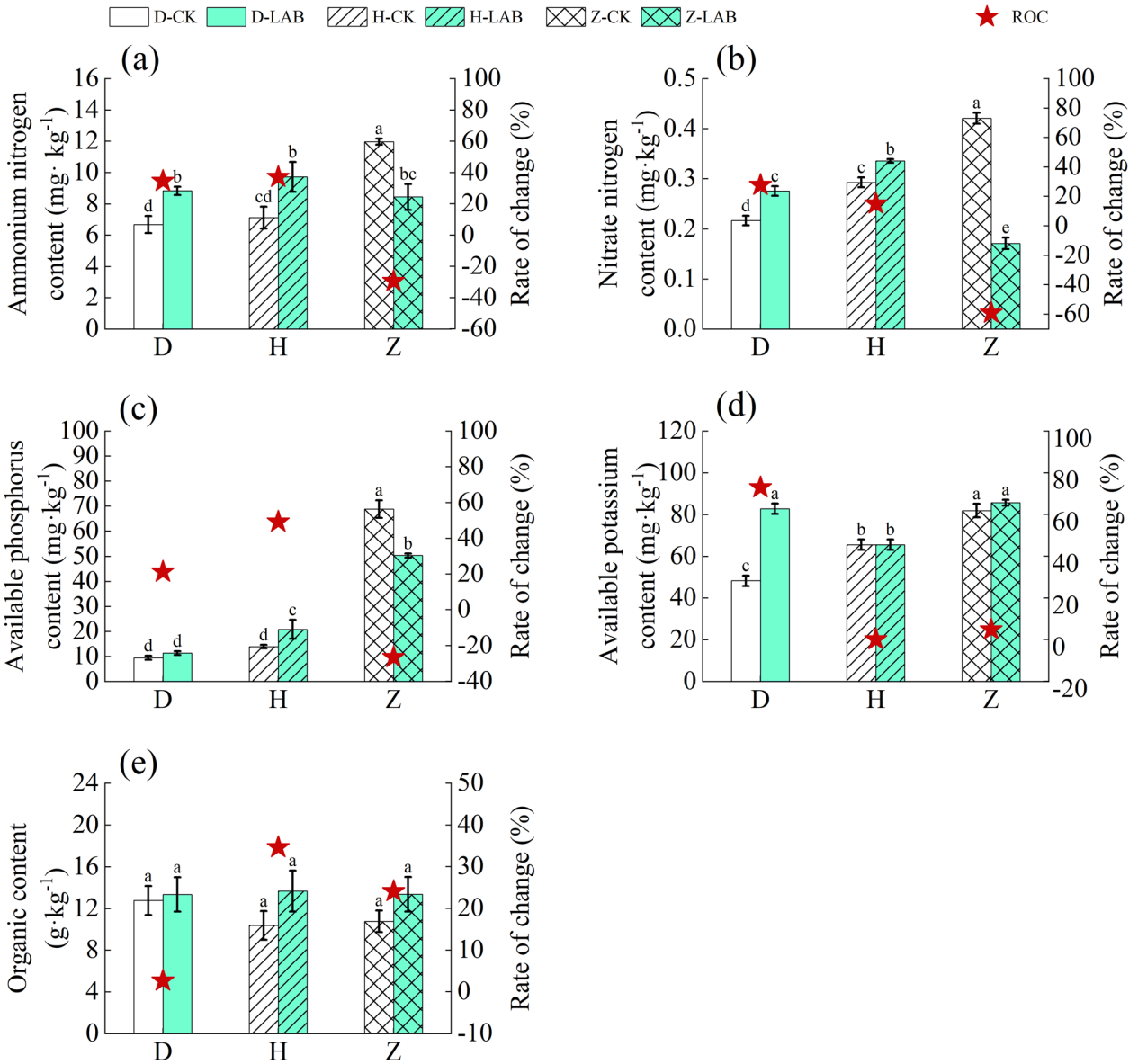


Fig. 3 Effects of LAB on the soil nutrient and organic matter content in rhizosphere soil of herbaceous peony and the rates of change. **a** Ammonium nitrogen content; **b** Nitrate nitrogen content; **c** Available phosphorus content; **d** Available potassium content; **e** Organic content. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

(100.00%), followed by 'Hongxiuqiu' (66.66%) and 'Zifengyu' (50.00%) (Fig. 7b). The CAT activity in the root systems of all the treated groups was higher than that of the control group and the promotion effect on 'Zifengyu' (296.80%) was better than that of 'Hongxiuqiu' (172.00%) and 'Dafugui' (87.30%) (Fig. 7c). The MDA content in the root system for 'Dafugui' decreased by 13.65%, while that of 'Hongxiuqiu' and 'Zifengyu' increased by 49.01% and 95.01%, respectively (Fig. 7d).

Changes in root vitality and osmotic adjustment substance content

After the application of LAB, the root vitality of 'Dafugui' and 'Hongxiuqiu' increased by 75.84% and 112.00%, respectively, while that of 'Zifengyu' decreased

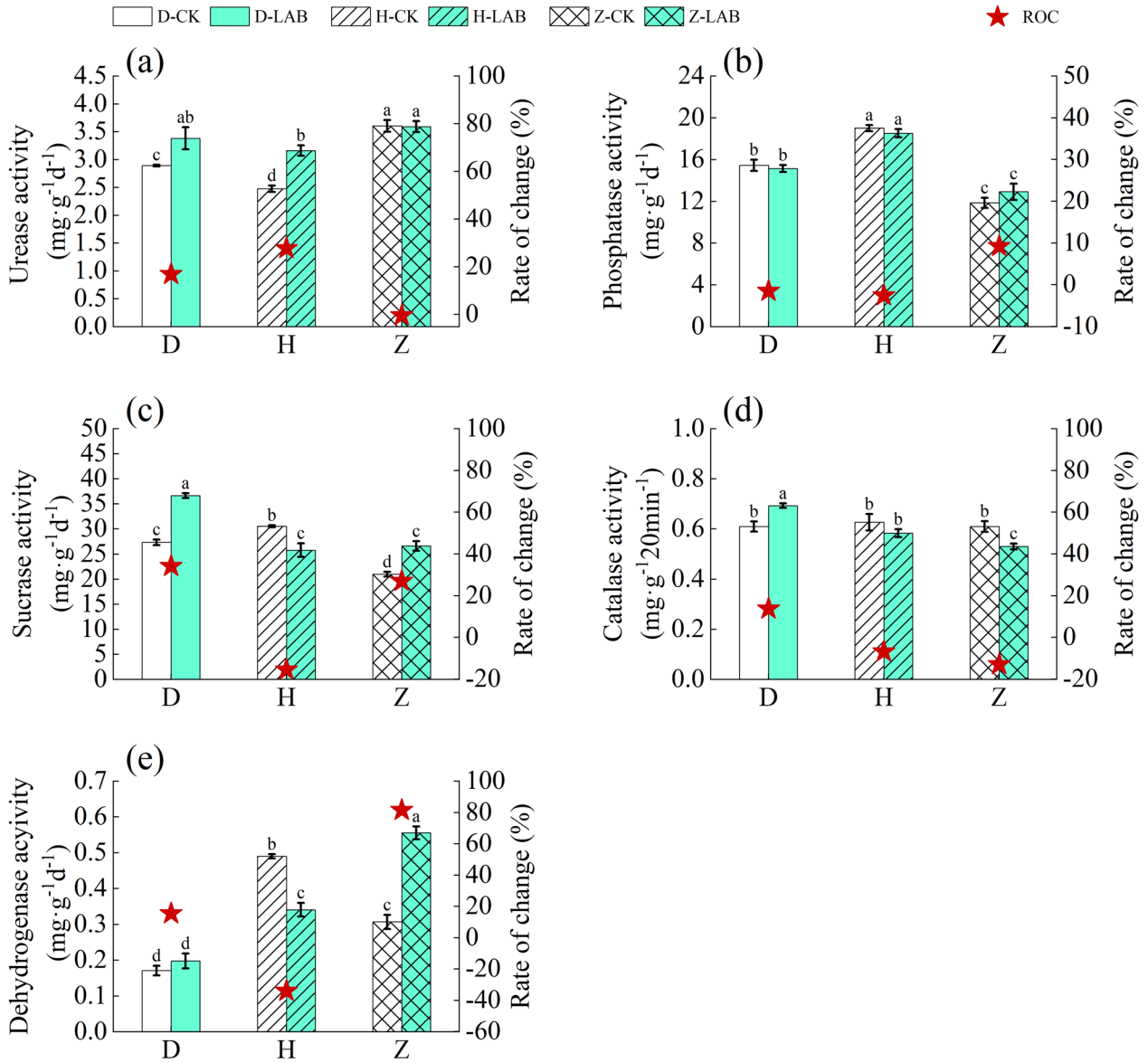


Fig. 4 Effects of LAB on the rhizosphere soil enzyme activity of herbaceous peony and the rates of change. **a** Urease activity; **b** Phosphatase activity; **c** Sucrase activity; **d** Catalase activity; **e** Dehydrogenase activity. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

by 7.26% (Fig. 8a). The content of soluble sugar in the roots of 'Dafugui' was significantly increased by 36.08%, 'Zifengyu' was no significant difference, 'Hongxiuqiu' decreased by 25.55% (Fig. 8b). The content of soluble protein in the roots of 'Zifengyu' increased significantly by 13.78%, 'Dafugui' was no significant difference, 'Hongxiuqiu' decreased by 26.5% (Fig. 8c). The proline content of 'Hongxiuqiu' (195.00%) showed the largest increase, followed by 'Dafugui' (113.00%) and 'Zifengyu' (108.00%) (Fig. 8d).

Changes in root system phytohormone and spermidine content

Following the LAB treatment, the IAA content in the roots of 'Hongxiuqiu' and 'Zifengyu' increased by 34.15% and 69.87%, respectively, while that of 'Dafugui' decreased by 11.95% (Fig. 9a). The ABA content in the root system decreased for all varieties, with 'Dafugui' (68.42%) showing the greatest decrease, followed by 'Hongxiuqiu' (16.48%) and 'Zifengyu' (6.39%) (Fig. 9b). The spermidine content in the root system also showed

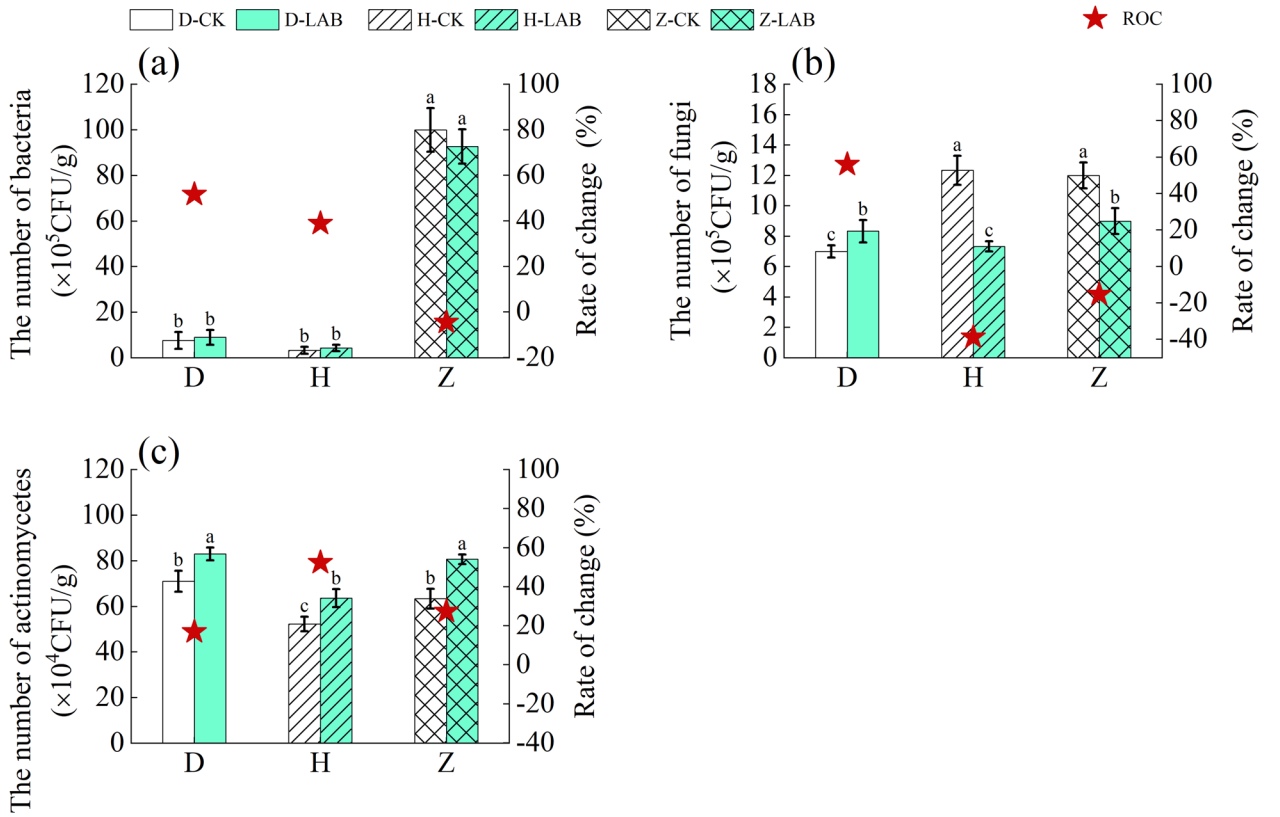


Fig. 5 Effects of LAB on the rhizosphere soil microorganism quantity of herbaceous peony and the rates of change. **a** The number of bacteria; **b** The number of fungi; **c** The number of actinomyces. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

a decreasing trend, with 'Dafugui' (26.33%) showing the greatest decrease, followed by 'Hongxiuqiu' (24.68%) and 'Zifengyu' (16.69%) (Fig. 9c).

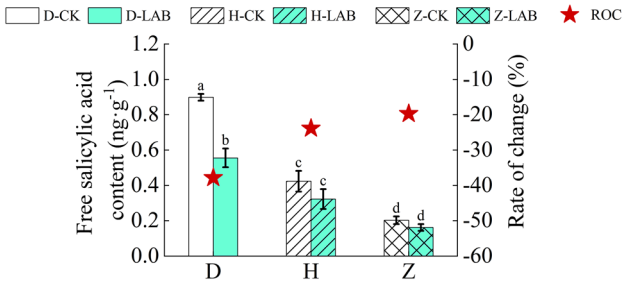


Fig. 6 Effects of exogenous LAB on the rhizosphere soil free salicylic acid content of herbaceous peony and the rates of change. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

Changes in the root system paeoniflorin content

After treatment with LAB, the content of paeoniflorin in the roots of 'Hongxiuqiu' and 'Zifengyu' had significant differences compared with the control group, the change of rates were 71.59% and 41.1%, respectively, and 'Dafugui' was no significant difference (Fig. 10).

Discussion

Influence of LAB on the morphology of herbaceous peony

Application of LAB is an important way to adjust the soil microecological environments and improve plant growth and development [24]. Microbial agents have been improves to accelerate the absorption and transformation of nutrients in plants, increase the length and dry weight of cucumber branches, promote plant growth and development [25–27], and significantly increase the plant height of wheat and sorghum [28, 29]. PGPR can increase promote the plant height and stem diameter in herbaceous peony [10]. In this study, the plant height and stem diameter of 'Dafugui' and 'Hongxiuqiu' were increased after the application of exogenous LAB, which

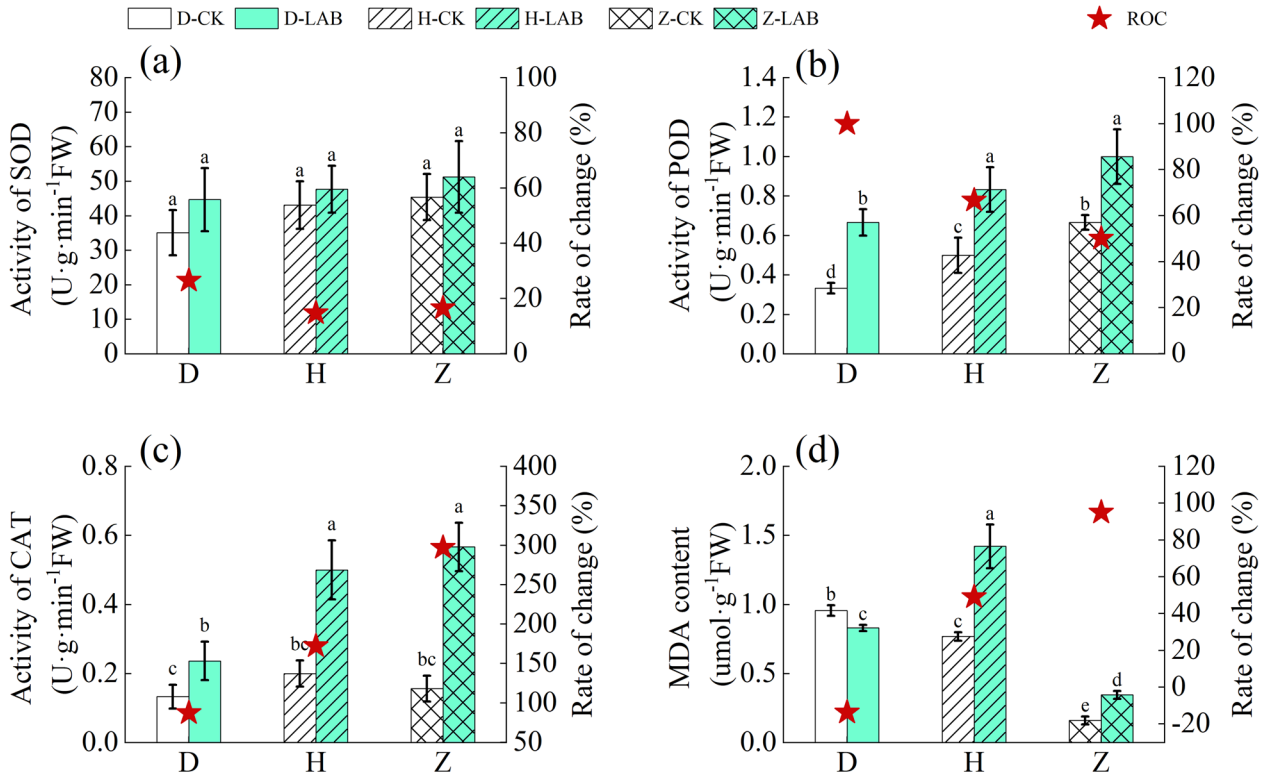


Fig. 7 Effect of LAB on the antioxidant enzymes activity and MDA content of herbaceous peony roots and the rates of change. **a** Activity of SOD; **b** Activity of POD; **c** Activity of CAT; **d** MDA content. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

had a significant promoting effect, which was the same as the aforementioned results. This indicates that the soil microenvironment is improved after the application of exogenous LAB, the soil nutrient content is increased, and the ability of roots to absorb nutrients was enhanced, which increased the plant height and stem diameter of herbaceous peony. The yield and fruit quality of tomatoes increased after the application of *Trichoderma microbicides* [30]. After the application of exogenous LAB, the planting soil of herbaceous peony was improved, the growth and development of the plants were promoted, the flowering rates of the three herbaceous peony varieties increased, and their ornamental value was improved. The maximum number of branches per plant and the number of leaves per plant increased after the application of *Trichoderma microbicides* and biochar to the soil [31]. In this study, the leaf length, leaf width and leaf area of 'Dafugui' and 'Hongxiuqiu' increased slightly, while there was no significant difference between seen 'Zifengyu' and the control group. Thus, the application of exogenous LAB can improve the light absorption ability of herbaceous peony, enhance photosynthesis, and produce more organic substances, all of which play a positive role in the growth of herbaceous peony. There were differences in the root–shoot ratio and plant water content among the three herbaceous peony varieties. It is speculated that this is mainly due to the different effects exogenous LAB treatment on the different herbaceous peony varieties. Therefore, the effects of exogenous LAB on the root–shoot ratio and water content of plants need to be further studied.

Influence of LAB on herbaceous peony rhizosphere soil

Influence of LAB on rhizosphere soil pH and conductivity

The rhizosphere refers to the microecosystem in which roots, soil, and microorganisms interact with each other. It is the most active area for material transformation and energy flow, and plays an important role in maintaining plant growth [28]. Soil pH is an important factors that affects the physical and chemical properties and fertility of soil. In this study, the pH of the rhizosphere soil increased after the application of LAB,

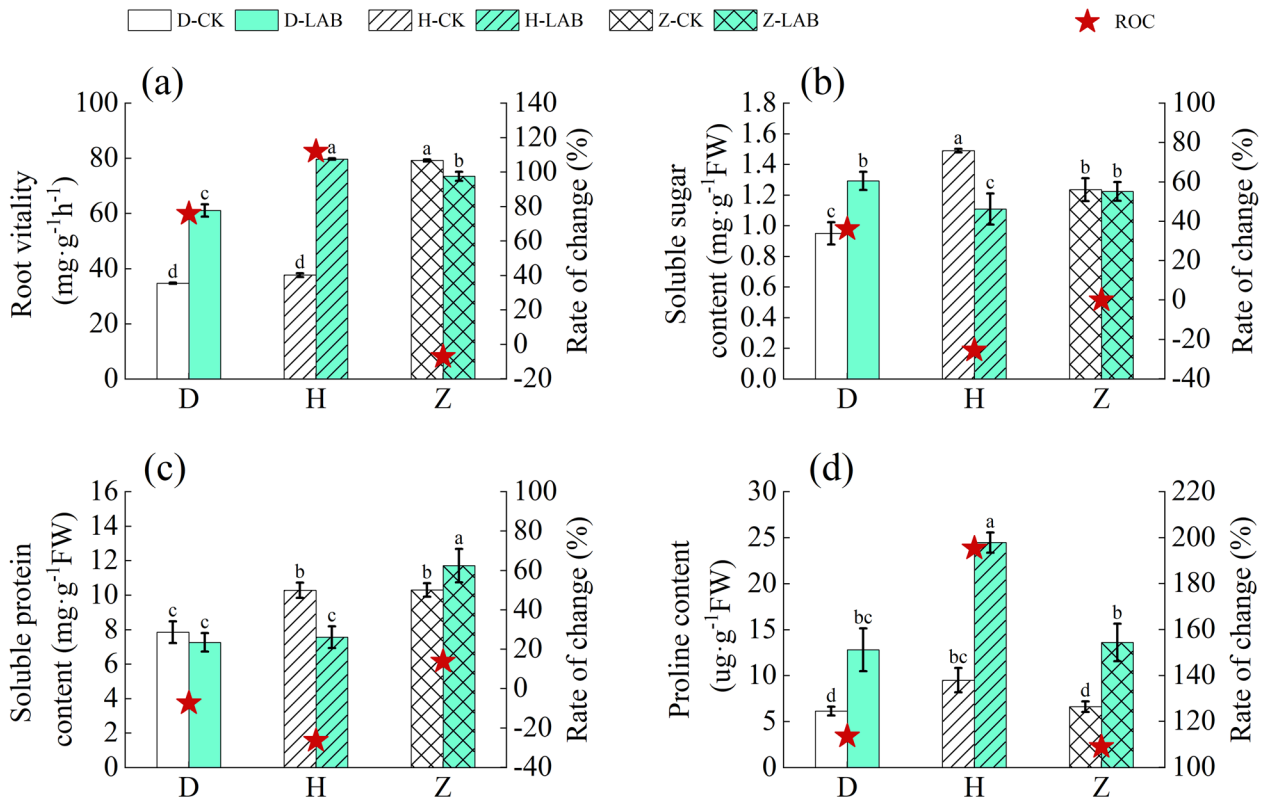


Fig. 8 Effects of LAB on the root vitality and osmotic regulatory substance content of herbaceous peony roots and the rates of change. **a** Root vitality; **b** Soluble sugar content; **c** Soluble protein content; **d** Proline content. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

but remained within the range suitable for the growth of herbaceous peony. Soil conductivity represents the salt ion content in the soil. The electrical conductivity is affected by both external and internal soil conditions in soil. The application of microbial agents can improve the agglomeration of saline soils and reduce the electrical conductivity [32]. In this study, application of LAB reduced the electrical conductivity of the rhizosphere in the soil of 'Hongxiuqiu' and 'Zifengyu', this means that *Lactobacillus plantarum* in LAB can utilise effectively nutrients and energy to degrade heavy metals by secreting organic acids, thus reducing the accumulation of salt in rhizosphere soil and improving the promotion of herbaceous peony plant growth.

Influence of LAB on rhizosphere soil nutrient and organic matter content

The reasonable application of bioorganic microbial agents is a key technical measure for improving soil quality and preserving soil structure [33]. Many studies have shown that filling with microbial agents and organic fertilisers can regulate the physical properties of rhizosphere soil and soil fertility and create a stable nutrient space for plant growth [14]. In this study, when the content of ammonium nitrogen in rhizosphere soil was high, the content of nitrate nitrogen was also high, the reasons for this are mainly that the ammonium nitrogen in the soil can be converted to nitrate nitrogen by nitrifying bacteria, the ammonium nitrogen content in soil has a positive correlation with the amount of nitrate nitrogen. Previous research results show that the content of available phosphorus and potassium in the rhizosphere soil of pepper significantly increased after the application of microbial agents [34]. LAB had different effects on the available P and K content in 'Dafugui', 'Hongxiuqiu', and 'Zifengyu' rhizosphere soil, where that of 'Dafugui' increased. The available P content in rhizosphere soil of 'Hongxiuqiu' increased, but the available K content did not change. The available K content in the rhizosphere

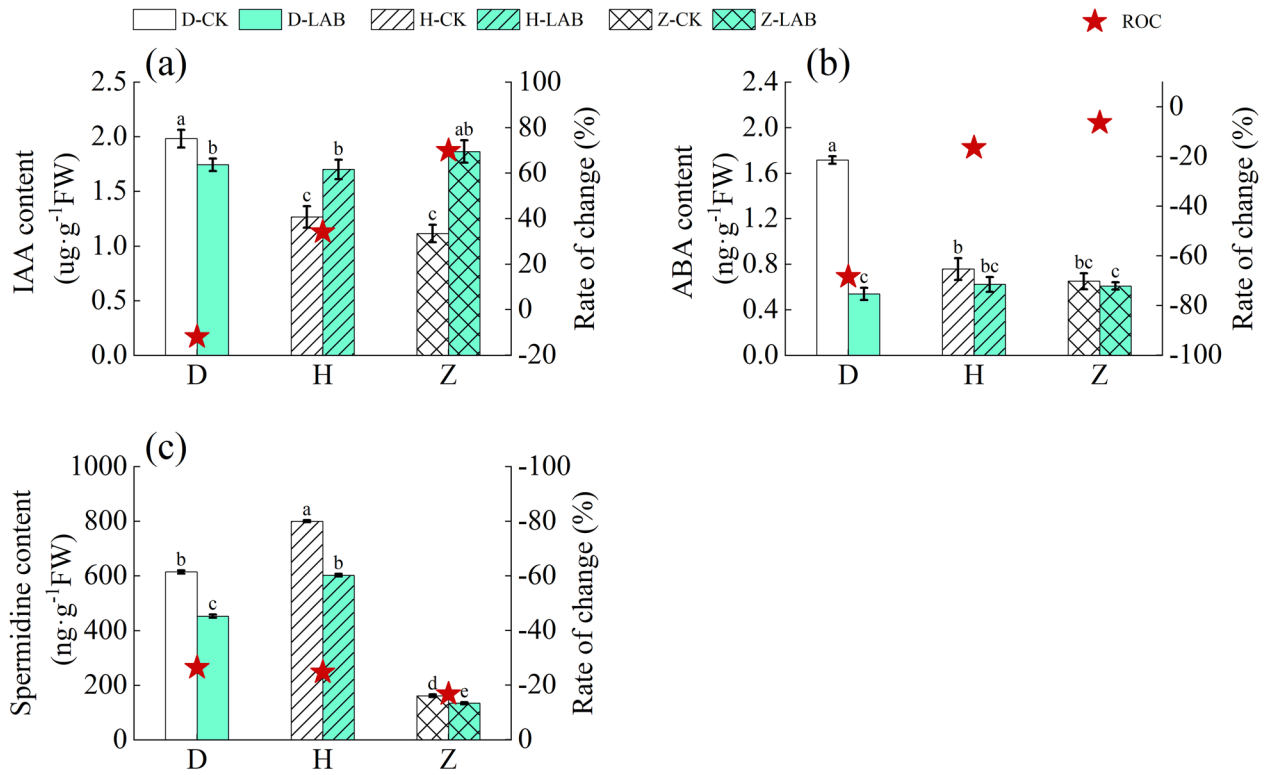


Fig. 9 Effects of LAB on the hormone and spermidine content of herbaceous peony roots and the rates of change. **a** IAA content; **b** ABA content; **c** Spermidine content. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

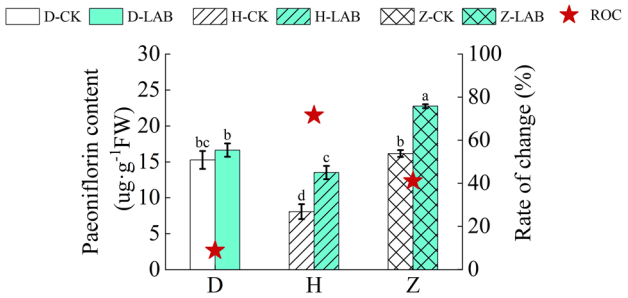


Fig. 10 Effects of LAB on the Paeoniflorin content in the roots of herbaceous peony and the rates of change. *D* 'Dafugui'; *H* 'Hongxiuqiu'; *Z* 'Zifengyu'. *D-CK* 'Dafugui' control group; *D-LAB* 'Dafugui' treatment group, *H-CK* 'Hongxiuqiu' control group; *H-LAB* 'Hongxiuqiu' treatment group, *Z-CK* 'Zifengyu' control group; *Z-LAB* 'Zifengyu' treatment group. Rate of change (ROC): the ratio of the amount of change (difference between the treatment and control groups) to the control group. All values were represented as the mean ± SE of three replicates (n = 3)

soil of 'Zifengyu' increased, while the available P content decreased. It is speculated that this is because the increase of phosphatase activity in the 'Zifengyu' rhizosphere soil accelerated the decomposition and transformation of available P, thus promoting a large absorption of P in the soil by plants, leading to the reduction in its content. The different growth characteristics of different herbaceous peony varieties led to differences in soil N, P and K contents. The application of organic fertilization by the digestate increased the content of organic matter in the rhizosphere soil [35]. In this study, the organic matter content of all three species of herbaceous peony was slightly increased by the application of LAB, the main reason is that *Lactobacillus plantarum* can promote the decomposition of soil organic matter, synthesize amino acids, vitamins and other physiologically active substances, and promote the growth of plants. Therefore, the application of microbial agents can achieve the purpose of improving soil, thus promoting the growth and development of herbaceous peony plants.

Influence of LAB on rhizosphere soil enzyme activity

Soil enzymes play an important role in the biochemical processes in soil and they promote the activation

and renewal of nutrients. Soil enzyme activity is an important index reflecting soil quality, with changes in enzyme activity affecting the absorption of effective nutrients by plants [36, 37]. Previous research results show that microbial agents can improve the activity of urease, phosphatase and catalase in watermelon continuous cropping soil, and promote the transformation of nutrient substances. Different types of microbial agents increased the activity of urease, phosphatase, and reductase in watermelon substrate soils [38]. In this study, it was found that the activities of urease, sucrase, catalase, and dehydrogenase activities in the rhizosphere soil of 'Dafugui' increased following LAB application, accelerate the transformation of nutrients in the soil, is conducive to the absorption of herbaceous peony. Studies have shown that in flue-cured tobacco, after treatment with different concentrations of compound microbial agents, with an increase in concentration, the enzyme activity will increase first increases and then decreases [39]. In this study, the activity of urease in the rhizosphere soil in the 'Hongxiuqiu' increased, the activities of phosphatase and catalase had no significant difference, while the activities for sucrase and dehydrogenase decreased; the activities for sucrase and dehydrogenase in the 'Zifengyu' rhizosphere soil increased, the activities for urease and phosphatase had no significant difference, while the catalase activity decreased. This result may be affected by the nutrient content and application concentration of rhizosphere soil, resulting in different enzyme activities.

Influence of LAB on rhizosphere soil microorganism quantity

Bacteria, fungi, and actinomyces are the main microbial communities in soil and have an important effect on plant nutrient absorption and soil quality. Changes in the soil microbial community structure and quantity are important indicators of soil fertility, nutrient conversion rates, and microbial activity [40]. Many studies have shown that inoculation with microbial agents can induce changes in rhizosphere microbial communities and improve the soil microecological environment [41, 42]. After the application of Bacillus subtilis SNB-86, the number of bacteria and actinomycetes in the rhizosphere soil of Malus hupehensis seedlings increased, and the number of fungi decreased significantly [43]. The results of this study showed that following LAB treatment, the bacteria and actinomycetes quantity increased in the rhizosphere soil of 'Hongxiuqiu', while the number of fungi decreased. This may be because *Lactobacillus plantarum* contains a large quantity of beneficial microbial flora, which improves the soil microbial community structure, activates insoluble substances in the rhizosphere soil, and increases the supply of nutrient elements, which in turn, it promotes plant growth and development. The richness and composition of rhizosphere microorganisms are closely related to soil properties, and the types and contents of metabolites in rhizosphere soil, the species and relative abundance of bacteria and fungi in rhizosphere soil of different varieties of the same plant are also different [4, 44], and the quantity of bacteria, fungi, and actinomyces in the 'Dafugui' rhizosphere soil increased, The actinomyces quantity increased in the 'Zifengyu' rhizosphere soil, while the number of bacteria and fungi decreased, it is consistent with the aforementioned research results, the concrete types of bacteria, fungi and actinomycetes in the rhizosphere soil of peony need to be further studied in the later stage.

Influence of LAB on rhizosphere soil free salicylic acid content

Salicylic acid is a common phenolic acid which has physiological functions such as regulating plant growth and aging [45]. When the phenolic acid content in the soil is too high, it affects root respiration, changes mineral absorption, and inhibits plant growth [46]. In our previous study, the rhizosphere soil of herbaceous peony contained secondary metabolites, such as benzoic acid, catechin, ferulic acid, and free salicylic acid, and the content and types of secondary metabolites produced by the roots of different varieties of herbaceous peony differed [3, 4]. In the present study, only free salicylic acid was detected in the soils of 'Dafugui', 'Hongxiuqiu', and 'Zifengyu', and its content in the treated groups was lower than that in the control group. It is speculated that the microorganisms in *Lactobacillus plantarum* promoted the degradation of free salicylic acid, reduced its content, increased the plant height and the flowering rate of herbaceous peony plants, and improved its ornamental value.

Influence of LAB on herbaceous peony root physiology

Influence of LAB on antioxidant oxidase activity and root MDA content

SOD, POD, and CAT are important protective enzymes of the defence system in plant defence systems. They can remove excessive reactive oxygen species from the plant body through coordination, maintain homeostasis, and protecting cells from damage [47]. In this study, the application of LAB increased the SOD, POD and CAT activities of herbaceous peony roots, because *Lactobacillus plantarum* can promote root growth, reinforce nutrient absorption capacity, and improve the resistance of herbaceous peony to adversity. MDA is a product of membrane lipid peroxidation, and its content is an important indicator of the degree of membrane lipid peroxidation and the response of plants to stress conditions [48]. Following LAB treatment, the MDA content in the root system of 'Dafugui' decreased, while that of 'Hongxiuqiu'

and 'Zifengyu' did not, indicating that different varieties of herbaceous peony require different concentrations of LAB and that the treatment had different effects on its accumulation.

Influence of LAB on root vitality and osmotic adjustment substance content

Plant roots are important for the fixation and absorption of nutrients. Root vitality reflects the absorption capacity of the roots and directly affects plants growth [49, 50]. The application of *Lactobacillus plantarum* mixture improves the germination and root growth of wheat, which has a positive effect on crop growth and development [51]. The results of this study, demonstrate improvement of root vitality of 'Dafugui' and 'Hongxiuqiu' following LAB application. The root vitality of 'Zifengyu' decreased, which may be caused by the low nitrogen content in the soil.

Under stress, osmotic adjustment substances such as proline and soluble sugars can actively accumulate organic or inorganic substances to increase cell fluid concentration, maintain cell water potential, and improve the ability of plants to withstand adverse stress [52, 53]. A previous study showed that the application of LAB could significantly increase the content of soluble sugar and soluble protein in Petunia [54]. In this study, after application of LAB, the content of soluble sugar in the roots of 'Dafugui' increased, and the content of soluble protein had little effect; the contents of soluble sugar and soluble protein in the roots of 'Hongxiuqiu' decreased; the content of soluble protein in the roots of 'Zifengyu' increased significantly, and the content of soluble sugar was less affected. The proline content of all three varieties increased significantly. The changes in the content of osmotic regulators in the roots of herbaceous peony after treatment with exogenous LAB were different from those in the above studies, which may have been caused by the different nutrient contents and the type and content of secondary metabolites in the microenvironments of different varieties of herbaceous peony.

Influence of LAB on root system phytohormone and spermidine content

Plant hormones and polyamines are compounds that are derived from important metabolic pathways in plants, which can not only regulate the synthesis and metabolism of substances to regulate plant growth, but also enhance plant stress resistance [55]. Previous studies have found that the application of nitrogen increased auxin content and reduced ABA content at the beginning of the young panicle stage [56]. This was observed with the increase of IAA content and decrease of ABA content in the root system of 'Hongxiuqiu' and 'Zifengyu' following the application of LAB in this study; however, the contents of IAA and ABA in the roots of 'Dafugui' decreased; and this shows that the effects of LAB on the root hormone content of different herbaceous peony varieties are different. Following the LAB treatment, the spermidine content in the roots of the three varieties decreased, and the application of LAB improved the physicochemical properties of the soil, making the soil more favourable for the growth of herbaceous peony, resulting in a positive effect on the growth of herbaceous peony at a lower concentration of spermidine in the root system. Further research is needed on the specific mechanism of spermidine action in the root system of herbaceous peony.

Influence of LAB on the root system paeoniflorin content

The accumulation and release of plant secondary metabolites are important factors that affect plant growth [57]. Paeoniflorin is the main active substance in the herbaceous peony root system, has anti-inflammatory, antioxidant, and antiviral effects. As a main component of Chinese medicine, its content is not only controlled by related genes, but also by the soil environment [58]. In this study, the content of paeoniflorin increased in all three species after LAB treatment, which not only enhanced the resistance of herbaceous peony to adversity, but also increased the medicinal value of herbaceous peony. Therefore, the value of using LAB to enhance the content of paeoniflorin in herbaceous peony roots for medical and other applications requires further research.

Conclusions

After root irrigation with LAB, herbaceous peony improved the planting soil and, therefore, alleviated replanting problems. Furthermore, microbial agents such as LAB have great potential to improve plant productivity and maintain green development in agriculture. The results showed that the application of exogenous LAB could increase the contents of ammonium nitrogen, nitrate nitrogen, available phosphorus, available potassium and organic matter in the 'Dafugui' and 'Hongxiuqiu' rhizosphere soil, and improve the enzyme activity, but had little effect on the 'Zifengyu'; the activity of antioxidant enzymes and the content of proline in the roots of the three varieties increased, the osmotic adjustment substances were accumulated to enhance the resistance of the plants to relieve environment stress; promote the growth and development of herbaceous peony plants and increase the flowering rate, among them, the morphological indexes of 'Hongxiuqiu' increased, and the promotion effect was better. However, there are some differences though in the effects of LAB on the rhizosphere soil and root physiology of different herbaceous peony varieties.

Further studies are required to determine the applicable concentration, times of application, and the changes in the beneficial and harmful microbial communities in rhizosphere soil. Therefore, this study provides a new biotechnology for resolving the replanting problems of herbaceous peony; it provides a reference for improving soil, promoting the cultivation and development of high-efficiency, high-quality, and high-yield herbaceous peony, reducing production costs, and solving replanting problems.

Abbreviations

LAB	Lactic acid bacteria
HPLC	High-performance liquid chromatography
ABA	Abscisic acid
IAA	3-Indoleacetic acid
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
MDA	Malondialdehyde
ROS	Reactive oxygen species
TCA	Trichloroacetic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00516-2.

Additional file 1. Experimental Specific Method - Support Information.

Author contributions

XY write conceptualization, methodology, writing—original draft preparation; LY, YS, FL, LD, CZ and DZ participate resources and formal analysis; LS, AX and XS are responsible for conceptualization, validation and supervision. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

This manuscript is an original paper and has not been published in other journals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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