BMC Plant Biology

RESEARCH Open Access

# Growth and selenium bioaccumulation in rape seedlings promoted by strain *Limosilactobacillus* sp. LF-17

Haifeng Li[1*], Fengjiao Li[1], Mengyu Wang[1], Caibo Hou[1], Feng Jia[1*], Xifeng Wang[1] and Mingjun Li[2]

**Abstract**

Selenium (Se) is an essential trace element that plays a critical role in human tissue formation, metabolism, and physiological functions. However, many individuals worldwide suffer from Se deficiency diseases. This study aims to evaluate the impact of Se-tolerant LF-17 agents and exogenous $Na_2SeO_3$ application on the growth, enzyme activity, and metabolic characteristics of rape seedlings. Treatment LF-3 (inoculation of Se-tolerant LF-17 agent and exogenous $Na_2SeO_3$, with the soil Se concentration of 5 mg/kg) led to a 38.62% increase in plant height and a 116.7% increase in fresh weight. And the Se-tolerant LF-17 agent in treatment LF-3 also reduced the oxidative stress induced by exogenous $Na_2SeO_3$ compared to that of treatment LF-2 (with the same amount exogenous $Na_2SeO_3$ only), as evidenced by the lower activities of SOD, POD, and CAT, as well as less content of malondialdehyde. Furthermore, the upregulation of metabolic pathways such as "cuticle, suberine, and wax biosynthesis" "flavonoid biosynthesis," and "terpenoid backbone biosynthesis" enhanced the plant's stress resistance as revealed by non-targeted metabolomics sequencing method. This approach offers promising applications for improving Se bioavailability in crops, mitigating Se toxicity, addressing global Se deficiency challenges and is expected to contribute to fulfilling the Se supplementation needs of the population.

**Clinical trial number**

Not applicable.

**Keywords** *Limosilactobacillus* sp., Selenium, Plant growth promotion, Metabolome

*Correspondence:
Haifeng Li
hfli@haut.edu.cn
Feng Jia
mrjiafeng@163.com
[1]College of Biological Engineering, Henan University of Technology, Zhengzhou 450001, China
[2]Henan Haochuang Agricultural Technology Group Company Limited, Zhengzhou 450001, China

## Introduction

As an essential trace element beneficial to plants and crucial for bacteria, green algae, animals, and humans [1], selenium (Se) typically exists in the human body in the form of over 25 selenoproteins and selenoenzymes [2]. The recommended daily intake of Se for adults ranges from 55 to 400 μg, as proposed by the Food and Nutrition Board of the Institute of Medicine (IOM, 2000). Se supplementation can reduce the risk such as cardiovascular diseases and thyroid disorders. Furthermore, Se 's beneficial effects on immunity enhancement and DNA repair, encompassing its oxidation resistance and other mechanisms, may aid in cancer prevention [3]. Se also can enhance antioxidant activity and inhibit the intrinsic and extrinsic pathways of inflammation, autophagy, and apoptosis [4]. Signaling pathway that affect cell survival, such as poly ADP-ribose polymerase pathway, is involved in the protective effects of Se [4]. Additionally, Se deficiency has been deemed to impact the health of approximately 1 billion individuals worldwide for several decades [5]. Microorganisms like lactic acid bacteria (LAB) are the key factors in the transformation of Se in soil [6], and the elemental or selenide forms of Se cannot be absorbed by plants. Microorganisms induce the absorption of Se in plants, affecting the health of plants and humans [7].

Compared to the inorganic Se forms of selenates and selenites, organic Se compounds tend to be more bioavailable and less toxic to humans. Se exerts an influence on growth and the secondary metabolite content of plants [8], so screening or cultivating of crops with high Se content is of great significance to supply Se in the daily life [9]. The total daily Se intake of local residents in Mingyueshan, Yichun, China, be regarded as Se-enriched region (approximately 50 μg), primarily attributed to rice and many leafy vegetables [9]. At low concentrations, $Na_2SeO_3$ facilitated the growth of *Tribonema minus* by enhancing chlorophyll content and the oxidation resistance; however, at higher levels, it caused obvious oxidative damage [10].

Excessive Se is capable of inhibiting the growth and metabolism of most organisms, yet certain microorganisms demonstrate the capacity to tolerate moderate concentrations of Se [10]. Microorganisms usually play a crucial role in the process of biotransformation of Se species through dissimilatory, assimilatory and detoxification metabolic pathways [11]. Se continues to exhibit beneficial effects on plants, improving the activity of related enzymes and enhancing resilience against cold, drought, and metallic stress [12, 13]. The optimal and toxic concentrations of Se varies significantly among different plant species. The catalase (CAT, EC 1.11.1.6) and superoxide dismutase (SOD, EC 1.15.1.11) was observed to increase in rice plants in response to the supply of $Na_2SeO_4$ (1.5 mM). Concomitantly, many primary metabolism products, such as total sugars, amino acids and nitrate increased, suggesting a possible defense mechanism for the osmotic regulation of rice plants to alleviate the toxicity caused by Se [14]. Foliar application of $Na_2SeO_3$ increased the accumulation of arginine and flavonoid compounds, promoting the growth and development of alfalfa (*Medicago sativa*) [15]. Similarly, studies on lettuce and tea trees indicated that Se application increases the content of amino acids such as L-aspartate acid and serine [16]. Therefore, to address the dual challenge of nutritional Se deficiency and its environmentally elevated concentrations, a deeper understanding of Se uptake and metabolism in plants is imperative [17]. Rape is cultivated globally as a vegetable and forage, and rape seed oil is also an important source of edible oil and condiments [18]. Moreover, rape is a kind of Se-enriched vegetables. It has a well-developed root system and abundant secretions, providing a favorable environment for microbial colonization and interaction. And the growth cycle of rape is relatively short, making it suitable for rapid validation of the effect of microbial agents.

Microbial agents are utilized as biological fertilizers, harboring numerous beneficial microorganisms capable of inhabiting crops and their rhizosphere soil, thereby enhancing soil fertility and fostering plant growth [19]. They further modify the microbial community in rhizosphere soil by reducing pathogenic fungi, significantly enhancing plant growth and yield [20]. Microorganisms enhance the bioavailability of Se in soil and promote its uptake by plants through altering soil properties, releasing secretions, and inducing the production of metabolites that facilitate Se absorption [21]. The application of microbial agents significantly elevates phosphorus and potassium contents in grains, as well as nitrogen content in the straw, during the stage of grain-filling, thereby providing more necessary nutrients for wheat grain growth [22].

Among numerous microorganisms, certain LAB that are generally recognized as safe (GRAS) are the preferred microorganisms for the synthesis of Se nanoparticles (SeNPs). They are widely used in various fields such as medicine, food, agriculture, and fisheries [23]. LAB with high probiotic efficacy and safety in food, are also considered promising candidates for sustainable agriculture as they enhance soil fertility, and promote plant growth [24]. Martinez et al. found that LAB can accumulate Se within the cells, produce SeNPs, and bind Se with selenocysteine (SeCys) [25]. Therefore, LAB is an ideal candidate for bioaugmentation of Se in crops. The utilization of beneficial microorganisms for Se bioaugmentation offers advantages of low cost and high safety, rendering it a prevalent approach for Se supplementation. Se-tolerant LAB possess the capability to convert highly toxic inorganic Se, including $Na_2SeO_3$, into organic Se [26]. Se-tolerant

LAB exhibits broad application prospects in agriculture [27]. The Se content in plants can be elevated through increased soil Se concentration. As a fertilizer, Se-tolerant LAB promotes biodegradation of soil organic matter, leading to the production of organic acids and microbial metabolites. Se-tolerant LAB exhibits tolerance to higher concentrations of Se-tolerant environments. However, the impact of the combination of Se-tolerant LAB and exogenous Se on the growth properties, Se assimilation, related activities of enzymes and metabolic properties of rape remains largely unexplored.

In this study, a LAB strain, *Limosilactobacillus* sp. LF-17, with promising Se-tolerant potential was selected for investigation. Following the determination of the strain's tolerance range for various $Na_2SeO_3$ concentrations on agar plates, a combination of Energy-dispersive X-ray spectroscopy (EDS), Scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) was employed to ascertain the external combination of Se on the cell walls as well as the effects of Se enrichment on the microstructure and infrared spectrum of LF-17. Subsequently, strain LF-17 was cultured for the production of microbial agents and fertilizer. Furthermore, the mitigating effects and metabolic mechanisms of Se-tolerant LAB agents against inorganic Se toxic stress in plants were explored via an untargeted LC-MS approach, aiming to establish a foundation for the enhanced utilization of Se-tolerant LAB agent in crop growth and Se enrichment. The application of the method in this study will further promote the growth and Se absorption of rape and other crops, thereby helping to increase Se intake by people.

## Materials and methods

### Materials

The Gram-positive, heterofermentative LAB *Limosilactobacillus* sp. LF-17 (CGMCC 1.62016, GenBank accession no. OP890628) was isolated from a traditional dough starter, Jiaozi, in Shangqiu City, Henan Province, China, using Man-Rogosa-Sharpe (MRS) plates [28]. The Se-tolerance of five LAB strains (*Lactobacillus plantarum* LB-5, *Lactobacillus brevis* LB-3, *Lactobacillus fermentum* LB-1, *Pediococcus pentosaceus* M-3, and *Limosilactobacillus* sp. LF-17) was compared on MRS plates containing 50 mg/L of $Na_2SeO_3$ [29]. And strain LF-17 was selected due to its highest Se-tolerance, because only strain LF-17 could grow after 24 h at 37 °C, while the other four strains did not grow well under the same conditions. *Limosilactobacillus* sp. LF-17 also exhibited good growth under initial pH ranging from 5.3 to 6.3 on MRS plates and demonstrated effective phosphate solubilization and plant growth promotion in the previous study [30]. After 24 h of incubation at 37 °C, the cells of the strain *Limosilactobacillus* sp. LF-17 reached the logarithmic phase.

The rape variety named "purple noble" used in this study was purchased from Nanjing Ideal Agricultural Science and Technology Co., Ltd. in China.

### Determination of $Na_2SeO_3$ tolerance of strain LF-17

The cells of strain *Limosilactobacillus* sp. LF-17 were cultured in MRS broth at 37 ℃ for 24 h [31]. Subsequently, the cells were inoculated into MRS plates containing 0, 500, 1000, 1500, 2000, and 3000 mg/L of $Na_2SeO_3$, respectively, to determine the Se tolerance capacity (colony formed or not) of *Limosilactobacillus* sp. LF-17 at 37 ℃ for 24–72 h.

### SEM observation and IFTR analysis of the cells under se stress

The surface morphology of *Limosilactobacillus* sp. LF-17 cells with or without Se stress was observed using SEM combined with EDX method. Fresh fermentation broth contained *Limosilactobacillus* sp. LF-17 cells, cultured in MRS medium with Se (2 mM) for 24–48 h, was used, with cells grown in Se-free medium serving as the control. After centrifugation at 4 °C and 5000 r/min for 10 min, the cell pellets were collected and fixed with 2.5% glutaraldehyde overnight in a refrigerator at 4 °C. Subsequently, the samples were rinsed for three times with phosphate-buffered saline (PBS) (0.1 M, pH 7.0), each for 15 min, and then added with 1% osmium tetroxide solution for 1–2 h. After the osmium acid waste liquid was gently removed, the cells were rinsed again with 0.1 M PBS (pH 7.4) for 15 min. Ethanol solutions with gradient concentrations of 30%, 50%, 70%, 80%, 90%, and 95% were used for dehydration of the samples. Finally, the samples were placed in 100% ethanol and dried in a freeze dryer (FD-1 A-50, Bo Yikang, Beijing). After being mounted on the sample stage with conductive carbon adhesive and sputter-coated with gold for 90 s, providing a sufficient gold layer thickness to ensure good electrical conductivity and obscuring the surface details of the sample or affect the EDX elemental analysis [32]. The surface morphology of the cells was observed by an SEM (Sigma 300, ZEISS, Germany), and the chemical constituent of the specific region was determined using EDS (Xplore 30, OXFORD, Britain) [33]. Three samples were taken from the same treatment group and measured separately, and the results were consistent.

After the preparation of the freezing-dried bacterial powders, both before and after Se enrichment, a compression method was employed to analyze the infrared spectral absorption characteristics of various bacterial cells. This analysis was conducted using a FTIR system (Nexus 670, Nicolet, USA) as described by Fan et al. [34]. The wavenumber range was set for 4000–400 $cm^{-1}$, with 32 scans and a resolution of 4 $cm^{-1}$. Three samples were

taken from the same treatment group and measured separately, and the results were consistent.

#### Preparation of the LF-17 bacterial agents

Single colonies of strain LF-17 were isolated and incubated in 5 mL of MRS broth at 37 °C for 24 h, enable bacteria to reach logarithmic growth phase. Subsequently, 3 mL of the suspension was added to 150 mL of MRS medium, incubated at 37 °C for 24 h, and centrifuged at 8000 r/min for 5–8 min. Following the removal of the supernatant, the cell pellet was resuspended in moderate amount of sterile water twice to achieve a final concentration of approximately $3.1 \times 10^7$ CFU/mL.

#### Design of the pot experiment

The rape seeds were surface-disinfection with a 1% solution of sodium hypochlorite (NaOCl) for 10 min and then rinsed thoroughly with sterile water. Subsequently, some plump rape seeds were placed on a petri dish lined with two layers of wet filter paper, covered with wet gauze, and incubated in the dark conditions for 24 h in a constant temperature incubator set at 25 ± 1 °C to induce germination. Each prepared pots (10 × 10 × 8.5 cm) was first filled with 500 g of farmland soil (pH 7.5–7.8, air-dried through a 60-mesh sieve). Meanwhile, 10 mL of sterile water was added to the pots of the control treatment (CK). Then the soil in the LF-1 and LF-3 treatments were supplemented with 10 mL of bacterial agent. The LF-2 and LF-3 treatments were added with $Na_2SeO_3$ solution to achieve a $Na_2SeO_3$ concentration of 5 mg/kg in soil (slightly higher than that of the average Se content in the soil of in China). Bacterial cells first grew and accumulated Se in MRS medium supplemented with 2 mM $Na_2SeO_3$ for 24 h (converted inorganic Se into organic Se forms by LAB) and was suspended in 10 mL sterile water after centrifugation (10000 r/min, 5 min) of 10 mL the broth. The suspension was then added to the soil in the pots of the LF-4 treatment. Then the Se-enrichment LAB agent was applied in the LF-4 treatment ($Na_2SeO_3$ was not added). Germinated rape seeds were selected and planted with 20 seeds per pot.

All the treatments were performed in quadruplicates. Pots were placed in a light incubator with a cycle of 12 h of darkness (20 °C) and 12 h of light (25 °C), at a relative humidity of 65%. Every 24 h, each pot was added with 30 mL tap water. To ensure uniformity of light, temperature, and other conditions, the positions of the pots in different treatment groups were rotated every 3 d.

#### Determination of the growth indicators and enzyme activity of the rape seedlings

After 35 d of planting, the rape seedlings were randomly picked and uprooted from the pot, ensuring the integrity of their roots. The shoots and roots of the 50 plants were individually washed with distilled water and dried on sterilized filter paper in an oven set at 50 ℃. The root length and height of each seedling were measured using a ruler, and their fresh weight was determined using a one-ten-thousandth precision electronic balance (FA1004, China).

The activities of CAT, peroxidase (POD, EC 1.11.1.7), SOD and malondialdehyde (MDA) content were measured according to the method reported by Dai et al. [35]. Fresh rape seedlings leaves (0.5 g) were taken and ground into a paste in 5 mL of 50 mM phosphate buffer solution under ice bath conditions and then centrifuged (10,000 r/min, 10 min). The supernatant was collected as the crude enzyme extract. The activity of SOD in plant leaves was measured using the nitroblue tetrazolium photoreduction method. After the addition of the crude enzyme extract to the photoreduction reaction for 20 min at 25 ℃, the absorbance at 560 nm was measured to calculate the activity. The CAT activity was measured by the absorbance at 240 nm after the reaction of the mixture of 0.2 mL of the crude enzyme extract and 2.8 mL of the assay agent (1.5 mL pH 7.0 phosphate buffer, 1 mL distilled water, and 0.3 mL 0.1 mol/L hydrogen peroxide). The activity of POD was determined using the guaiacol colorimetric method. 1 mL of the crude enzyme extract was mixed with 3 mL of the assay agent and react for 30 s at 25 ℃. The absorbance at 470 nm was then measured to calculate the POD activity. The MDA content was measured using the thiobarbituric acid method as follows: 0.5 g of rape seedlings leaves was first weighed and 5 mL of 5% trichloroacetic acid was added, then the sample was ground into a homogenate and centrifuged at 3, 000 r/min for 10 min. 2 mL of the supernatant was then transferred to a mixture with 2 mL of 0.67% thiobarbituric acid. The mixture was combined and boiled in a water bath at 100 °C for 30 min before cooling and centrifuging again, and the absorbance values of the supernatant were measured at 600, 532, and 450 nm, respectively. The determination of Se content in the rape seedling plants was quantitatively analyzed in accordance with fluorescence spectrometry [36]. The chlorophyll content in the leaves of different seedlings was determined using the 95% ethanol-grinding method. 0.5 g of small pieces rape seedlings leaves was taken and placed into a 10 mL centrifuge tube. Then, 5 mL of 95% ethanol was added, and the mixture was kept in the dark at room temperature for 24 h to allow the chlorophyll to be completely extracted. The absorbance values at 665 nm and 649 nm were then measured to calculate the chlorophyll content [37].

#### Metabolic analysis of the rhizosphere of rape seedlings

Rhizosphere soil samples were carefully collected from the plants, air-dried, and passed through a 60-mesh sieve. The metabolic properties of the rape rhizosphere

soil in each treatment were analyzed using an untargeted metabolomics method as described by Zhang et al. [38]. Approximately 0.05 g of the freeze-dried rhizosphere soil was put into a 2 mL centrifuge tube and extracted with 500 µL of an extraction solution (methanol: water = 4: 1, containing 2% L-2 chlorophenylalanine) for 30 s [39]. After being ground with magnetic beads in a frozen tissue grinder (Sakezi×48, sakezi, China) at 50 Hz for 3 min at -20 °C. To this, 200 µL chloroform was added, and the mixture was ground again at -20 ℃, 50 Hz, for 3 min. Following ice water bath ultrasonic extraction for 30 min at 5 ℃ and 40 kHz, the sample was allowed to stand at -20 ℃ for 30 min, and then centrifuged at 13,000 r/min at 4 ℃, for 15 min. The supernatant was then collected and transferred to a glass derivative bottle, where it was dried using nitrogen gas. Subsequently, 80 µL of a methoxyamine hydrochloride pyridine solution (15 mg/mL) was added, and the mixture was vortex-mixed for 2 min. It was then placed in a shaking incubator set at 37 ℃ for an oximation reaction lasting 90 min. Thereafter, 80 µL of a trifluoroacetamide solution (containing 1% trimethylchlorosilane) was added, and the mixture was vortex-mixed for 2 min. The reaction was carried out at 70 ℃ for 60 min [40]. After the samples were cooled to room temperature and stabilized, they were analyzed on a gas chromatography-mass spectrometry (GC-MS) platform (Agilent 8890B, Agilent Technologies) equipped with a quadrupole mass spectrometer (Xevo TQ-S Cronos, Waters, USA). During the analysis process, a quality control sample was conducted after every 5–15 samples.

### Data analysis and statistics

The experimental data were organized and analyzed using Microsoft Excel 2019; The data were subjected to analysis of variance (ANOVA) using SAS 9.2 software; Significant differences were compared using Fisher's least significant difference (LSD) test; Graphs were plotted using Origin 2021 software.

## Results and discussion

### Tolerance to different $Na_2SeO_3$ concentrations of strain LF-17 on plates

The growth status of strain LF-17 on plates containing various concentrations of $Na_2SeO_3$ is depicted in Fig. 1. As the $Na_2SeO_3$ concentrations increased from 0 to 500 mg/L, the color of the colonies of strain LF-17 grown on MRS plates changed from white to pink and then gradually deepened to red until reaching 2000 mg/L. Under the conditions with $Na_2SeO_3$ concentration of 2500 mg/L, the growth of cells were inhibited, and they were unable to form colonies on plates supplemented with 3000 mg/L of $Na_2SeO_3$. Simultaneously, the Se content accumulated in the strain was determined after 48 h of growth in liquid MRS medium with an initial $Na_2SeO_3$ concentration of 1 mM, according to the method specified in the National Environmental Protection Standard of the People's Republic of China (HJ 811–2016). The concentration of $Na_2SeO_3$ in the supernatant (centrifuged at 8000 r/min for 10 min) decreased by 90%, and the color of the bacterial cells also changed from white to red, indicating that the elemental Se in the liquid was absorbed into the cells. Additionally, when the test strains were cultured on Se-containing plates, all colonies exhibited a color change from white to orange-red, because Se-tolerant LAB can convert $Na_2SeO_3$ into elemental Se or organic Se [41, 42]. And the elemental Se could be released outside the cells, which may display an orange-red color. After Se enrichment under appropriate concentration range, LAB can enhance the ability of antioxidation [43], but higher concentrations of Se salts may inhibit the growth of the bacterial cell [44]. Zan et al. [29] inoculated LAB on MRS solid medium containing Se concentrations ranging from 0 to 40 mg/L to screen for Se-tolerant strains. The results of Chen et al. [43] indicate that only the isolated strain P2 was able to grow in a solution containing 30 mg/L $Na_2SeO_3$. The strains with higher Se-tolerant capacity was aimed to obtain in this study, thus the concentration of $Na_2SeO_3$ was increased to about 3000 mg/L. Strain LF-17 was not able to form colonies on plates supplemented with 3000 mg/L $Na_2SeO_3$, and the cell growth was inhibited,

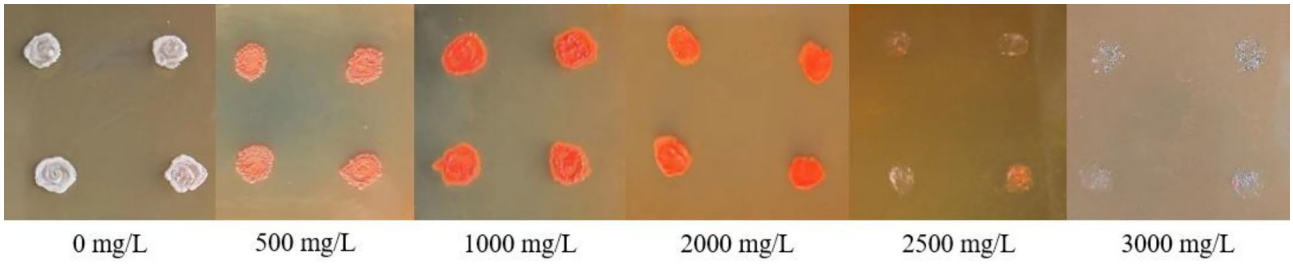


**Fig. 1** The colony status of *Limosilactobacillus* sp. LF-17 grow on MRS plates added with different concentrations of $Na_2SeO_3$

so the concentration of 3000 mg/L might be the tolerance limit for strain LF-17.

### Se accumulation on the LF-17 cell surface determined by IFTR, SEM and EDS

As shown in Fig. 2A, it is evident that the infrared spectrum absorption of strain LF-17 exhibits a notable change following Se accumulation. Prior to Se accumulation, the -OH stretching vibration peak is observed at 3300 $cm^{-1}$, whereas after Se accumulation, it shifts to 3419 $cm^{-1}$, suggesting an association with Se enrichment on the cell surface. In addition, after Se enrichment, the absorption peaks of LF-17 at the original positions of 2930, 1654, 1537, and 1233 $cm^{-1}$ marginally shifted to the lower wavenumbers of 2927, 1651, 1536, and 1232 $cm^{-1}$. Within the low-frequency region (1350–400 $cm^{-1}$), in addition to the stretching vibrations of single bonds, deformation vibrations also contribute to the formation of spectral bands. The absorption in this region is influenced by the molecular composition, and the resulting differences are subtle and intricate. The shift in the absorption peak suggests that Se absorbed by the.

LAB may interact with polysaccharides, intracellular proteins and nucleic acids, potentially influencing the corresponding chemical bonds [45].

From the images taken by SEM in Fig. 2B and C, the bacterial cells of LF-17 displayed a smooth surface and a typical bacillus morphology prior to Se accumulation, with no detection of Se by EDS. Following Se accumulation, the cell surface exhibited adhesions (indicated by red circle markings), suggesting the attachment of Se to the bacterial cells. Se attached to the surface of bacteria can form elemental Se aggregates either alone or in combination with extracellular polysaccharides and proteins, serving as a cellular detoxification mechanism [44]. Wang et al. [46] also confirmed that SeNPs synthesized by LAB exhibit strong antioxidant and antimicrobial activities. Previous studies by Zan et al. [29] found that under conditions with 10 mg/L of Se, the overall morphology of *L. plantarum* 6076 remained unaltered, however, detailed SEM imaging of the cell surface of the strain revealed the presence of pits and an augmentation in surface deposits, suggesting potential alterations in the morphology and composition of the cell surface.

### Effects of different treatments on the growth indicators of rape seedlings

As demonstrated in Table 1, the growth status of rape seedlings in the LF-1 and LF-3 groups was excellent, showing a significant increase in plant height and fresh weight compared to that of the CK and LF-2 groups ($P<0.05$). So the inoculation of LF-7 agent was beneficial for the rape seedlings in LF-1 group, while the presence of inorganic Se alone in the LF-2 treatment group inhibited the growth of rape seedlings, likely attributed to the toxicity of the added $Na_2SeO_3$. Gupta et al. have summarized that Se might induce toxicity through mechanisms of the formation of abnormal selenoproteins and inducing oxidative stress [12] and the treatment of LF-3 and LF-4 in this study might mitigate the toxicity of inorganic Se in the soil. But an average increase of 38.62% in plant height and 116.7% in fresh weight was shown in the plants in LF-3 group addition with both LF-17 agent and $Na_2SeO_3$, indicating the helpful effect of the strain LF-17 about reducing the toxicity of $Na_2SeO_3$. Se-tolerant LAB may convert inorganic Se into organic Se, which is more readily for plants to bind [47], thereby stimulating a variety of physiological processes. Upon the addition of bacterial agents after Se accumulation in the LF-4 group, the root length of rape seedlings also exhibited a significant enhancement ($P<0.05$). It is plausible that the secretion of organic acids, plant hormones, or organic Se transformed by the strain LF-17 contributes to the observed phenomenon, as LAB have the potential to stimulate the activation of rhizosphere soil material elements and promote plant growth [48]. Therefore, Se-tolerant LF-17 could promote the growth of plant, although the application of the LF-17 agents might be affected by the natural climatic conditions such as temperature and drought, etc.

### Effect of bacterial agents on the rhizosphere soil enzyme activity related to oxidant stress of rape seedlings

In plants, under adverse environmental conditions, there is typically an increased development of reactive oxygen species (ROS), including superoxide anions and hydrogen peroxide, which subsequently triggers the activation of plant defense responses [49]. Ascorbic acid, glutathione (GSH), and their related enzymes, alongside enzyme systems such as POD, SOD and CAT, serve as crucial protective components within these antioxidant systems [50]. Plants under stress usually produce a large amount of ROS within their cells, which can cause damage to cell structure and function. Plants always activate their antioxidant enzyme system, including SOD, CAT, among others to counteract the harm of ROS [51]. SOD can convert the harmful superoxide radical ($O_2^-$) into hydrogen peroxide ($H_2O_2$), which is then transformed into water by POD and CAT [52]. Therefore, the enzyme activity of the antioxidant system can indicate the ability of the plants to resist injury stress [53].

As depicted in Fig. 3A, the SOD activities in the leaves of rape seedlings significantly increased ($P<0.05$) in the LF-2, LF-1, and LF-4 treatment groups, in comparison to the CK group. This increase is likely attributed to the addition of $Na_2SeO_3$ and the metabolism of LF-17 agents (both Se-enriched and non-Se-enriched) in the soil. SOD enzyme activities were observed to increase under Se application on the RB966928 variety of sugarcane

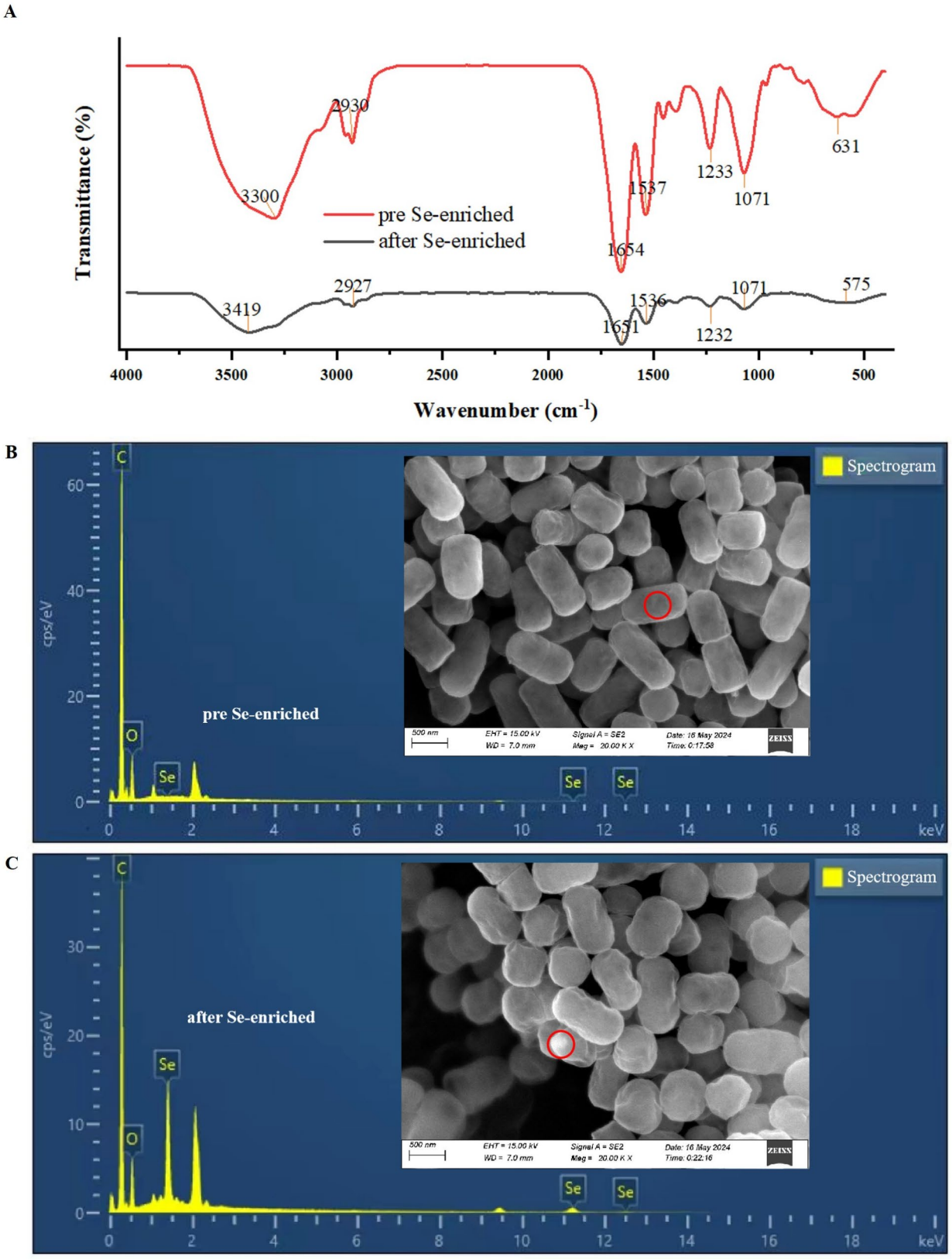


**Fig. 2** (**A**) IFTR spectra of strain LF-17 before and after Se-enriched, (**B**) SEM images and EDS analysis of strain LF-17 pre Se-enriched, (**C**) SEM images and EDS analysis of strain LF-17 after Se-enriched

**Table 1** Pot experiment design and the names of each treatment

| Treatment | Bacterial agent | Organic Se | Inorganic Se |
|---|---|---|---|
| CK | - | - | - |
| LF-1 | + | - | - |
| LF-2 | - | - | + |
| LF-3 | + | - | + |
| LF-4 | + | + | - |

(-) Indicate without addition. (+) Indicate addition

(*Saccharum* sp.), as reported by Araujo et al. [54]. However, in the LF-3 treatment group, where both $Na_2SeO_3$ and LF-17 agents (without Se-enriched) were applied, the SOD activities in rape leaves were the lowest. The results may be influenced by the metabolic activity of strain LF-17. This suggests the presence of the least abiotic and biotic stress in this treatment, potentially due to the accumulation of Se by the cells of strain LF-17. The rape seedlings in LF-3 treatment exhibited the lowest SOD activities and MDA content under $Na_2SeO_3$ application, which may be attributed to the metabolic activities of the cells in bacterial LF-17 agent. Due to the intake of Se by LAB, the SOD activities in the cells can be enhanced, whereas the production of MDA was diminished, ultimately leading to improved cell viability [42].

As shown in Fig. 3B, the rape seedlings in the LF-4 treatment exhibited the highest MDA content, representing a 77.16% increase compared to the CK group, while the LF-3 treatment exhibited the lowest MDA content. The results were similar to the trends observed in SOD activity in Fig. 3A, indicating that ROS exerted similar effects in the different treatments. Notably, the environmental stress in the LF-4 treatment was particularly intense, potentially attributed to the distinct metabolic characteristics of the Se-tolerant LF-17 agents in the soil. In the study conducted by Dai and Jia [55], the MDA content in the roots of three alfalfa varieties was observed to increase under Se treatment, compared to the control plants.

As can be seen in the Fig. 3C, the POD activity in the leaves of rape seedlings in the LF-2 group was the highest, compared to that in the CK group, potentially attributed to the elevated stress pressure induced by inorganic Se. The POD activity in the LF-1 treatment exhibited a significant decrease ($P<0.05$), suggesting the unique effect of LF-17 agents (without Se-enrichment). As shown in Fig. 3D, the CAT activity was highest in the LF-4 treatment, followed by the LF-2 treatment, whereas it significantly decreased in the LF-3 and LF-1 treatments compared to the LF-4 group ($P<0.05$). The antioxidant enzyme system, including GSH reductase and thioredoxin reductase, along with SeNPs and selenomethionine (SeMet), primarily contribute to the excellent antioxidant activity [56]. Se also may regulate the expression of genes related to the enzymes in antioxidant systems. Se influenced the expression of genes encoding POD, SOD and CAT in mustard, leading to an increase in the concentrations of specific components in the cell wall, such as pectin, hemicelluloses and lignin [57]. Additionally, Se enhanced the activity of SOD, POD, and glutathione peroxidase (GPX) [58, 59].

Kieliszek et al. [60] found a significant increase in the activity of the antioxidant enzyme system in the presence of SeNPs. Many studies have reported that SeMet effectively inhibits lipid peroxidation by antagonizing the formation of free radicals, and GPX converts $H_2O_2$ to alleviate lipid peroxidation [61]. Se-tolerant LAB exhibit excellent antioxidant activity, primarily attributable to their antioxidant enzymes and the presence of SeNPs. Drutel et al. [62] reported that Se is a component of several antioxidant enzymes, including GPX and iodothyronine deiodinase, which aid in resistance to the harmful impacts of free radicals produced during the process of oxiation. Consequently, it is inferred that the activity of these antioxidant enzymes within cells may be significantly enhanced by Se stimulation in the soil [33].

As shown in Fig. 3E, a significant difference in the chlorophyll content of rape leaves among treatment groups was observed ($P<0.05$), with the CK treatment exhibiting the highest total content. The purple coloration of the rape leaves in the experiment, as shown in Fig. 4, indicates the impacts of different treatments on the synthesis of chlorophyll and anthocyanins, which is variety-dependent. Moulick et al. [63] soaked rice seeds in a 0.5–1.5 mg/kg $Na_2SeO_3$ solution and observed significant increases in plant height, chlorophyll content, and grain weight, which ultimately enhanced the quality and yield of the rice. Se had a minimal impact on chlorophyll levels, yet it increased the concentration of anthocyanins. The chlorophyll content was higher in the rape seedlings from CK group (without Se addition), but it decreased in the plants from the other treatments, possibly due to the replacement of sulfur atoms in key enzymes of chlorophyll synthesis by Se, which disrupted their configurations, reduced their activity, and severely hindered the synthesis of chlorophyll [63]. High levels of Se may also lead to the loss of thylakoid membrane integrity, damaging the photosynthesis of the plants. Excessive Se, potentially in the LF-2 treatment, may exert toxicity by inhibiting growth and chlorophyll synthesis, leading to the production of ROS that induce the oxidative stress [35].

As shown in Fig. 3F, the Se content in the plant of rape seedlings subjected to the LF-2 and LF-3 treatments significantly increased upon the addition of $Na_2SeO_3$ ($P<0.05$), with the highest levels observed in conjunction with the LF-17 agent, averaging 105.74 mg/kg dry weight (DW) and accompanying improved plant growth

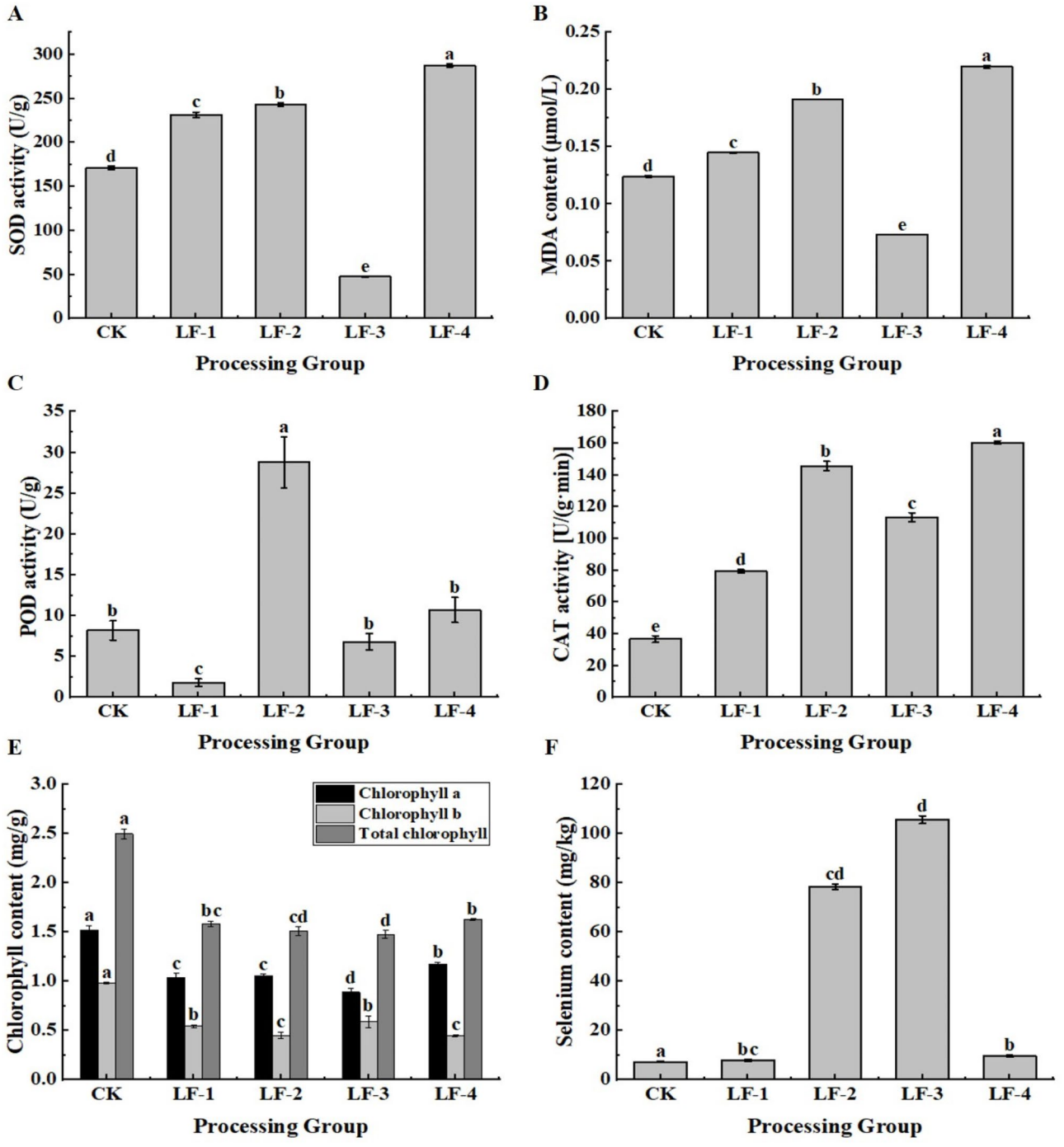


**Fig. 3** The physiological index of the rape seedlings leaves in different treatments: (**A**) SOD activity, (**B**) MDA content, (**C**) POD activity, (**D**) CAT activity, (**E**) chlorophyll content, and (**F**) Se content

characteristics. The Se content in the plants of rape seedlings increased by 7.65% under the influence of the LF-17 agent, even in the LF-1 treatment soil where no Se was applied. Dai et al. [55] found that *M. sativa* and *V. alfalfa* exhibited the highest Se-accumulation in the leaves and the stem, up to 17.19 and 28.43 mg/kg DW, respectively, when supplied with 900 μM Se. The provision of Se exhibited positive effects on plant growth, albeit at high concentrations, it adversely impacted growth. The application of SeNPs fermentation broth resulted in a significant elevation of Se content in wheat and rice grains [13]. Araujo et al. [54] investigated two sugarcane varieties (RB966928 and RB867515) across four Se application rates (0, 5, 10, and 20 μM) applied as $Na_2SeO_3$ in a

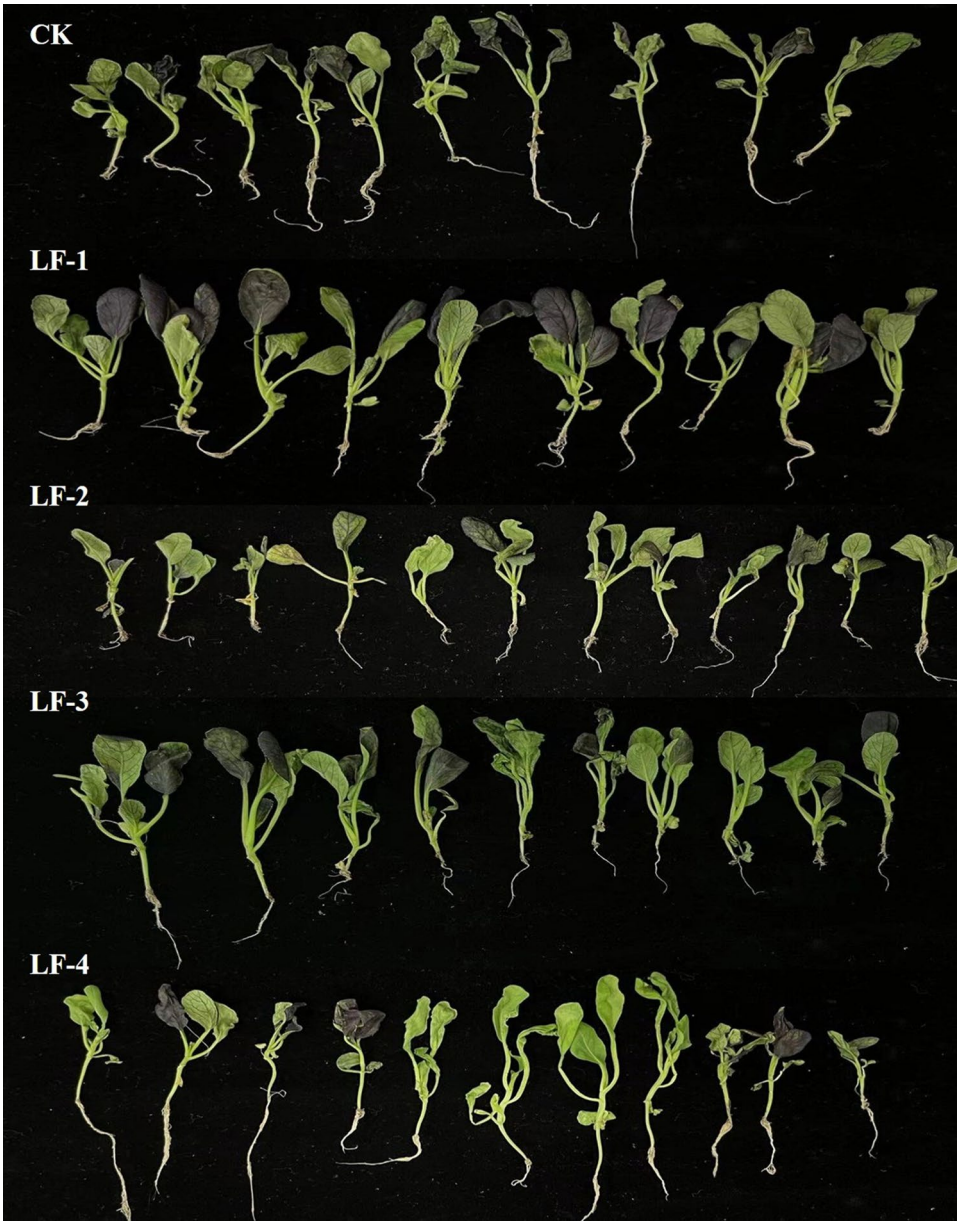


**Fig. 4** Photos of the rape seedlings in different treatments

nutrient solution, finding that both varieties exhibited increased leaf Se concentrations under Se application. The content of Se in plants reached from 0.38 to 5.76 mg/kg DM after exposure to Se(IV) and 2.09 to 45.73 mg/kg DM for exposure to Se(VI) [64]. Se-enriched microorganisms can enhance the availability of Se; thus, effective management of these Se-rich biofertilizers may bolster soil resilience, safeguard biodiversity, and foster practices conducive to sustainable agriculture.

### Rhizospheric metabolites in response to the effects of different treatments

Plants typically adapt to external environmental changes by producing secondary metabolites [65]. Changes in metabolic pathways, as influenced by significantly differentiated metabolites and KEGG topology analysis, are depicted in Fig. 5. Specifically, the hypergeometric distribution algorithm was applied to the 1209 metabolites annotated in the KEGG database to identify pathways enriched in differential metabolic concentrations, with *P*-values corrected using the Benjamini-Hochberg (BH) method. Comparison of metabolic topology maps between LF-3 and the other treatment groups revealed

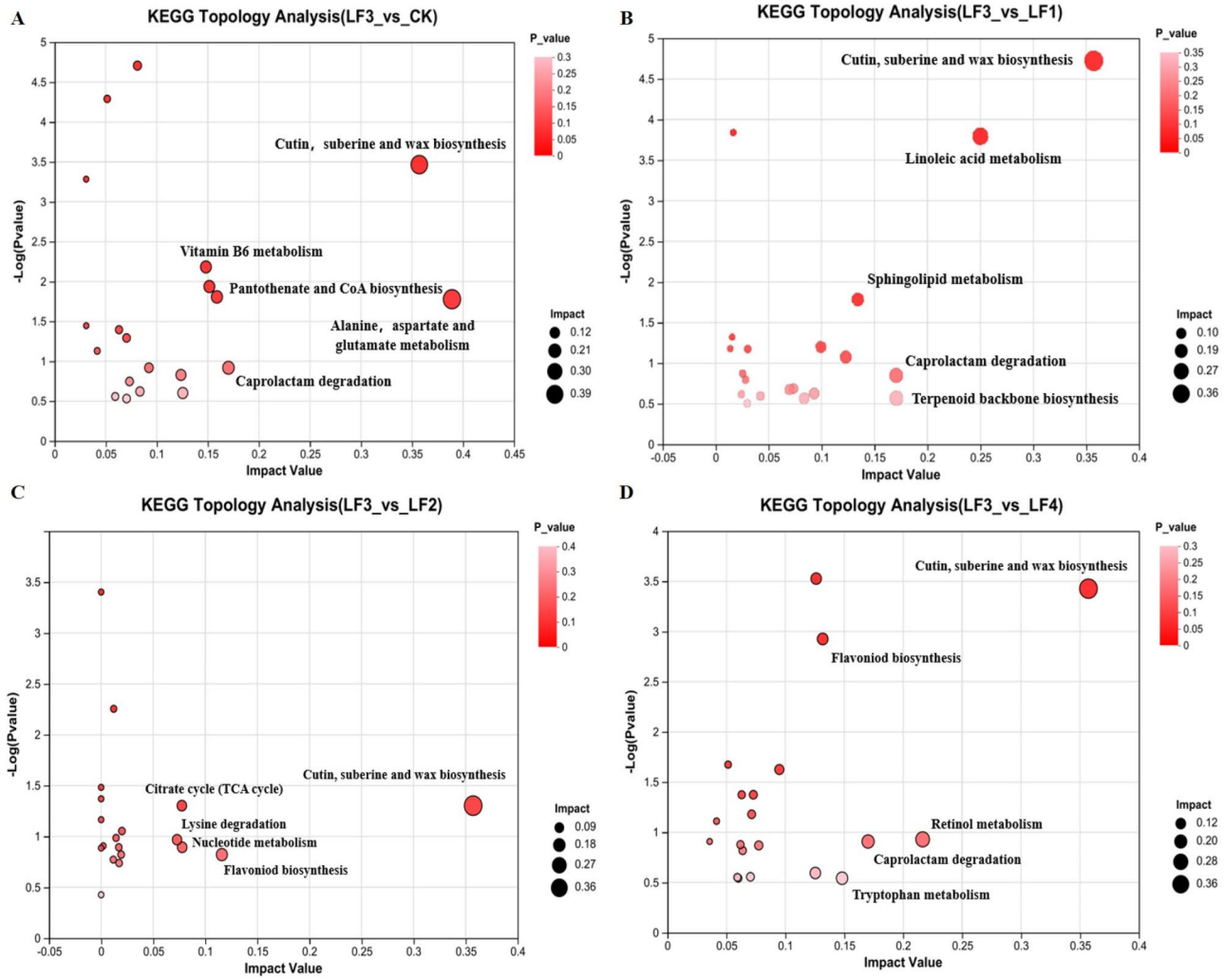


**Fig. 5** Comparison of KEGG topology analysis results between LF-3 and other treatments: (**A**) LF-3 and CK, (**B**) LF-3 and LF-1, (**C**) LF-3 and LF-2, (**D**) LF-3 and LF-4. Note: Each bubble in the topology analysis Figure represents a KEGG pathway. The horizontal axis indicates the relative importance of metabolites within the pathway, as measured by the "Impact Value". The vertical axis represents the enrichment significance of metabolites involved in the pathway, as indicated by -log10 (*P*-value). The size of the bubble corresponds to the "Impact Value"; the larger the bubbles, the greater the importance of the pathway

that the LF-3 treatment induced up-regulation of the "cutin, suberine, and wax biosynthesis" pathways. The lipid composition of the stratum corneum, which covers the plant's surface, is referred to as cuticular wax, and it is essential for enhancing plant longevity and protecting plants against biotic and abiotic stressors [66]. So the addition of both exogenous $Na_2SeO_3$ and LF-17 agent might alter the composition and structure of the cutin, preventing water loss and promoting the transport of nutrients. In the case of suberine, its deposition is induced by various environmental stresses, including injury, drought, and salinity [67]. A recent study has shown that the synthesis and degradation of suberin in the root dermis are both responses to nutritional stress, mediated by ethylene and abscisic acid [68]. Therefore, the exogenous $Na_2SeO_3$ and LF-17 agent both in LF-3 treatment might upregulate the metabolic pathway of "cutin, suberine, and wax biosynthesis" in the system compared to that of the other treatment groups, supporting protection to rape against the Se stress.

As shown in Fig. 5A, the metabolic processes in the rape rhizosphere soil under the LF-3 treatment exhibited up-regulation of the pathways involved in "$VB_6$ metabolism", "alanine, aspartate and glutamate metabolism" and "pantothenate and CoA biosynthesis", as compared to the CK treatment. $VB_6$ is essential for all organisms and is recognized as a critical cofactor necessary for numerous enzymatic reactions [69]. Several studies have demonstrated that $VB_6$ acts as an effective antioxidant, possessing ROS-quenching capabilities comparable to those of tocopherol and ascorbic acid [70]. As a ROS scavenger, $VB_6$ can enhance an organism's resistance to both biotic

and abiotic stressors [71]. This may be the reason for the lower antioxidant related enzyme activity in LF-3 treatment. Coxon et al. [72] confirmed the significant value of pantothenate overproduction in plants, as it influences various physiological processes, including plant growth, development, and stress resistance to both abiotic and biotic factors [73]. In plants, glutamic acid and aspartic acid serve as precursors for up to 11 amino acids, derived from the secondary metabolite precursors of the coumarin CoA pathway. And this pathway produces alkaloid derivatives such as coumarin, flavonoids, suberine, which are related to the cellular antioxidant capacity [74]. Consequently, the combined treatment of Se-tolerant LF-17 agent and exogenous $Na_2SeO_3$ (in LF-3 treatment) might promote the growth of rape and resist the stress induced by Se better. The results are consistent with the data shown in Table 2.

As shown in Fig. 5B, the metabolic processes within the rape rhizosphere soil under the LF-3 treatment were found to up-regulate the pathways related to "linoleic acid (LA) metabolism", "sphingolipid metabolism", "caprolactam degradation" and "terpenoid backbone biosynthesis" in contrast to the LF-2 treatment. In plants, an increased LA content contributes to the maintenance of membrane integrity and fluidity, thereby facilitating adaptation to environmental stress [75]. Sphingolipids are crucial signaling molecules involved in signal transduction pathways, essential for maintaining membrane integrity, tolerance, and stress response [76], and have been demonstrated to mediate the formation of plant cell walls and the differentiation of cell types under biotic and abiotic stresses [77]. Lipophilic diterpenoids are particularly complex due to the involvement of multiple rate-limiting enzymes [78], which are pivotal in plant growth, related metabolism, and defense process against natural enemies, pathogens, and other competitive organisms [79]. Therefore, compared to the treatment without exogenous Se stress (LF-1), the presence of exogenous Se stress (in LF-3 treatment) might enhance the adaptability of rape to the environment and strengthen its ability to resist enemies and pathogens.

As shown in Fig. 5C, compared with that in LF-2 treatment, the metabolism in the rape rhizosphere soil in LF-3 treatment was found to up-regulate the pathways of "cutin, suberine, and wax biosynthesis", "citrate cycle (TCA cycle)", "lysine degradation", "nucleotide metabolism", and "flavonic biosynthesis". TCA cycle is a central element in carbon metabolism in higher plant species [80]. During stressful conditions, enzymatic and non enzymatic components of the antioxidant resistant system jointly clear ROS [81]. Hypoxia-induced oxidation-reduction inhibition of aconitine oxidase during hypoxia, leading to the accumulation of CA, is considered a stress adaptation strategy that can induce metabolic changes [82]. Stress responses in plants by activating their enzyme defense system, which is facilitated by the accumulation of CA [83]. Flavonoids are suggested to play crucial roles in plant responses to environmental signals, especially under biotic and abiotic stress. Plants may substantially change their physiological response to Se [17]. The Se-tolerant LF-17 agent and exogenous $Na_2SeO_3$ in LF-3 treatment might enhance the "TCA cycle" and "Flavonoid biosynthesis" pathways in the plant or microorganisms.

As shown in in Fig. 5D, it is evident that the pathways for "Cutin, suberine, and wax biosynthesis", "Flavonic biosynthesis", "Retinol metabolism", "Caprolactam degradation", and "Tryptophan metabolism" were up-regulated in the LF-3 treatment compared to that in the LF-4 treatment. Multiple mechanisms have evolved in plants to adapt to abiotic and biotic stresses, such as the reshaping of primary and secondary metabolic pathways associated with carbohydrates, proteins, amino acids, nucleic acids, lipids, and plant hormones [84].

The top 30 metabolites with the highest metabolic abundance in CK, LF-1, LF-2, LF-3, and LF-4 are shown in Fig. 6. Higher abundance of three acid substances is observed in LF-3, namely urochoic acid, cyclopassifloic acid D, and glycocholic acid. It has been emphasized in multiple studies that plants can enhance their tolerance to heavy metal toxicity and promote root absorption of heavy metals through the secretion of a range of chelating agents, including low molecular weight organic acids [85]. Organic acids can dissolve metal oxides, thereby improving their bioavailability [86]. Furthermore, the abundance of tryptophan in LF-3 treatment is observed to be higher compared to LF-2, which is consistent with the upregulated tryptophan metabolism observed in LF-3 in Fig. 5D. It has been confirmed by Moreno-Martin et al. [87] that some LAB can accumulate Se salts and biotransform them into Se amino acids and SeNPs. L-tryptophan has also been found to elicit physiological reactions, increase nutrient absorption, and thereby enhance plant growth and development [88]. The KEGG database could not contain complete metabolic pathways of plant and the Microorganisms in soil, which may result in the neglect the information of certain pathways or enzyme functions in interpreting complex soil-plant

**Table 2** Growth indicators of the rape seedlings in different treatment groups after grow for 35 d

| Treatment groups | Plant height (cm) | Root length (cm) | Fresh weight (g) |
|---|---|---|---|
| CK | $5.10 \pm 0.13^{d}$ | $2.7 \pm 0.14^{ab}$ | $0.12 \pm 0.01^{d}$ |
| LF-1 | $8.72 \pm 0.19^{a}$ | $2.75 \pm 0.48^{ab}$ | $0.38 \pm 0.04^{a}$ |
| LF-2 | $5.20 \pm 0.28^{d}$ | $2.40 \pm 0.12^{b}$ | $0.13 \pm 0.01^{d}$ |
| LF-3 | $7.07 \pm 0.31^{b}$ | $2.73 \pm 0.21^{ab}$ | $0.26 \pm 0.02^{b}$ |
| LF-4 | $6.30 \pm 0.55^{c}$ | $2.98 \pm 0.23^{a}$ | $0.20 \pm 0.04^{c}$ |

Note: The data is expressed as the mean ± standard deviation, and different letters in the same column indicate the significance of the difference ($P<0.05$)

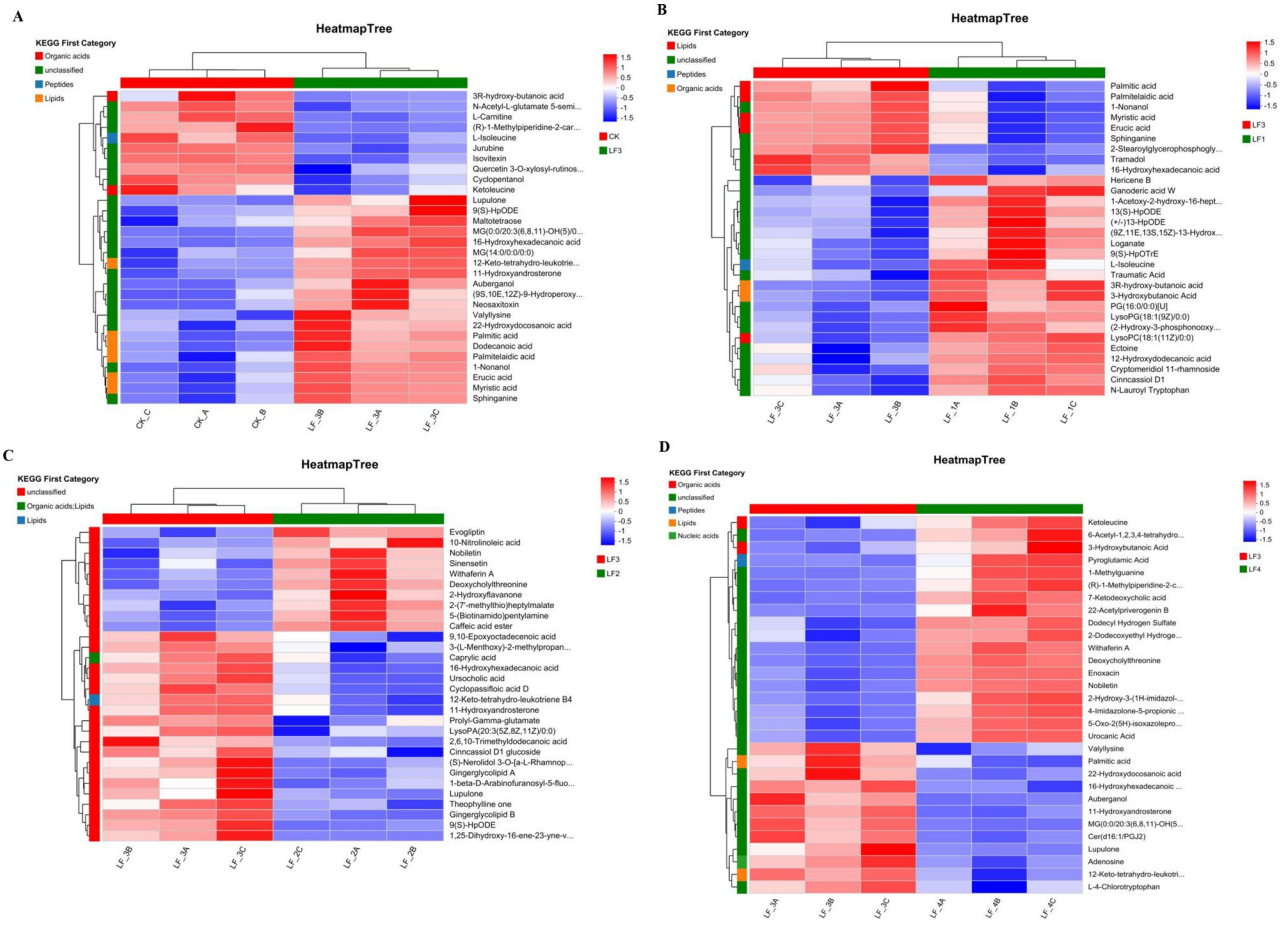


**Fig. 6** Top 30 metabolic products abundance heatmaps of the rhizosphere soil in different treatments: (**A**) LF-3 and CK, (**B**) LF-3 and LF-1, (**C**) LF-3 and LF-2, and (**D**) LF-3 and LF-4. Note: Each column in the Figure represents a sample, and each row represents a kind of metabolite. The color in the Figure indicates the relative expression level of the metabolite in the samples. The specific trend in expression level can be seen in the numerical annotations on the right of the color scale on the upper right. The different metabolite clustered on the left of the Figure, and the names of the them are on the right. The closer two branches of the metabolites are, the more similar their expression levels are. The closer two sample branches are, the more similar the expression patterns of all metabolites in these two samples, indicating a closer trend in metabolite expression levels

interactions. Therefore, it is necessary to combine the data from the metabolome sequencing method and other information to analyze the effects of different treatments on the growth and Se absorption of plant.

The findings of this study could provide beneficial reference for the application of LAB LF-17 agent in the growth of rape seedlings and Se biofortification. However, further investigation is needed to explore the impact of strain LF-17 on the growth of other crops and food quality. For subsequent research, it is planned to conduct the further experiment in the field to verify the effects of the LF-17 agent under natural conditions. In the future, Se-enriched LAB might be applied to daily diets (such as yogurt and fermented plant protein beverages) and in the agricultural sector, such as feed additives and Se-enriched fertilizer. So they could provide a safe and controllable Se nutrition supplementation for the people in Se-deficient areas.

## Conclusion

Strain LF-17 exhibited a higher tolerance to $Na_2SeO_3$ concentrations of 2500 mg/L on MRS plates, with the Se element detectable on the cell surface of LF-17 as determined by a combination of FTIR, SEM, and EDX analyses after Se accumulation cultivation. Significant increases ($P<0.05$) in growth and Se content of rape seedlings were observed upon treatment with Se-tolerant LF-17 agent and $Na_2SeO_3$, while oxidative stress was significantly reduced following the enrichment of metabolic pathways including "cutin, suberine and wax biosynthesis", "flavoniod biosynthesis" and "terpenoid backbone biosynthesis".

### Author contributions

H. L.: Funding acquisition, Conceptualization, Writing-review and editing. F. L.: Investigation, Data curation, Writing-original draft. M. W.: Investigation, Data curation, Methodology. C. H.: Software, Validation, Methodology. F. J.: Visualization, Writing-review and editing, Funding acquisition. X. W.: Software, Investigation, Data curation. Mi. L.: Investigation, Methodology.

**Funding**
This research was supported by the Henan Provincial Science and Technology Research Project (242102310322), Natural Science Foundation of Henan Province (252300420168) and Cultivation Project of Tuoxin Team in Henan University of Technology.

**Data availability**
Data is provided within the manuscript or supplementary information files.

## Declarations

**Ethical approval**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 11 December 2024 / Accepted: 27 March 2025
Published online: 04 April 2025

**References**

1. Huang S, Yu K, Wen L, Long X, Sun J, Liu Q et al. Development and application of a new biological nano-selenium fermentation broth based on *Bacillus subtilis* SE201412. Sci Rep. 2023;13(1):2560.
2. Sun Y, Shi Y, Jia H, Ding H, Yue T, Yuan Y. Biosynthesis of selenium nanoparticles of *Monascus purpureus* and their Inhibition to *Alicyclobacillus Acidoterrestris*. Food Control. 2021;130:108366.
3. El-Sayed HS, El-Sayed SM, Youssef AM. Designated functional microcapsules loaded with green synthesis selenium nanorods and probiotics for enhancing stirred yogurt. Sci Rep. 2022;12(1):14751.
4. Shalihat A, Hasanah AN, Mutakin, Lesmana R, Budiman A, Gozali D. The role of selenium in cell survival and its correlation with protective effects against cardiovascular disease: A literature review. Biomed Pharmacother. 2021;134:111125.
5. Cunha MLO, Oliveira LCAd, Silva VM, Montanha GS, Reis AR. Selenium increases photosynthetic capacity, Daidzein biosynthesis, nodulation and yield of peanuts plants (*Arachis Hypogaea* L). Plant Physiol Biochem. 2022;190:231–9.
6. Wang Z, Huang W, Pang F. Selenium in soil-plant-microbe: A review. BECT. 2022;108(2):167–81.
7. Khanna K, Kumar P, Ohri P, Bhardwaj R. Harnessing the role of selenium in soil–plant-microbe ecosystem: ecophysiological mechanisms and future prospects. Plant Growth Regul. 2022;100(2):197–217.
8. Liu K, Li S, Han J, Zeng X, Ling M, Mao J, et al. Effect of selenium on tea (*Camellia sinensis*) under low temperature: changes in physiological and biochemical responses and quality. Environ Exp Bot. 2021;188:104475.
9. Li K, Luo Y, Zeng C, Zhong Q, Xiao Z, Mao X, et al. Selenium accumulation in plant foods and selenium intake of residents in a moderately selenium-enriched area of Mingyueshan, Yichun, China. J Food Compos Anal. 2023;116:105089.
10. Wang F, Li Y, Yang R, Zhang N, Li S, Zhu Z. Effects of sodium selenite on the growth, biochemical composition and selenium biotransformation of the filamentous microalga *Tribonema minus*. Bioresour Technol. 2023;384:129313.
11. Li K, Xu Q, Gao S, Zhang S, Ma Y, Zhao G, et al. Highly stable selenium nanoparticles: assembly and stabilization via Flagellin FliC and Porin OmpF in *Rahnella aquatilis* HX2. J Hazard Mater. 2021;414:125545.
12. Gupta M, Gupta S. An overview of selenium uptake, metabolism, and toxicity in plants. Front Plant Sci. 2017;7:2074.
13. Bhadwal S, Sharma S. Selenium alleviates carbohydrate metabolism and nutrient composition in arsenic stressed rice plants. Rice Sci. 2022;29(4):385–96.
14. Gouveia GCC, Galindo FS, Dantas Bereta Lanza MG, Caroline da Rocha Silva A, Pereira de Brito Mateus M, Souza da Silva M, et al. Selenium toxicity stress-induced phenotypical, biochemical and physiological responses in rice plants: characterization of symptoms and plant metabolic adjustment. Ecotox Environ Safe. 2020;202:110916.
15. Wang F, Yang J, Hua Y, Wang K, Guo Y, Lu Y, et al. Transcriptome and metabolome analysis of selenium treated alfalfa reveals influence on phenylpropanoid biosynthesis to enhance growth. Plants (Basel). 2023;12(10):2038.
16. Jiang X, Zhou W, Li D, Wang H, Yang Y, You J, et al. Combined transcriptome and metabolome analyses reveal the effects of selenium on the growth and quality of *Lilium lancifolium*. Front Plant Sci. 2024;15:1399152.
17. Raina M, Sharma A, Nazir M, Kumari P, Rustagi A, Hami A, et al. Exploring the new dimensions of selenium research to understand the underlying mechanism of its uptake, translocation, and accumulation. Physiol Plant. 2020;171(4):882–95.
18. Xiao M, Wang H, Li X, Mason AS, Fu D. Rapeseed as an ornamental. Horticulturae. 2021;8(1):27.
19. Thomas L, Singh I. Microbial biofertilizers: types and applications. Biofertilizers for sustainable agriculture and environment. Soil Biology2019. pp. 1–19.
20. Ahsan T, Tian P, Gao J, Wang C, Liu C, Huang Y. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping system. J Adv Res. 2023;23:367–73.
21. Yang D, Hu C, Wang X, Shi G, Li Y, Fei Y, et al. Microbes: a potential tool for selenium biofortification. Metallomics. 2021;13(10):54.
22. Li Y, Wei J, Ma L, Wu X, Zheng F, Cui R, et al. Enhancing wheat yield through microbial organic fertilizer substitution for partial chemical fertilization: regulation of nitrogen conversion and utilization. J Soil Sci Plant Nutr. 2024;24(1):935–43.
23. Stabnikova O, Khonkiv M, Kovshar I, Stabnikov V. Biosynthesis of selenium nanoparticles by lactic acid bacteria and areas of their possible applications. World J Microb Biot. 2023;39(9):230.
24. Wang B, Zhao N, Li J, Xu R, Wang T, Guo L, et al. Selenium-enriched *Lactobacillus plantarum* improves the antioxidant activity and flavor properties of fermented *Pleurotus eryngii*. Food Chem. 2021;345:128770.
25. Martinez FG, Moreno-Martin G, Pescuma M, Madrid-Albarran Y, Mozzi F. Biotransformation of selenium by lactic acid bacteria: formation of seleno-nanoparticles and seleno-amino acids. Front Bioeng Biotechnol. 2020;8:506.
26. Nie X, Yang X, He J, Liu P, Shi H, Wang T, et al. Bioconversion of inorganic selenium to less toxic selenium forms by microbes: A review. Front Bioeng Biotechnol. 2023;11:1167123.
27. Wen C, He X, Zhang J, Liu G, Xu X. A review on selenium-enriched proteins: preparation, purification, identification, bioavailability, bioactivities and application. Food Funct. 2022;13(10):5498–514.
28. Li L, Feng L, Yi J, Hua C, Chen F, Liao X, et al. High hydrostatic pressure inactivation of total aerobic bacteria, lactic acid bacteria, yeasts in sour Chinese cabbage. Int J Food Microbiol. 2010;142(1–2):180–4.
29. Zan L, Chen Z, Zhang B, Zou X, Lan A, Zhang W, et al. Screening, characterization and probiotic properties of selenium-enriched lactic acid bacteria. Fermentation. 2024;10(1):39.
30. Li H, Chen S, Wang M, Shi S, Zhao W, Xiong G, et al. Phosphate solubilization and plant growth properties are promoted by a lactic acid bacterium in calcareous soil. Appl Microbiol Biotechnol. 2023;108(1):17.
31. Shu G, Mei S, Chen L, Zhang B, Guo M, Cui X, et al. Screening, identification, and application of selenium-enriched *Lactobacillus* in goat milk powder and tablet. J Food Process Preserv. 2020;44(6):e14470.
32. Arunachalam K, Davoodbasha M. Imaging bacteria and biofilm by field emission scanning electron microscopy. analytical methodologies for biofilm research. Springer Protocols Handbooks2021. pp. 205– 22.
33. Yang, Li Y, Zhang L, Fan M, Wei X. Response surface design for accumulation of selenium by different lactic acid bacteria. 3 Biotech. 2017;7(1):1–14.
34. Fan Y, Li J, Ren X, Wang D, Liu Y. Preparation, characterization, bacteriostatic efficacy, and mechanism of zinc/selenium-loaded sodium humate. Appl Microbiol Biotechnol. 2023;107(24):7417–25.
35. Dai H, Shan CJ, Lu C, Jia GL, Wei AZ, Sa WQ, et al. Response of antioxidant enzymes in Populusxcanescens under cadmium stress. Pak J Bot. 2012;44(6):1943–9.
36. Smrkolj P, Stibilj V. Determination of selenium in vegetables by hydride generation atomic fluorescence spectrometry. Anal Chim Acta. 2004;512(1):11–7.
37. Arnon D. Copper enzymes in isolated chloroplasts-polyphenoloxidase in beta-vulgaris. Plant Physiol. 1949;24(1):1.
38. Zhang SJ, Yan W, Li T, Li R, Wang J, et al. Regulation of rhizospheric microbial network to enhance plant growth and resist pollutants: unignorable weak electric field. Sci Total Environ. 2023;855:158888.

39. Liu L, Lin Y, Lei S, Zhang Y, Zeng H. Synergistic effects of Lotus seed resistant starch and sodium lactate on hypolipidemic function and serum nontargeted metabolites in hyperlipidemic rats. J Agric Food Chem. 2021;69(48):14580–92.
40. Pang S, Zhou Y, Yang L, Zhou J, Li X, Xia S. Simultaneous removal of nitrate and ammonium by hydrogen-based partial denitrification coupled with anammox in a membrane biofilm reactor. Bioresour Technol. 2023;369:128443.
41. Xia SK, Chen L, Liang JQ. Enriched selenium and its effects on growth and biochemical composition in *Lactobacillus bulgaricus*. J Agric Food Chem. 2007;55(6):2413–7.
42. Liao J, Wang C. Factors affecting selenium-enrichment efficiency, metabolic mechanisms and physiological functions of selenium-enriched lactic acid bacteria. J Future Foods. 2022;2(4):285–93.
43. Chen Y, Li Q, Xia C, Yang F, Xu N, Wu Q, et al. Effect of selenium supplements on the antioxidant activity and nitrite degradation of lactic acid bacteria. World J Microb Biot. 2019;35(4):1–13.
44. Martínez FG, Moreno-Martin G, Pescuma M, Madrid-Albarrán Y, Mozzi F. Biotransformation of selenium by lactic acid bacteria: formation of seleno-nanoparticles and seleno-amino acids. Front Bioeng Biotechnol. 2020;8:506.
45. Sun Y, Wang H, Zhou L, Chang M, Yue T, Yuan Y, et al. Distribution characteristics of organic selenium in Se-enriched *Lactobacillus* (*Lactobacillus paracasei*). LWT. 2022;165:113699.
46. Wang Q, Wang C, Kuang S, Wang D, Shi Y. Biological selenite reduction, characterization and bioactivities of selenium nanoparticles biosynthesised by *pediococcus acidilactici* DSM20284. Molecules. 2023;28(9):3793.
47. Yin H, Qi Z, Li M, Ahammed GJ, Chu X, Zhou J. Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in *Oryza sativa* L. Ecotox Environ Safe. 2019;169:911–7.
48. Yang YS, Lin Y, Zhang W, Wang Y, Wang R, et al. Activation of potassium released from soil by root-secreted organic acids in different varieties of tobacco (*Nicotiana tabacum*). Funct Plant Biol. 2020;47(4):318–26.
49. Garg N, Manchanda G. ROS generation in plants: Boon or Bane?? Plant Biosyst. 2009;143(1):81–96.
50. Tahi H, Wahbi S, El Modafar C, Aganchich A, Serraj R. Changes in antioxidant activities and phenol content in tomato plants subjected to partial root drying and regulated deficit irrigation. Plant Biosyst. 2008;142(3):550–62.
51. Hasanuzzaman M, Parvin K, Bardhan K, Nahar K, Anee TI, Masud AAC, et al. Biostimulants for the regulation of reactive oxygen species metabolism in plants under abiotic stress. Cells. 2021;10(10):2537.
52. Liang Y, Li D, Chen Y, Cheng J, Zhao G, Fahima T, et al. Selenium mitigates salt-induced oxidative stress in durum wheat (*Triticum durum* Desf.) seedlings by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. 3 Biotech. 2020;10(8):368.
53. Meddich A. Biostimulants for resilient agriculture—improving plant tolerance to abiotic stress: a concise review. Gesunde Pflanzen. 2022;75(4):709–27.
54. Araujo MAd M, AARd, Silva VM, Reis ARd. Selenium enhances ROS scavenging systems and sugar metabolism increasing growth of sugarcane plants. Plant Physio Biochem. 2023;201:107798.
55. Dai H, Jia G. Effects of se on the growth, tolerance, and antioxidative systems of three alfalfa cultivars. Environ Sci Pollut Res. 2017;24(17):15196–201.
56. Xiao D, Li T, Huang X, Zhu K, Li Z, Dong Y, et al. Advances in the study of selenium-enriched probiotics: from the iInorganic se into se nanoparticles. Mol Nutr Food Res. 2023;67(23):2300432.
57. Liu Y, Lv H, Yang N, Li Y, Liu B, Rensing C, et al. Roles of root cell wall components and root plaques in regulating elemental uptake in rice subjected to selenite and different speciation of antimony. Environ Exp Bot. 2019;163:36–44.
58. Renwei F, Wei C, Tu S. The roles of selenium in protecting plants against abiotic stresses. Environ Exp Bot. 2013;87:58–68.
59. Lanza MGDB, Reis AR. Roles of selenium in mineral plant nutrition: ROS scavenging responses against abiotic stresses. Plant Physiol Biochem. 2021;164:27–43.
60. Kieliszek M, Bierla K, Jiménez-Lamana J, Kot AM, Alcántara-Durán J, Piwowarek K, et al. Metabolic response of the yeast *Candida utilis* during enrichment in selenium. Int J Mol Sci. 2020;21(15):5287.
61. Kumar BS, Kunwar A, Singh BG, Ahmad A, Priyadarsini KI. Anti-hemolytic and Peroxyl radical scavenging activity of organoselenium compounds: an in vitro study. Biol Trace Elem Res. 2010;140(2):127–38.
62. Drutel A, Archambeaud F, Caron P. Selenium and the thyroid gland: more good news for clinicians. Clin Endocrinol. 2013;78(2):155–64.
63. Moulick D, Ghosh D, Chandra Santra S. Evaluation of effectiveness of seed priming with selenium in rice during germination under arsenic stress. Plant Physiol Biochem. 2016;109:571–8.
64. Mechora S, Placido Torres D, Bruns RE, Skof M, Ugrinovic K. Effect of selenium treated broccoli on herbivory and oviposition preferences of *Delia radicum* and *Phyllotreta* spp. Sci Hortic. 2017;225:445–53.
65. Pant P, Pandey S, Dall'Acqua S. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. Chem Biodivers. 2021;18(11):e2100345.
66. Ahmad HM, Rahman MU, Ali Q, Awan SI. Plant cuticular waxes a review on functions, composition, biosyntheses mechanism and transportation. Life Sci J. 2015;12(4s):60–7.
67. Kolattukudy. Polyesters in higher plants. Adv Biochem Eng Biotechnol. 2001;71:1–49.
68. Li-Beisson Y, Verdier G, Xu L, Beisson F. Cutin and Suberin polyesters. Encycl Life Sci. eLS2016. pp. 1–9.
69. Hellmann H, Mooney S. Vitamin $B_6$: A molecule for human health?? Molecules. 2010;15(1):442–59.
70. Samsatly J, Bayen S, Jabaji SH. Vitamin $B_6$ is under a tight balance during disease development by rhizoctonia Solani on different cultivars of potato and on *Arabidopsis Thaliana* mutants. Front Plant Sci. 2020;11:509412.
71. Chamoun R, Jabaji S. Expression of genes of *Rhizoctonia Solani* and the biocontrol *Stachybotrys elegansduring* mycoparasitism of hyphae and sclerotia. Mycologia. 2017;103(3):483–93.
72. Coxon KM, Chakauya E, Ottenhof HH, Whitney HM, Blundell TL, Abell C, et al. Pantothenate biosynthesis in higher plants. Biochem Soc Trans. 2005;33(4):743–6.
73. Hildebrandt Tatjana M, Nunes Nesi A, Araújo Wagner L, Braun H-P. Amino acid catabolism in plants. Mol Plant. 2015;8(11):1563–79.
74. Alfosea-Simón M, Simón-Grao S, Zavala-Gonzalez EA, Cámara-Zapata JM, Simón I, Martínez-Nicolás JJ, et al. Physiological, nutritional and metabolomic responses of tomato plants after the foliar application of amino acids aspartic acid, glutamic acid and Alanine. Front Plant Sci. 2021;11:581234.
75. Dar AA, Choudhury AR, Kancharla PK, Arumugam N. The $FAD_2$ gene in plants: occurrence, regulation, and role. Front Plant Sci. 2017;8:285984.
76. Haslam TM, Feussner I, Nakamura Y. Diversity in sphingolipid metabolism across land plants. J Exp Bot. 2022;73(9):2785–98.
77. Liu Y, Wang L, Li X, Luo M. Detailed sphingolipid profile responded to salt stress in cotton root and the GhIPCS1 is involved in the regulation of plant salt tolerance. Plant Sci. 2022;316:111174.
78. Ma Y, Yuan L, Wu B, Li Xe, Chen S, Lu S. Genome-wide identification and characterization of novel genes involved in terpenoid biosynthesis in *Salvia miltiorrhiza*. J Exp Bot. 2012;63(7):2809–23.
79. Bohlmann J, Keeling CI. Terpenoid biomaterials. Plant J. 2008;54(4):656–69.
80. Schnarrenberger C, Martin W. Evolution of the enzymes of the citric acid cycle and the glyoxylate cycle of higher plants. Eur J Biochem. 2002;269(3):868–83.
81. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010;48(12):909–30.
82. Gupta, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, et al. Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot. 2012;63(4):1773–84.
83. Tahjib-Ul-Arif M, Zahan MI, Karim MM, Imran S, Hunter CT, Islam MS, et al. Citric acid-mediated abiotic stress tolerance in plants. Int J Mol Sci. 2021;22(13):7235.
84. VanWallendael A, Soltani A, Emery NC, Peixoto MM, Olsen J, Lowry DB. A molecular view of plant local adaptation: incorporating stress-response networks. Annu Rev Plant Biol. 2019;70(1):559–83.
85. Qiao D, Han Y, Zhao Y. Organic acids in conjunction with various oilseed sunflower cultivars promote cd phytoextraction through regulating microenvironment in root zone. Ind Crops Prod. 2022;183:114932.
86. Onireti OO, Lin C, Qin J. Combined effects of low-molecular-weight organic acids on mobilization of arsenic and lead from multi-contaminated soils. Chemosphere. 2017;170:161–8.
87. Moreno-Martin G, Pescuma M, Pérez-Corona T, Mozzi F, Madrid Y. Determination of size and mass-and number-based concentration of biogenic senps

synthesized by lactic acid bacteria by using a multimethod approach. Anal Chim Acta. 2017;992:34–41.
88. Abbas SH, Sohail M, Saleem M, Mahmood T, Qamar M, Majeed A, et al. Effect of L-tryptophan on plant weight and pod weight in Chickpea under rainfed conditions. Sci Tech Dev. 2013;32(4):277–80.

## Publisher's note