Effect of GR24 on the growth and development of licorice under ~~different~~low phosphorus ~~concentrations~~stress

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Abstract:

Background. Glycyrrhiza ~~is,~~ a perennial herbaceous medicinal plant, ~~which~~ is ~~widely used~~extensively utilized in the pharmaceutical industry. The growth of Glycyrrhiza is ~~often limited~~frequently constrained by ~~the~~soil phosphorus ~~effectiveness~~availability, as a significant portion of ~~the soil because most of the~~ arable land in China ~~is in a~~suffers from phosphorus ~~deficit~~deficiency.

Method. ~~In this~~This study~~,~~ utilized Ural *Glycyrrhiza uralensis* Fisch ~~was used~~ as the ~~research object,~~subject and ~~GR24 (a synthetic Srigolactones) was applied under three environments, simulating no phosphorus (P1), low phosphorus (P2), normal phosphorus supply (P3), to investigate~~examined the ~~effect of GR24 on the growth and development of licorice and to get~~application of GR24, a synthetic strigolactone, under three phosphorus conditions: none (P1), low (P2), and normal (P3). The research aimed to ascertain the optimal concentration of GR24 ~~application, which can provide~~for promoting licorice growth and development, thereby providing a theoretical ~~basis for the cultivation of licorice.~~ foundation for its agricultural management.

Results. The optimal GR24 concentration ~~of GR24 under~~for P3 and P2 ~~treatments~~conditions was identified as G3, which ~~led to increased~~enhanced growth ~~indices, organ~~metrics, chlorophyll a and b ~~content, as well as enhanced~~ levels, and while also boosting antioxidant enzyme ~~activity~~activities in licorice. ~~Moreover,~~Specifically under P3 ~~treatment, it significantly promoted the accumulation of~~ , significant increases in liquiritigenin and glycyrrhizic acid~~, while under P2 treatment, it promoted the accumulation of isoliquiritigenin~~ levels were observed. Under P2, increases were noted in isoliquiritigenin, liquiritigenin, and liquiritin~~. It was shown by the transcriptome~~ levels. Transcriptome analysis ~~that there were~~revealed differential expression, with 137 and 270 ~~cases of lsoliquirtin, liquirtrigenin, liquirtin under P3 and P2 137 and 294~~ genes ~~were~~ up-regulated and 77 and 294 genes ~~were~~ down-regulated ~~under~~in the P3 and P2 treatments, respectively. GO functional enrichment ~~revealed that~~identified 132 and 436 ~~DEGs were annotated,~~differentially expressed genes for P1 and P2 respectively, ~~and~~while KEGG ~~was mainly~~pathways were predominantly enriched in ~~the pathways of phytopathogen~~plant-pathogen interactions and phenylpropanoid ~~synthesis, respectively. The effects of P1 treatment spraying with GR24 on the *Glycyrrhiza uralensis* Fisch growth index, phosphorus content accumulation, chlorophyll a and b content, and antioxidant enzyme activity promotion was~~biosynthesis. Application of GR24 in P1 conditions did not ~~significant,~~significantly affect growth indices but ~~significantly promoted the accumulation of~~did enhance

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glycyrrhetic acid, ~~Isoliquirtin, liquirtin,~~isoliquiritin, and liquiritin accumulation. Transcriptome ~~analysis~~profiling in this treatment identified 465 up-regulated ~~genes~~ and 1,109 down-regulated genes. GO ~~function~~ annotation ~~involving~~involved 1,108 ~~DEGs, while~~differentially expressed genes, and KEGG analysis ~~mainly~~was primarily enriched in the plant-pathogen interaction pathway. ~~In addition, analysis of the changes in~~ Furthermore, transcription ~~factors showed that there were changes~~factor analysis revealed alterations in the C2H2, NAC, and MYB families ~~related to~~, which are associated with phosphorus response.

Keywords: G*lycyrrhiza uralensis* Fisch; Strigolactones; antioxidant enzymes; medicinal constituents; transcriptome

Phosphorus~~, as~~ is an essential nutrient ~~for plant growth,~~ that plays a ~~crucial~~pivotal role in nearly all metabolic processes within plants (Kayoumu M et al., 2023). ~~Nevertheless~~However, the concentration of effective phosphorus ~~concentration~~ in the soil ~~is far from meeting~~often fails to meet the demands of normal ~~demand of~~ plant growth (Qiu et al., 2020). ~~However, the effective concentration of phosphorus in the soil is far from enough to meet the normal demand of plant growth. If the~~Insufficient phosphorus supply ~~is insufficient, the~~ can lead to significant changes in both the external and root morphology of ~~the plant will change, and~~plants, as well as transformations in their physiological characteristics ~~will be transformed~~ (Li et al., 2006). ~~Phosphorus is~~ As a non-renewable resource, ~~with~~ global phosphorus reserves ~~being~~are limited~~. Soil~~, making soil phosphorus availability ~~constitutes~~ the primary limiting factor ~~affecting~~for high agricultural yields in China (Tian, 2001). ~~Soil phosphorus availability is the primary limiting factor affecting agricultural productivity in China.~~ The phosphorus required for plant growth and development is ~~mainly obtained through the~~ primarily sourced from soil phosphorus ~~reservoir, or~~ reserves and fertilization ~~so that plants can absorb enough phosphorus. Increasing~~, ensuring adequate absorption by plants. The practice of increasing phosphorus fertilizer ~~is~~use, however, represents a "high input, low output" ~~pathway to solve the problem of phosphorus nutrition to maintain~~strategy. Consequently, maintaining high crop yields while protecting the environment has become a ~~worldwide~~critical area of global research ~~task~~ (Yuan et al., 2024). Additionally, ~~at the same time,~~ phosphorus stress ~~is also an important factor limiting~~significantly limits the production of medicinal plants~~-~~, further emphasizing its crucial role in agricultural productivity (Vance ey al., 2002). ~~Moreover, phosphorus stress is also an important factor limiting the production of medicinal crops.~~

Glycyrrhiza uralensis Fisch ~~is,~~ a ~~plant belonging to~~member of the genus *Glycyrrhiza Linn* within the *Leguminosae* family, ~~which has the effects of relieving~~is recognized for its medicinal properties, including pain~~, expelling~~ relief, phlegm ~~and~~expulsion, cough~~, benefiting the vital~~ suppression, energy ~~and tonifying the~~enhancement, spleen tonification, and ~~regulating~~the modulation of various ~~medicines, etc~~pharmaceuticals (Gao et al., 2009). ~~Moreover, it also serves as an important~~ Additionally, this species plays a crucial role as a sand-fixing plant in the desert and semi-desert ~~areas~~regions of western China (Du, 2007). ~~However,~~Nevertheless, over~~-~~extraction has severely depleted wild licorice ~~has been damaged by over-excavation and its~~ populations, rendering their resources ~~are seriously~~critically scarce. Cultivated licorice, ~~as~~despite being a mainstream commodity, ~~suffers from the problems of plant~~faces challenges such as inhibited growth, quality degradation, ~~yield reduction, etc. which makes it difficult to meet~~ and reduced yields, issues that complicate adherence to the quality standards ~~stipulated~~established in the Chinese Pharmacopoeia (Gao, 2019). ~~The quality~~Furthermore, the biochemical composition of licorice ~~does not meet the standards outlined in the Chinese Pharmacopoeia. Licorice contains a variety~~includes a diverse array of terpenoids (Zhang et al., 2015). Phosphorus ~~is both~~plays a critical role in the ~~synthesis~~biosynthesis of terpene precursors ~~via~~through the MVA pathway ~~(~~, involving acetyl-CoA, ATP, and NADPH~~) and phosphorus synthesis via~~, as

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well as through the MEP pathway (involving glyceraldehyde phosphate and pyruvate) were found to be, both of which are significant (KAPOOR for plant metabolic functions (Kapoor R et al., 2017).

Strigolactones ((SLs) is a), sesquiterpene hormonehormones derived from beta-carotene (Omoarelojie et al., 2021). This phytohormone is thought, are believed to play a keycrucial role in the regulation ofregulating both aboveground plant conformationarchitecture and root development (Omoarelojie et al., 2021; Ma N et al., 2017; Marzec et al., 2016). GR24, a synthetic Srigolactonesstrigolactone, is known to participaterecognized for its involvement in the responseresponses to abiotic stresses and isacts as a positive regulator of the response to adversitystress responses (Shi et al., 2024). Specifically, GR24, identified as a positive regulator of stress response, has been demonstratedshown to enhance drought and salt tolerance in *Arabidopsis thaliana* (Jumyong Y et al., 2023) and interactsto interact with other hormones to promote lateral root growth in oilseed rape (MAMa et al., 2020). MoreoverAdditionally, it has also been showndemonstrated that exogenous GR24 can improve the metabolism of antioxidant enzyme systems, phenylpropanoid, phenylpropanoids, nitric oxide (NO), and H_2Shydrogen sulfide (H2S) in strawberry to maintainstrawberries, thereby maintaining fruit quality during storage (Huang et al., 2021). The results of this study are summarized in the following table. Meanwhile, it has also been shown that Moreover, dulcitolactone canhas been shown to regulate the plant type and phytoplasma of medicinal plants, therebythus influencing the growth and development of medicinal parts in a targeted manner, and achieving the purpose of regulating the to achieve the desired "optimal type" (Cao et al., 2023).

Therefore, inIn the present study,we aimed our aim was to screenidentify a more favorableeffective regimen for the growth of *Glycyrrhiza uralensis* Fisch in a low -phosphorus environment as well astoenvironments and to investigate the effect of exogenously appliedeffects of the exogenous application of monocotyledonin lactone on the accumulation of medicinal components.

1. Experimental methodology

1.1 Overview of the pilot area

The testexperimental material wasconsisted of *Glycyrrhiza uralensis* Fisch seedlings, each with four true leaves, and the. These seedlings were maintainedcultivated and treated in the Wenjiang District, of Chengdu (, located at coordinates 30°36′-30°52′ N, and 103°41′-103°55′ E), which has. This region is characterized by a subtropical monsoon climate with, which offers a favorable, temperate climate, a longenvironment, extended summer period, a shortseasons, brief winter periodperiods, and a longprolonged frost-free periodintervals (Deng, 2022).

1.2 Experimental material

The plant material used for the test was For the experiment, uniform and fully developed seeds of *Glycyrrhiza uralensis* Fisch, full and uniformly textured seeds were selected for. These seeds were treated by soaking and mixing within 98% sulfuric acid for half an hour and rinsedthirty minutes followed by extensive rinsing (more than three times) with plenty of distilled water (Deng, 2022; Liang et al., 2016). The test substrate for the test was a 1:1homogeneous mixture of field soil and sand, in a 1:1 ratio. The culture

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containers used were autoclaved ~~(121°C, 2h)~~ hydroponic boxes (~~70 boxes),~~ 121°C for 2 hours) and 30×25 cm plastic pots, totaling 70 boxes.

1.3 Drugs and reagents

The synthetic analog of strigolactones, GR24 ~~of the test Strigolactones,~~ was ~~purchased~~procured from Beijing Kulaibo Science and Technology Co., Ltd~~.~~., with a CSA No~~.~~. of 76974-79-3. Initially, 1 mg of GR24 was ~~taken~~dissolved in ~~advance, and~~ a small ~~amount~~volume of acetone ~~was added to dissolve it~~, and then diluted with water ~~was added~~ to a volume of 335.2 ~~μ~~μL to ~~obtain the~~prepare a 10 mM ~~GR24 mother~~stock solution (Zhu et al., 2022~~), which was then configured into~~). This stock solution was further diluted to create standard solutions at concentrations of 0 ~~μ~~μmol/L, 1 ~~μ~~μmol/L, 10 ~~μ~~μmol/L, 100 ~~μmol/L. The GR24 mother solution was then reconstituted into 0 μmol/L, 1 μmol/L, 10 μmol/L, 100 μ~~μmol/L, and 1000 ~~μmol/L aqueous solution, 1000 μmol/L aqueous GR24 solution~~μmol/L for experimental use.

1.4 Experimental design

The ~~test began~~ experiment commenced on April 10, 2023, ~~first~~with the sterilization of ~~all, the~~ licorice seeds ~~were sterilized, and petri dish~~followed by their germination~~, 7~~ in Petri dishes. Seven days ~~after the seed~~ post-germination, seedlings with a high top cover ~~moved~~ were transferred into ~~the~~ hydroponic ~~box seedling~~boxes (70 boxes, each ~~box of~~containing 9 plants, ~~a total of~~totaling 630 seedlings~~) hydroponie~~). These were cultivated hydroponically until the ~~licorice grows out of~~seedlings developed 4-5 ~~pieces of~~ true leaves ~~(probably about~~, approximately 20 days~~),~~ later. Subsequently, seedlings exhibiting similar growth were selected ~~the growth of the similar licorice seedlings moved into the diameter of 30 cm, the height of the 25 cm~~ and relocated to plastic pots~~, the pots~~ measuring 30 cm in diameter and 25 cm in height. The cultivation substrate ~~for the Mixed and~~in these pots consisted of a sterilized mixture of garden soil and sand ~~(~~in a 1:1~~),~~ ~~3~~ ratio. Three seedlings were transplanted into each pot, ~~totaling~~ resulting in a total of 120 pots. After ~~transplanting, all~~transplantation, the pots were ~~placed in the~~ initially stored inside a building~~, and one~~. One week later, they were ~~placed in the~~moved to an outdoor experimental field~~, which was sheltered~~ protected from rain. ~~At the beginning of soil~~Soil cultivation~~,~~ 1/4 of ~~the~~ began with the application of 1/4 strength Hoagland nutrient solution ~~was used to slow cultivate for 5 days, after that, 1/2 of the~~to gradually acclimate the seedlings over five days, followed by 1/2 strength for the next five days. Full-strength Hoagland ~~nutrient~~ solution was ~~used to cultivate for 5 days, and~~ then ~~the full amount of Hoagland nutrient solution was~~ used for ~~normal~~regular management~~, and then~~. When the seedlings ~~were starved for five days when they grew~~bore 6-8 true leaves, ~~and~~ they underwent a five-day starvation period during which they were moderately rehydrated ~~during the period~~ (Zhang et al., 2023). ~~After the end of~~Following the starvation treatment, different phosphorus concentrations were ~~supplied, and three phosphorus concentrations~~administered. Three treatments were ~~set: the~~established: full ~~amount of~~ strength Hoagland ~~nutrient~~ solution as the normal phosphorus supply ~~treatment~~ (P3), ~~the~~one-third the original $NH_4H_2PO_4$ concentration ~~of~~in the Hoagland ~~nutrient~~ solution ~~$NH_4H_2PO_4$ was reduced to 1/3 of the original as~~for the low phosphorus treatment (P2), and ~~the~~a phosphorus-free Hoagland ~~nutrient~~ solution ~~was set as~~for the zero phosphorus ~~free~~ treatment (P1~~), and regular fertilizer application was carried out i.e., once a week~~). Regular fertilization involved weekly watering~~,~~ with 500 ml ~~at a time,~~of solution, supplemented by moderate ~~supplemental~~additional watering~~.~~ as necessary. Foliar spraying ~~of different~~with various concentrations of GR24 ~~treatments was started~~commenced on June

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10~~, the~~. The treatment ~~group~~groups included a control sprayed with GR24 (G1~~), as a blank control at~~) and four ~~concentration levels (1 μM,~~additional groups treated with GR24 at concentrations of 1 μM (G2), 10 ~~μ~~μM~~;~~ (G3), 100 ~~μ~~μM~~;~~ (G4), and 1000 ~~μM) i.e., G2, G3, G4, and~~ μM (G5~~, and spray every~~). Spraying was performed each evening until the leaves ~~solidify into~~retained water droplets ~~and do not drop, with a frequency of once a week. The pots of all treatment groups were randomly placed at a distance between the pots to avoid shading each other. The pots were randomly switched every two weeks to~~without dripping, repeated weekly. To minimize ~~the effect of~~ environmental ~~factors on them. At the end~~biases, the placement of pots within each treatment group was randomized, and their positions were systematically rotated every two weeks. At the conclusion of the growing season in October 2023, ~~Uralic sweetgrass started to be measured for~~ the morphological ~~indexes as well as various~~ and physiological ~~indexes~~indices of Glycyrrhiza uralensis were systematically measured.

1.5 Indicators and methods

1.5.1 Measurement of growth indicators

~~All plants were harvested at~~At the end of October 2023, all plants were harvested, and the adhering soil ~~on the plants~~ was ~~slowly washed away~~gently rinsed off with running water. ~~Five~~In each treatment group, five plants were ~~collected in each group.~~sampled. The length of the underground ~~part~~portion was measured ~~with~~using a steel tape, while the diameter of the main root was ~~measured~~gauged with a vernier caliper~~, and the~~. The fresh ~~weight~~weights of the aboveground and underground parts of *Glycyrrhiza uralensis* Fisch ~~was weighed with~~were determined using a balance. ~~Take a picture~~A photograph of each specimen, with a ruler ~~as a reference, upload the picture~~for scale, was taken and uploaded to Image J for root area projection analysis~~, get the projected area of the root, dry it~~. The samples were then dried in an oven at 75~~°C to~~°C until a constant weight was achieved, and ~~weigh~~ the dry ~~weight~~weights of the aboveground and underground parts ~~of *Glycyrrhiza uralensis* Fisch with a balance~~were subsequently weighed.

1.5.2 Determination of chlorophyll content

~~Five~~For each treatment, five fresh leaves were collected ~~from each treatment, ~~, washed, and dried~~, the~~. The veins were ~~cut off~~removed, and ~~cut into pieces, 0.1g~~the remaining leaf tissue was ~~weighed,~~chopped. A sample weighing 0.1 g was used for the extraction and ~~the~~ quantification of chlorophyll a and ~~B were detected by~~ b using the acetone method (Yang, 1996~~), and then the~~). The concentrations of chlorophyll a and ~~chlorophyll~~ b were calculated ~~according to~~using the ~~formula~~appropriate formulas.

1.5.3 Antioxidant enzyme assay

~~Five~~From each treatment group, five plants were randomly selected ~~from each treatment, and the~~. The fully expanded apical leaves were ~~collected~~harvested, washed ~~and~~, dried, and weighed ~~to determine~~. The activities of superoxide dismutase (SOD) and peroxidase (POD) were measured spectrophotometrically ~~by~~using a Solarbio kit~~, and catalase~~. Catalase (CAT) ~~by~~ activity was determined using a Shimadzu UV-2041 ultraviolet spectrophotometer (Shimadzu, Japan) (Xu et al., 2022~~), model UV-2041 spectrophotometer (Shimadzu, Japan~~).

1.5.4 Materials and methods for the determination of pharmaceutical ingredients

1.5.4.1 Pharmaceutical ingredient measurement materials

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The medicinal constituents of *Glycyrrhiza uralensis* Fisch were ~~determined~~quantified using High-Performance Liquid Chromatography (HPLC ~~()~~ as detailed by Xu et al~~.,~~ . (2021~~), and the~~). Standards for glycyrrhetic acid ~~standard was purchased~~were sourced from Chengdu Pufide Biotechnology Co~~. Lsoliquirtin standard was purchased~~., isoliquiritin from Shanghai McLean Company, and ~~the rest of the standard was purchased~~other standards from Chengdu Kangbang Biotechnology Co. Ltd~~, with batch~~. Batch numbers ~~of~~for these standards include Glycyrrhizic acid (21080201), Glycyrrhetic acid (20041002), ~~LsoliquirtigeninIsoliquiritigenin~~ (21101901), ~~LsoliquirtinIsoliquiritin~~ (C11602211), Glabridin (21032701), Liquirtigenin (22110902~~)~~), and Liquirtin (21041301~~) (see annex for~~). Further details~~)~~. are provided in the annex.

1.5.5 Transcriptome assays

The root tips of *Glycyrrhiza uralensis* Fisch ~~was dug out and the root tips~~ were ~~picked and~~excavated, washed to remove ~~the~~ soil, and ~~quickly put into~~immediately immersed in liquid nitrogen for ~~quick~~rapid freezing ~~and then transferred into a~~. Subsequently, samples were stored at -80~~°C refrigerator,~~°C and sent to Beijing Baimaike Company for ~~testing~~transcriptome analysis.

1.6 Data processing

~~The data~~Data were analyzed using SPSS 25 software ~~and the level of~~ . The significance of ~~difference~~differences was ~~analyzed~~assessed using Duncan's multiple ~~comparisons of Duncan's~~ range test and LSD ~~in~~test within a one-way ANOVA ~~(one-way ANOVA) analysis, and the experimental results~~framework. Results were ~~plotted~~graphically represented using Origin2022 software.

2. Results and analysis

2.1 Effect of GR24 on growth ~~indexes~~indices of *Glycyrrhiza uralensis* Fisch under different phosphorus concentrations

~~As shown in~~ Table 1~~,~~ demonstrates that under no phosphorus stress, the fresh weight, dry weight, root length, basal stem diameter, and root projected area of *Glycyrrhiza uralensis* Fisch ~~under no phosphorus stress~~ were ~~smaller than~~ lower compared to those ~~of~~observed under low phosphorus stress and normal phosphorus supply treatments~~, fresh weight, dry weight, root length and basal stem of *Glycyrrhiza uralensis* Fisch~~. Furthermore, these indices under low phosphorus stress were ~~smaller than those under~~ also reduced when compared to the normal phosphorus supply ~~treatments~~conditions, although ~~the~~these differences were not statistically significant. ~~And the difference~~The variation in root projected area, however, was significant. ~~Under~~ There were no significant differences in any indices among the GR24 concentration treatments under no-phosphorus stress~~, there was no significant difference between all indexes of GR24 concentrations~~ when compared to the G1 concentration treatments. ~~However,~~Under low phosphorus stress, the G3 concentration ~~significantly increased~~notably enhanced the fresh weight, dry weight, root length, and projected root area of licorice by 53.8%, 38.2%, 20.1%, and 28.3%, respectively. ~~The~~In the normal phosphorus supply treatment, the G3 concentration significantly increased the fresh weight, dry weight, root length, and basal stem diameter of *Glycyrrhiza uralensis* Fisch by 78.57%, 82.1%, 36%, and 45.8%, respectively.

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~~Additionally~~Moreover, the root projected area ~~of *Glycyrrhiza uralensis* Fisch was~~ significantly increased by 46.1% and 36.1% under G3 and G5 concentrations, ~~i.e. by 46.1% and 36.1%,~~ respectively.

2.2 Effect of GR24 on phosphorus content of roots, stems, and leaves of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

As depicted in Figure 1 ~~illustrates that,~~ under no phosphorus stress conditions, ~~under no-phosphorus stress treatment,~~ phosphorus accumulation in the roots, stems, and leaves of licorice treated with GR24 was lower than that ~~of the~~ observed under low- phosphorus stress ~~treatment~~. Similarly, ~~under low-phosphorus stress treatment,~~ phosphorus accumulation ~~in roots, stems, and leaves of treated *Glycyrrhiza uralensis* Fische~~ was ~~lower than that of the~~reduced under low phosphorus stress compared to normal phosphorus ~~supplying treatment~~ supply conditions.

Under normal phosphorus supply ~~treatment~~, the ~~accumulation of~~ phosphorus content in the roots ~~of licorice~~ treated with the G3 concentration was significantly higher than that ~~of~~ treated with the G1 concentration ~~by~~, showing an increase of 23.1%. ~~Conversely, normal phosphorus supply treatment, G3, G5 concentration treatment of *Glycyrrhiza uralensis* Fisch stem~~For the stems, phosphorus content ~~accumulation~~under treatments with G3 and G5 concentrations was significantly higher than under the G1 concentration treatment, ~~respectively increased by~~ with increases of 98.33% and 138.33%.%, respectively. Furthermore, under no-phosphorus stress ~~treatment~~, the ~~accumulation of~~ phosphorus content in the leaves ~~of licorice under~~ treated with the G4 concentration ~~treatment~~ was significantly higher than that ~~of~~in the G1 ~~concentration~~ treatment, ~~which increased by~~exhibiting an increase of 68.66%, ~~and under~~%. Under low phosphorus stress ~~treatment~~, the ~~accumulation of~~ phosphorus content in the leaves ~~of *Glycyrrhiza uralensis* Fisch~~ under G2 and G3 concentration treatments was significantly higher than that ~~of~~under the G1 ~~concentration~~ treatment, ~~which increased by~~with increases of 29.31% and 30.46%, respectively. ~~And the~~Additionally, under normal ~~supply of~~ phosphorus ~~treatment~~supply conditions, the G3 treatment group ~~had~~exhibited a significant increase in leaf phosphorus content of 19.35% compared to the G1 group.

2.3 Effect of GR24 on antioxidant enzyme activities of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

Figure 2 ~~demonstrates~~illustrates the impact of phosphorus stress on the activities of ~~SOD (~~superoxide dismutase~~), POD (~~ (SOD), peroxidase (POD), and ~~CAT (~~catalase (CAT). The activities of SOD, POD, and CAT ~~all became larger~~ increased with ~~the aggravation of~~escalating phosphorus stress~~, and the activities~~ levels. The activity levels of SOD and POD ~~of *Glycyrrhiza uralensis* Fisch~~ under no phosphorus stress were higher than those ~~of~~in the low phosphorus stress group and the normal phosphorus supply treatment group. ~~Additionally, the~~ Furthermore, CAT activities ~~of CAT~~ were significantly higher than those ~~of~~seen in the low phosphorus stress group and the normal phosphorus supply treatment group~~, and the activities of~~. Also, SOD activities were higher in the no phosphorus stress group than ~~those of~~in the normal phosphorus supply treatment group, ~~and the activities of~~ whereas POD and CAT activities were significantly higher ~~than those of the normal phosphorus supply treatment group at the~~ in the low phosphorus stress~~. POD and CAT activities were both significantly higher than those of~~ group compared to the normal phosphorus supply ~~treatment~~ group.

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Under no-phosphorus stress treatment, the G2 concentration of GR24 treatment significantly increased theSOD activity of SOD i.e.by 15.76% increase, but the effect of%, although the G3 concentration treatment group was notshowed no significant, and there effect. There was no increase, and thealso a significant increase in POD and CAT activities ofin the GR24 concentration treatments compared withto the G1 concentration treatment. Under low phosphorus stress treatment, The POD activity of G3 concentration treatment was higher than that of G1 concentration treatment, which with a significant increased by 19.6%, while CAT activity was not improved and significantly increased in each concentration treatment group. Theresulted in a significant 19.6% increase in POD activity compared to the G1 concentration treatment. Similarly, SOD activity ofin the G3 concentration treatment was significantly higher than that ofin the G1 concentration treatment and , with a significant increased byan increase of 38.5%. The POD activity of the G3 concentration treatment was discarded higher than that of the G1 concentration treatment, withalso showed a significant increase of 22.9%.% over the G1 treatment. However, there was no improvement andwere no significant increaseimprovements in CAT activity in eachacross any concentration treatment groupgroups.

2.4 Effect of GR24 on the content of medicinal components of *Glycyrrhiza uralensis* Fisch atunder different phosphorus concentrations

Based on theThe experimental results, it was known indicated that the G3 concentration had the best effect on the regulation ofwas most effective in regulating *Glycyrrhiza uralensis* Fisch, so the treatment with G3; therefore, this concentration was chosenselected for the subsequent experiments. Analysis fromof Figure 3 revealed that there wasshowed no significant differencedifferences in the contentslevels of glycyrrhetic acid, liquirtigenin, liquirtin, and glabridin between the treatments with different levels ofacross varying phosphorus supplylevels and different GR24 concentrations. TheHowever, the content of lsoliquirtigeninisoliquirtigenin in the low phosphorus stress treatment was significantly higher than that in the no-phosphorus stress treatment at both G1 and G3 concentrations, and the content of lsoliquirtigenin in the normal phosphorus supply treatment was significantly higher than that in the no-phosphorus stress treatment at both G1 and G3 concentrations. Furthermore, contentSimilarly, isoliquirtigenin levels in the normal phosphorus supply treatment were significantly elevated compared to those in the no-phosphorus stress treatment at both concentrations. Additionally, the levels of liquirtigenin in both the normal phosphorus supply treatment and low phosphorus stress treatment wastreatments were lower than that ofthose in the no phosphorus stress treatment, and the content of . Specifically, in the G3 concentration treatment, the contents of liquirtigenin and glycyrrhizic acid in G3 concentration treatment was increased significantly increased by 72.2% and 21.26%, respectively. The lsoliquirtigenin content of the Under low phosphorus stress treatment was significantly higher than that of the no-phosphorus stress treatment under the G1 concentration treatment, and that of the G3 concentration treatment was higher than that of the no-phosphorus stress treatment but not significant. Lsoliquirtigenin, the levels of isoliquirtigenin, liquirtigenin, and liquirtin were significantly higher in the G3 treatment than in the G1 treatment under low phosphorus stress treatment by, showing increases of 131.29%, 118.79%, and 145.83%, respectively. UnderIn the phosphorus-free treatment, the G3 treatmentconcentration significantly increased lsoliquirtinboosted the contents of isoliquirtigenin and liquirtin by 164.52% and 23.94%, respectively.

2.5 Effect of GR24 on the expression of *Glycyrrhiza uralensis* Fisch genes under different phosphorus concentrations

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2.5.1 Transcriptome sequencing quality assessment

In this ~~experiment, the~~study, RNA sequencing analysis was performed on 18 eukaryotic ~~reference transcriptome (RNA-seq) analysis of 18~~ samples ~~was completed, and~~, yielding a total of 122.60 Gb ~~Clean Data was obtained, and the Clean Data of~~ of clean data, with each sample ~~reached~~providing approximately 5.82 Gb (See Appendix Table 3). ~~In this study, the~~ The quality of the sequencing was substantiated by a Q30 base percentage ~~of Q30 bases was~~exceeding 95.17% ~~and above~~ (See Appendix Table 4), ~~indicating that the sequencing base recognition was~~reflecting reliable and accurate~~. In this study, the Clean Reads of~~ base recognition. The clean reads from each sample were ~~sequenced~~mapped against the designated reference genome~~, and the~~ with a matching efficiency ~~ranged~~ranging from 70.36% to 91.75%.~~–~~

2.5.2 Differential gene expression analysis

~~In this study, differential~~Differential gene expression analysis was conducted for the comparisons AvsB, DvsE, and GvsH ~~was conducted~~ using ~~Fold Change ≥~~a fold change threshold of ≥2 and a FDR ~~<~~ of ≤0.01 ~~as screening criteria~~ (Fig. 4). The statistical power ~~value of~~for AvsB ~~is~~was 0.7422, ~~that of~~ DvsE ~~is~~was 0.4688, and ~~that of~~ GvsH ~~is~~was 0.506. A total of 1,574 differential gene expressions were identified in AvsB, ~~of which~~comprising 465 ~~were~~ up-regulated ~~genes~~ and 1,109 ~~were~~ down-regulated genes~~, a total of~~. In DvsE, 214 differential ~~gene~~ expressions were identified ~~in DvsE, of which~~, with 137 ~~were~~ up-regulated ~~genes~~ and 77 ~~were~~ down-regulated genes~~, and a total of~~. For GvsH, 588 differential ~~gene~~ expressions were ~~identified in GvsH. 137~~ noted, evenly split with 294 up-regulated ~~genes~~ and ~~77~~294 down-regulated genes~~, GvsH identified a total of 588 differentially expressed genes. of which 294 were up-regulated genes and 294 were down-regulated genes.~~.

2.5.3 GO enrichment analysis

The GO functional enrichment analysis of ~~differentially expressed genes (~~DEGs~~)~~ in the *Glycyrrhiza uralensis* Fisch transcriptome ~~(Fig.~~, as depicted in Figure 5~~),~~, revealed that 1,108, 132, and 436 DEGs were annotated in the GO database ~~under~~for the comparative analyses of AvsB, DvsE, and GvsH, respectively. In the AvsB ~~was distributed in~~comparison, DEGs were classified into 17 ~~classes~~categories of Biological ~~process~~Processes (BP), 3 of Cellular Components (CC), and 12 of Molecular Functions (MF). DvsE showed distributions across 16 BP classes, 3 CC classes ~~of Cellular component (CC),~~ and ~~12~~11 MF classes ~~of Molecular function (MF), DvsE was distributed in 16~~, whereas GvsH had 19 BP classes ~~of BP~~, 3 CC classes ~~of CC~~, and 11 MF classes ~~of MF, and GvsH was distributed in 19 classes of BP, 3 classes of CC and 11 classes of MF. AvsB was distributed in 17 categories of BP, 3 categories of CC, and 12 categories of~~, with an additional category in MF~~, DvsE was distributed in 16 categories of BP, 3 categories of CC, and 11 categories of MF, and GvsH was distributed in 19 categories of BP, 3 categories of CC and~~ bringing the total to 13 ~~categories of MF~~. Further ~~analysis~~scrutiny of the ~~top~~highest-ranked GO terms ~~revealed that the BP was mainly concentrated~~indicated a primary concentration in BP~~, especially~~ under the AvsB treatment ~~of AvsB. BP is mainly~~. Notably, the cellular process was significantly enriched ~~in cellular process~~ with 509 ~~changes, of which~~ alterations, comprising 139 ~~are~~ up-~~regulated~~regulations and 370 ~~are~~ down-~~regulated, and~~regulations. Similarly, the cellular anatomical entity exhibited 560 modifications, with ~~560 changes, of which~~ 138 ~~are~~ up-~~regulated~~regulations and 423 ~~are~~ down-~~regulated, and MF is mainly enriched~~regulations, aligning with the enrichment in CC ~~with~~, which also displayed 560 changes~~, of which 138 are up-regulated and 423 are down-regulated. In DvsE, there were 67 changes in~~. In the DvsE set, the metabolic process ~~enriched~~ in BP~~, of which~~ included 67 changes (38 ~~were~~ up-regulated and 29 ~~were~~ down-regulated~~,~~), and ~~there were 69 changes~~

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~~in~~ the cellular anatomical entity ~~enriched in MF, of which 69 were up-regulated and 29 were down-regulated. There were 69 changes in anatomical entity enriched in MF, of which 43 were up-regulated and 26 were down-regulated. 71 changes in~~in MF showed 69 changes with an equal number of up-regulations. Additionally, the catalytic activity ~~enriched~~ in CC~~, of which~~ showed 71 changes, with 40 ~~were~~ up-~~regulated~~regulations and 31 ~~were~~ down-~~regulated.~~regulations. For GvsH, the analysis reflected 204 changes ~~were found~~ in the cellular process ~~enriched in BP in GvsH, of which 38 were up-regulated and 29 were down-regulated. In GvsH, there were 204 changes in BP, of which~~ of BP (107 ~~were~~ up-regulated and 97 ~~were~~ down-regulated~~,~~), 217 changes in ~~MF~~ (the cellular anatomical entity~~),~~ of ~~which~~ MF (112 ~~were~~ up-regulated and 105 ~~were~~ down-regulated~~,~~), and in CC, binding was characterized by 216 ~~changes in CC (binding), of which~~ alterations (91 ~~were~~ up-regulated and 125 ~~were down-regulated, and 216 changes in GvsH (cellular process), of which 91 were up-regulated and 125 were down-regulated. There were 216 changes in binding enriched in CC, of which 91 were up-regulated and 125 were down-regulated.~~down-regulated).

2.5.4 KEGG pathway enrichment analysis

KEGG pathway enrichment analysis was ~~performed~~conducted on DEGs~~. Figure 8 shows~~ to identify the most significant metabolic and regulatory pathways affected. As illustrated in Figure 6, the analysis highlighted 20 pathways with the highest enrichment ~~in~~levels across the ~~comparison~~comparisons of AvsB, DvsE, and GvsH~~, where~~. In the AvsB and DvsE ~~were most abundant in~~comparisons, the plant-pathogen interaction pathway was predominant, with 96 and 18 entries, respectively~~.~~. This was followed by the GvsH comparison in the ~~MARK~~MAPK signaling pathway-plant ~~pathway~~ with 36 entries and ~~7, and GvsH in~~ the Phenylpropanoid biosynthesis (PBP) pathway with 14~~, followed by~~ entries. Additionally, the DNA replication pathway was notable with 8~~. In addition to this, we found that~~ entries. AvsB ~~was significantly enriched in~~ showed a significant enrichment of four genes in the Flavone and flavonol biosynthesis pathway~~,~~. Similarly, in GvsH, there was ~~significantly enriched in~~a notable enrichment of four genes in the Flavonoid biosynthesis pathway~~, Terpenoid backbone biosynthesis, and GvsH was significantly enriched in~~ and two genes in the Terpenoid backbone biosynthesis pathway, ~~and GvsH was significantly enriched in two~~with another five and four genes enriched in the ~~Terpenoid backbone biosynthesis~~latter pathway~~. backbone biosynthesis) pathway, GvsH was significantly enriched in 5 and 4 genes,~~ respectively.

2.5.5 Analysis of differential transcription factors

~~A total of 1495 Unigene were annotated as transcription factors in~~The *Glycyrrhiza uralensis* Fisch glabra transcriptome data~~, and as can be seen~~ revealed a total of 1495 unigenes annotated as transcription factors. As demonstrated in Table 2, ~~there were~~ 24 types of transcription ~~factor types with different~~factors exhibited varied expression patterns ~~of~~across the ~~same transcription factor in~~ AvsB, DvsE, and GvsH comparisons~~, with the highest number of differentially expressed~~ . The C2H2, NAC, WRKY, MYB, and GRAS~~. The top 3~~ transcription factors showed the highest numbers of differentially expressed genes. Specifically, the most up-regulated transcription factor families ~~with the most up-regulated expression~~ in AvsB ~~were~~included AP2/ERF-ERF, C2H2, and MYB, ~~which were~~with up-~~regulated~~regulations noted for 6, 2, and 2 genes, respectively. ~~The top 3 transcription factor families with the~~ The most down-regulated ~~expression~~families were C2H2, NAC, and WRKY, with 10, 9, and 8 genes down-regulated, respectively. ~~the top 3 transcription factor families up-regulated in~~In DvsE~~,~~ the top up-regulated transcription factor families were WRKY, MYB, and bHLH, each with increases in 2, 1, and 1 genes ~~up-regulated~~, respectively~~, and the~~; no down-regulations were observed. For GvsH, the most up-regulated transcription factor families were ~~all~~

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0. The top 3 transcription factor families up-regulated in GvsH were C2H2, bHLH, and HSF, all up-regulatedeach by 1 gene, andwhile the top 3most down-regulated transcription factor families wereincluded AP2/ERF-AP2, MYB, and AP2/ERF-ERF, down-regulated bywith decrements of 4, 3, and 3 genes, respectively.

3. DISCUSION

Phosphorus plays a critical role in is crucial for various physiological pathways of plant growth and is one of the indispensable elementsan essential element for plant growth (Santoro V et al., 2024), and). Deficiency in phosphorus deficiency will affectcan significantly impact the morphological, physiological, and biochemical characteristics, physiology, and biochemistry of plants. SLs have been demonstrated to positively regulateshown to enhance plant growth indicators, participate in contribute to the stress response of plants (Marzec et al., 2016), and regulate thesupport positive growth of plants in adversity (under adverse conditions (Andreo-Jimenez et al., 2015; Hong et al., 2020; HA C V L-G M A et al., 2014). It has also been shown that Additionally, GR24 can directionally, a synthetic analog of SLs, has been found to specifically regulate the medicinal partscomponents of medicinal plants (Cao et al., 2023; Wani K I et al., 2022). Despite extensive research on theinto GR24's ability of GR24 to mitigate plant stress, there are no reports on the alleviation of in plants, its effects on phosphorus stress in *Glycyrrhiza uralensis* Fisch by GR24have not yet been reported.

3.1 Effect of GR24 on growth indexesindices of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

Since the Phosphorus in soil will fix the phosphorusis predominantly in a form that can beplants cannot directly absorbed by the plant causing it to be difficult to move, the main phosphorus that can be absorbed by the plant is the effective phosphorus that can be accessed by the rootsabsorb, leading to its immobilization and limited availability to roots, which can only access what is termed 'effective phosphorus' (Péret et al., 2011; Li et al., 2023), so the). Consequently, root system indexes such as the length of the plant roots and the absorbing area can be used as an indicator of the efficiency of phosphorus uptake by the plantindices (Ding et al., 2008). The root system indicators such as root length and absorption area can be usedserve as indicators of plant phosphorus absorptionuptake efficiency (Ding et al., 2008). . In thisThis study, it was found revealed that phosphorus stress significantly impedes the growth morphology of *Glycyrrhiza uralensis* Fisch was significantly inhibited under phosphorus stress, and the; specifically, fresh weight, dry weight, root length, basal stem diameter, and root projected area of the plant were significantly markedly reduced compared to those in plants supplied with that of the normal phosphorus supplying group, and the decline of the above indexes was levels. These declines in growth indices were more obvious with the aggravation of pronounced as phosphorus stress intensified. Under no phosphorus stress, and under conditions of low phosphorus stress, sprayingavailability, the application of GR24 at the G3 concentration of GR24 significantly increasedenhanced the fresh weight, dry weight, root length, and root projection area of *Glycyrrhiza uralensis* Fisch compared withto the G1 treatment group. For instanceSupporting this observation, Tang (2019) reported that GR24 treatment improved the morphological indexesindices of *Oryza sativa L.* seedlings under phosphorus stress, and. Additionally, Tai et al. (2017) et al. found that GR24 treatment could promote thepromoted biomass accumulation ofin *Panicum virgatum L.* seedlings under cadmium stress, which is

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~~consistent with~~corroborating our findings~~, indicating~~ that ~~SLs~~strigolactones can ~~allocate~~mitigate the ~~damage~~detrimental effects of low ~~phosphoris~~phosphorus stress ~~to plant~~in plants. These results ~~indicated~~indicate that ~~strigolactone could regulate~~strigolactones can enhance the growth of *Glycyrrhiza uralensis*, Fisch under low phosphorus stress~~,~~ and ~~can~~ improve the ~~ability of *Glycyrrhiza uralensis Fisch* seedlings~~seedlings' adaptability to ~~adapt to low~~ phosphorus ~~environment~~ deficient environments. Similarly, Pang (2020~~) found that the use of GR24 could promote the increase of~~) discovered that GR24 application promoted increases in body length and the number of lateral roots ~~of~~in *Astragalus membranaceus var. mongholicus* (Bunge) P.K.~~Hsiaomain root, and in~~Hsiao. In this study, we also ~~found~~observed that under a normal phosphorus supply ~~treatment~~, the G3 concentration of GR24 significantly enhanced the fresh weight, dry weight, root length, basal stem diameter, and ~~-~~root ~~projection~~projected area of *Glycyrrhiza uralensis* Fisch~~,~~ ~~It can be inferred~~This suggests that GR24 can ~~also act~~function as a plant hormone to regulate ~~plant~~ growth under non-stress ~~free~~ conditions as well (Zhou, 2016). Under conditions of complete phosphorus ~~free stress treatment, spraying~~deprivation, however, application of GR24 did not significantly improve the ~~above indicators~~aforementioned growth indices of licorice, indicating that ~~spraying~~ GR24 can only partially alleviate the ~~damage caused by~~adverse impacts of stress ~~to a certain extent~~.

3.2 Effect of GR24 on chlorophyll content of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

Chlorophyll ~~plays a crucial role in~~ is essential for photosynthesis ~~by~~, absorbing ~~(Fromme et al., 2003)~~ and transferring light energy for primary photochemical reactions and other processes (~~TREVOR~~Fromme et al., 2003; Trevor G et al., 2009). ~~Chlorophyll~~ The chlorophyll content in plant leaves ~~can reflect~~reflects the photosynthetic capacity of ~~plant leaves~~the plants. Low phosphorus stress inhibits photosystem activity (Tang et al., 2005) and chloroplast membrane development, ~~thus inhibiting~~thereby reducing photosynthesis (Li et al., 2018). ~~Plants accumulate biomass through photosynthesis, and photosynthetic pigment content influences photosynthetic performance(Li et al., 2013), and in this~~ This study~~,~~ found that chlorophyll a and b in *Glycyrrhiza uralensis* Fisch ~~decreased~~diminished with increasing ~~forest~~phosphorus stress, indicating a ~~decrease~~decline in photosynthetic pigments and ~~a decline in the~~ photosynthetic performance ~~of licorice~~ under low phosphorus ~~stress. Furthermore,, it was also found that~~ conditions. However, the exogenous application of GR24 at the G3 concentration significantly increased the content of chlorophyll a and b under low phosphorus stress. ~~It has been~~ Previous studies have shown that GR24 can ~~increase~~enhance the chlorophyll content ~~of~~in *Triticum aestivum L.* under drought stress (Fang et al., 2021), which ~~is consistent~~aligns with the ~~results~~findings of this study. This suggests that ~~monocotyledonin lactone can participate~~strigolactones may play a role in the photosynthetic regulation of plants (Li et al., 2017) and ~~exogenous~~that the addition of GR24 can ~~increase the~~improve chlorophyll content in plant leaves under phosphorus stress ~~and alleviate~~, thereby mitigating the ~~effect~~adverse effects of low phosphorus ~~stress~~ on ~~the~~ photosynthetic performance ~~of plants~~ (Seiji et al., 2014). Additionally, ~~the present~~this study ~~also found~~observed that ~~the~~a normal supply of phosphorus ~~to the plant can increase the~~inherently increases chlorophyll content. ~~In addition, this study also found that the~~The application of an appropriate concentration of GR24 (~~i.e.,~~ specifically, the G3 concentration) under normal phosphorus ~~supply could~~conditions also ~~increase~~elevated the ~~content of~~ chlorophyll a and b content in *Glycyrrhiza uralensis* Fisch ~~and improve~~, enhancing the photosynthetic performance of ~~*Glycyrrhiza uralensis* Fisch photosynthetic system.~~the plant. Contrarily, Tian (2018) ~~found~~reported that ~~different~~various concentrations of strigolactone ~~have different~~had differing effects on the leaves of *Bambusa oldhamii Munro*, ~~and~~noting that a high concentration (5 μmol/~~l~~L GR24) ~~has an inhibitory~~

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~~effect on~~inhibited the green content of leaves~~, which is contrary to this study, which~~. This discrepancy may be ~~because~~due to the optimal concentration of GR24 ~~is~~varying among different ~~for different plants~~plant species.

3.3 Effect of GR24 on antioxidant enzyme activities of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

Phosphorus ~~is,~~ a ~~key~~critical component of cell membranes, ~~and the cell membrane~~ significantly influences their structure ~~of plants will be affected~~, particularly under conditions of low phosphorus stress (Jiang et al., 2024). ~~The to~~To mitigate ~~the~~oxidative damage ~~of~~induced by reactive oxygen ~~radicals (Gill et al., 2010),~~species, plants ~~will reduce~~regulate the ~~damage of reactive oxygen radicals on~~activities of SOD, POD, and CAT. This regulation is essential for protecting proteins, nucleic acids, and membrane systems ~~by regulating the activities of SOD, POD, and CAT to ensure their~~ , thereby supporting normal growth (Wang et al., 2022~~,~~; Foyer et al., 2013~~,~~; Wei et al., 2018). Research by Tang (2019) ~~showed~~demonstrated that the application of GR24 ~~applied~~ under low phosphorus stress ~~could reduce~~not only reduces the accumulation of reactive oxygen species~~, increase~~ in *Oryza sativa L.* seedlings but also enhances the activities of protective enzymes ~~(~~such as SOD, POD, ~~CAT)~~ and ~~alleviate~~CAT, thereby alleviating the ~~effects~~impacts of phosphorus stress ~~on *Oryza sativa L.* seedlings~~. Further studies by NI M et al. (2020) and ~~also showed~~Li et al. (2023) found that exogenous ~~application of~~ GR24 ~~could~~ significantly ~~alleviate the effects~~mitigates oxidative stress damage in cotton and *Malus pumila Mill.* seedlings under conditions of low-temperature ~~stress~~ and alkali stress ~~on cotton seedlings (NI M et al., 2020) and *Malus pumila Mill.* seedlings (Li et al., 2023) oxidative stress damage and increase the activities of SOD, POD and CAT.~~ , respectively, by increasing the activities of these antioxidant enzymes. This study also ~~found~~revealed that ~~with the aggravation of~~as phosphorus stress intensifies, the activities of SOD, POD, and CAT in the leaves of *Glycyrrhiza uralensis* Fisch ~~leaves gradually increased~~progressively increase, indicating ~~that *Glycyrrhiza uralensis* Fisch can alleviate~~an inherent mechanism by which the plant alleviates the adverse effects of phosphorus ~~stress by increasing the activity of antioxidant enzymes. Under low phosphorus treatment~~deficiency. Specifically, the application of GR24 at G2, G3, and G4 concentrations ~~can increase~~enhances SOD activity, with the G3 concentration proving most effective. Similarly, GR24 at the G3 concentration notably boosts the activities of both POD and CAT. Furthermore, under normal phosphorus conditions, the application of GR24 still enhances the activity of antioxidant enzymes in plants. At the G3 concentration, there is a notable increase in the activities of SOD ~~in plants. G3 treatment has the best effect~~and POD, while ~~the application of GR24 at G3 concentration can increase the~~ treatments at G2, G3, and G4 concentrations elevate CAT activity ~~of POD and CAT in plants. This indicates~~. These findings suggest that ~~the~~GR24 application ~~of GR24 can alleviate~~at the G3 concentration optimally mitigates the adverse effects of phosphorus stress~~, and it can be concluded that the application of GR24 at G3 concentration has the best effect. Under normal phosphorus supply treatment, the application of GR24 can also increase the~~ and enhances overall plant resilience by augmenting antioxidant enzyme ~~activity of plants. GR24 at G3 concentration can increase the activity of SOD and POD, while GR24 treatment at G2, G3, and G4 concentrations can increase the activity of CAT~~activities.

3.4 Effect of GR24 on the content of medicinal components of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

~~Both triterpenoids~~Triterpenoids and flavonoids ~~are~~constitute the ~~main~~primary active components of *Glycyrrhiza uralensis* Fisch ~~medicinal constituents. Glycyrrhizic acid~~, with glycyrrhizic acid and glycyrrhetic acid ~~are~~being structurally similar triterpenoids, and liquirtigenin, liquirtin, ~~lsoliquirtigenin, lsoliquirtin~~isoliquirtigenin, isoliquirtin, and glabridin ~~are~~categorized as flavonoids (Liu et al., 2013; Sheng et al., 2022). Phosphorus plays a pivotal role as it is ~~an important element that constitutes~~involved in the initial substrates of the terpenoid synthesis pathway, acetyl ~~-~~CoA, and glyceraldehyde-3-phosphate (Zeng et al., 2013~~) so~~). Consequently, phosphorus stress ~~will affect~~impacts the production and accumulation of active ingredients in medicinal plants. ~~The~~Our experimental results ~~of this experiment revealed~~indicate that the contents of ~~lsoliquirtigenin~~isoliquirtigenin, liquirtigenin, glycyrrhizic acid, ~~lsoliquirtin~~isoliquirtin, and liquirtin were ~~higher~~elevated under low phosphorus ~~treatment than~~conditions compared to those in the ~~group with~~ no phosphorus treatment~~, and~~ group. Moreover, the ~~contents~~content of ~~lsoliquirtin were~~isoliquirtin was higher ~~than those in the group with no phosphorus stress~~ in both the normal ~~supply of phosphorus~~ and low phosphorus stress~~. Studies have found~~ conditions than in the no phosphorus stress group. This aligns with findings by Hu et al. (2018), who reported that the synthesis of dihydroflavone and flavonols such as naringenin, rutin, and taxifolin~~, etc. can~~ could be ~~significantly~~notably inhibited under phosphorus ~~deficiency~~-deficient conditions ~~(Hu et al., 2018), which is consistent with our results. The~~ . Notably, the content of ~~lsoliquirtin decreases with the aggravation of~~isoliquirtin decreased as phosphorus stress~~. However, contrary to~~ intensified, diverging from the ~~research~~ results of Winkel-Shirley (2002), ~~it shows that the~~ which suggests multifaceted influences on flavonoid production ~~of flavonoids by~~ in plants ~~is affected in many ways. In this~~ . Additionally, our study~~, it was also~~ found that the application of GR24 ~~could increase the content of~~ under various phosphorus conditions could enhance the content of several terpenoids and flavonoids. Specifically, under no-phosphorus stress, GR24 increased the levels of glycyrrhetic acid, glycyrrhizic acid, glabridin, ~~lsoliquirtin~~isoliquirtin, and liquirtin~~ under no~~. Under normal phosphorus ~~stress,lsoliquirtigenin~~supply, the increases were noted in isoliquirtigenin, liquirtigenin, and glycyrrhizic acid, while under ~~normal~~ low-phosphorus ~~supply, and lsoliquirtigenin~~stress, GR24 elevated the contents of isoliquirtigenin, liquirtigenin, glycyrrhizic acid, ~~lsoliquirtin~~isoliquirtin, and liquirtin ~~under low-phosphorus stress treatments, which It indicated~~. This indicates that GR24 application ~~could~~can variably promote the accumulation of terpenoid and flavonoid contents ~~of~~in *Glycyrrhiza uralensis* Fisch ~~to different degrees~~ at different phosphorus concentrations. ~~Previous~~Such effects are consistent with previous research ~~supports the idea~~indicating that GR24 ~~can promote~~enhances the accumulation of anthocyanins in *Arabidopsis thaliana (L.) Heynh* (Cao et al., 2023) ~~which is similar to the results of this study. It has also been shown that the induction of *Tripterygium wilfordii Hook. f.* suspension cells with GR24 inhibits~~and suggests that GR24 may also modulate the production of terpenoids, as evidenced by its inhibition of diterpene secondary metabolites such as regolith methylesterase and strigolactone ~~to varying degrees~~ in *Tripterygium wilfordii Hook. f.* suspension cells (Wu et al., 2019~~), suggesting that GR24 application may also inhibit terpenoid production.~~).

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3.5 Effect of GR24 on the transcriptome of *Glycyrrhiza uralensis* Fisch at different phosphorus concentrations

Plants exhibit a ~~complex~~sophisticated response to adversity ~~involving~~that encompasses physiological, biochemical, and metabolic ~~process that involves~~processes. This response is mediated through the synergistic ~~action~~actions of multiple genes and a complex mechanism of co-regulation (Li et al., 2024; ~~PANT~~Pant BD et al., 2015; Sun et al., 2016). ~~The results of~~In this study, we identified ~~that 1298~~1,298, 163, and 513 DEGs ~~were found~~ under conditions of no-phosphorus stress, low-phosphorus stress, and normal phosphorus supply

~~treatments~~, respectively. Notably, the lowest number of DEGs was ~~found in response to~~ observed under low-phosphorus stress ~~compared~~, aligning with ~~the other two treatments, which was consistent with the study of~~ findings by Guan (2016). ~~The~~ GO enrichment ~~results indicated~~analysis revealed that the DEGs ~~under~~across the three treatments were ~~mainly enriched in the functions of~~predominantly associated with cellular processes, metabolic processes, cellular anatomical entities, and catalytic activity~~, and among the DEGs..~~ KEGG pathway ~~results revealed~~analysis showed that the no-phosphorus and low-phosphorus stress ~~treatment and low phosphorus stress treatment~~treatments were significantly enriched in ~~pathways such as~~ plant-pathogen interaction and signaling pathways, ~~and~~respectively. Conversely, the normal phosphorus supply treatment was significantly enriched in pathways such as phenylpropanoid biosynthesis~~,~~ and DNA replication~~, etc., whereas the~~ . The phenylpropanoid biosynthesis pathway ~~is not only an important pathway~~, crucial for ~~the synthesis of~~synthesizing secondary metabolites, ~~but~~ also plays ~~an important~~a vital role in plant growth ~~and~~, development, and environmental adaptation (Zhong et al., 2009~~),, which suggests that GR24 application can regulate the growth of~~), thereby underscoring the regulatory effects of GR24 on *Glycyrrhiza uralensis* Fisch. ~~In addition, the~~Moreover, all three treatments ~~were also enriched~~showed enrichment in ~~the~~pathways related to secondary metabolite production ~~pathway, suggesting that GR24 application had~~, indicating a positive effect of GR24 on the synthesis of medicinal components in Ural *Glycyrrhiza uralensis* Fisch.

~~It has been shown~~Our findings demonstrate that transcription ~~factor genes~~factors such as bHLH, AP2, MYB, WRKY, and NAC are responsive to phosphorus deprivation~~, among which the~~. Specifically, bHLH (Wang et al., 2023) and AP2 (Zhao et al., 2018) transcription factors ~~are~~ not only ~~involved~~participate in plant growth and development but also respond to secondary metabolic processes and abiotic stresses ~~in plants~~ (Zhang, 2023)~~. In this study, we identified that bHLH and ERF transcription factors are not only involved in plant growth and development but also respond to plant secondary metabolic processes and abiotic stresses. In this study, we found that under~~ Under no phosphorus stress, the ~~highest number of~~ most differentially expressed transcription factors ~~identified was~~ were from the C2H2 family, followed by the NAC family, ~~and~~ all of ~~them~~which were down-regulated ~~suggesting~~. This suggests that GR24 application ~~could improve~~may enhance tolerance to phosphorus deficiency ~~tolerance in Uralia glycyrrhiza. Additionally,~~in *Glycyrrhiza uralensis* Fisch. Under low phosphorus stress, the ~~highest number of~~WRKY family was the most differentially expressed ~~transcription factors identified was the WRKY family~~ and ~~was~~predominantly up-regulated ~~(~~, consistent with observations by Huang et al.~~,~~ . (2012~~). Showed)~~ that the WRKY family ~~was expressed in different~~ exhibits varied expression patterns in response to ~~various abiotic stresses, and most of them were up-regulated in Uralia glycyrrhiza (Guan et al., 2023). in Ural *Glycyrrhiza uralensis* Fisch, which is consistent with the present study.~~ different abiotic stresses. Under normal phosphorus supply ~~treatment,~~ the AP2/ERF-ERF family was the most differentially expressed transcription factor ~~family~~group and was down-regulated. ~~The AP2 family of transcription factors is often associated with the growth hormone signaling pathway in plants (Feng et al., This~~2020; Gu et al., 2017; Ritonga et al., 2021), and the down-regulation ~~of this family of transcription factors may because the application~~might be attributed to the effect of GR24 ~~affected the~~on growth hormone regulation ~~of~~in *Glycyrrhiza uralensis* Fisch, impacting associated signaling pathways (Feng et al., 2020; Gu et al., 2017; Ritonga et al., ~~resulting in down-regulation of the signaling pathway being affected.~~2021).

CONCLUSIONS

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~~In general, spraying~~ Overall, the application of GR24 ~~improved~~ameliorates the ~~negative~~adverse effects of phosphorus stress in *Glycyrrhiza uralensis* Fisch ~~under phosphorus stress, increased the~~ by increasing biomass ~~of *Glycyrrhiza uralensis* Fisch, increased the accumulation of~~ , ~~enhanced the~~ photosynthetic ~~capacity, that is, the content of photosynthetic pigments (chlorophyll a and b) increased,~~pigment levels, and while reducing the content of antioxidant enzymes ~~decreased,~~ and ~~increased~~enhancing the accumulation of ~~some~~certain triterpenoids and flavonoids ~~in *Glycyrrhiza uralensis* Fisch, so that~~ . Differential expression genes (DEGs) were ~~mainly~~primarily enriched in pathways ~~conducive~~favorable to the growth ~~and~~, development ~~of *Glycyrrhiza uralensis* Fisch and the accumulation of~~ , and secondary ~~metabolites, and also upregulated~~metabolite accumulation in *Glycyrrhiza uralensis* Fisch, with the upregulation of the WRKY family related to phosphorus stress~~, with~~ response. The G3 concentration ~~being the best~~was found to be most effective, indicating that ~~the~~GR24 application ~~of GR24 improved~~improves the phosphorus deficiency tolerance of *Glycyrrhiza uralensis* Fisch. ~~In addition, the application of GR24~~ Moreover, under normal phosphorus ~~supply treatment can promote the~~conditions, GR24 promotes plant growth and development ~~of *Glycyrrhiza uralensis* Fisch, increase its~~, increases biomass~~,~~ and ~~accumulation.~~, elevates chlorophyll content, ~~reduce the content of~~decreases antioxidant ~~enzymes~~enzyme levels, and ~~improve~~enhances the accumulation of ~~some~~ medicinal ~~ingredients~~components. DEGs in this context are mainly enriched in ~~the~~ pathways related to plant growth regulation, and ~~upregulate~~elevate the AP2 family of transcription factors ~~related to~~ associated with the plant growth hormone signaling pathway, ~~indicating~~demonstrating that ~~gr24 can~~GR24 not only ~~improve the~~improves growth ~~of *Glycyrrhiza uralensis* Fisch~~ under low phosphorus~~,~~ conditions but also ~~have a positive impact on~~positively impacts the growth and development of *Glycyrrhiza uralensis*, Fisch under normal phosphorus nutrition.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

Yuting Jing conceived and designed the experiments, performed the experiments, analyzed the data, performed the computation work, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Man Li authored or reviewed drafts of the paper, contributed to the analysis of the mathematical properties, and approved the final draft.

Yong Wu , Chengming Zhang and Chengshu Qiu contributed to the experimental design and participated in the revision of the manuscript.

Hengming Zhao assisted with some of the experiments and recorded data.

Li Zhuang and Hongling Liu was involved in conceptualizing the experimental design and suggested revisions to the experiment and the completion of the article and approved the final draft.

Supplemental Information

Transcriptom data information for this article can be found online at PRJNA1112769. The remaining raw data can be found in the attachment.

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