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Enhancing Glycyrrhiza (Licorice) stress tolerance and productivity: Insights from environmental factors, microbial interactions, and phytohormone regulation

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Abstract
Glycyrrhiza (Licorice) holds cultural and medicinal significance in China and is widely used in traditional Chinese medicine. The paper highlights the impact of drought stress on plant growth and metabolism, including reduced chlorophyll content and hindered photosynthesis. It emphasizes the importance of suitable environmental conditions such as temperature, precipitation, and altitude for optimal growth and quality of licorice. The study also focuses on strategies to improve the adaptability and productivity of Glycyrrhiza plants. It discusses the role of beneficial bacteria and dark septate endophytes in enhancing drought tolerance and promoting plant development. Additionally, the positive effects of mycorrhizal fungi on *Glycyrrhiza glabra* L. plants under heavy metal stress are examined, highlighting increased root weight and antioxidant activity. Furthermore, the application of methyl jasmonate (MeJA) is explored to alleviate salt stress by enhancing antioxidant activities and regulating metabolic enzymes. The paper stresses the significance of considering these stress factors and implementing appropriate strategies to improve the growth and resilience of Glycyrrhiza plants.
The review concludes by emphasizing the importance of understanding the mechanisms underlying stress tolerance and optimizing licorice cultivation practices. It highlights the need to consider factors such as temperature, precipitation, altitude, and soil characteristics for successful cultivation. These findings contribute to the knowledge of stress tolerance mechanisms in Glycyrrhiza plants and provide insights for improving their productivity and quality in challenging environments.

Keywords: Glycyrrhiza, drought stress, adaptability, microbial infection, stress tolerance mechanism

Introduction
Licorice, known as Gan-Cao in Chinese, is highly valued in China for its cultural significance and medicinal properties. It has a rich history in traditional Chinese medicine (TCM) and is widely utilized as a medicinal plant. First documented in Shen Nong's Herbal Classic during the Han dynasty, licorice has been traditionally used in TCM prescriptions to synergize the effects of other herbal medicines. Around the world, liquorice encompasses nearly thirty different species, with eight of them found in China. (Cheng *et al*.,) However, the Chinese Pharmacopoeia recognizes only three species - *Glycyrrhiza uralensis* Fisch., Glycyrrhiza inflata Bat., and *Glycyrrhiza glabra* L. - as the official sources of licorice (Cheng *et al*., 2021) [4]. According to, licorice is believed to enhance the vital energy known as "qi," alleviate pain, and serve as an effective antidote (Liu *et al*.,2015) [18]. It is a prominent ingredient in herbal formulations, and there is a saying that "nine out of ten formulas contain licorice," emphasizing its importance in reducing toxicity and enhancing the efficacy of other herbal medicines when combined (Jiang *et al*., 2020) [10]. In Japan, licorice finds diverse applications due to its chemical constituents. Glycyrrhizin, found in licorice, is utilized in approximately 70% of food products in Japan. Glabridin, another compound present in licorice, is employed in around 26% of medicinal cosmetics. Licorice extracts also contribute to approximately 4% of tobacco products and are utilized in other chemical products (Wang *et al*.,2020) [15].

Factors affecting growth of glycyrrhiza
Drought stress has a significant detrimental effect on plant growth and yield in arid and semiarid regions. It strongly influences metabolic pathways, particularly carbon (C) and nitrogen (N) metabolism, which are highly sensitive to drought conditions (Kessler, 2015). Drought stress results in reduced chlorophyll content, hindered photosynthesis, modified gene expression, and altered enzyme activities.

One of the main processes affected is photosynthesis, a crucial aspect of C metabolism, leading to decreased production of sucrose and other essential metabolites. Overall, drought stress disrupts plant metabolism, impairs photosynthesis, and negatively impacts the production of primary metabolites, thereby affecting plant productivity (Zhang *et al*., (2012) [20].

Suitable environmental conditions greatly affect the yield and quality of medicinal materials, with temperature and precipitation being crucial factors. For instance, when cultivating licorice in the Hexi Corridor and Inner Mongolia, higher temperatures negatively correlate with the content of glycyrrhizic acid, glycyrrhizin, and isoglycyrrhizin. Conversely, altitude and average annual precipitation positively correlate with these beneficial components. In summary, temperature, precipitation, and altitude play significant roles in determining the growth and quality of medicinal plants like licorice (Cui *et al*., 2023) [6].

Glycyrrhiza uralensis plants have significant roles in arid and semiarid regions, serving as windbreaks, aiding in sand fixation, and contributing to soil formation (Egamberdieva *et al*., 2016) [7]. However, the cultivated G. *uralensis* varieties currently dominating the market supply exhibit lower quality and stress tolerance compared to their wild counterparts. Consequently, there is a pressing economic and social importance in identifying effective strategies to improve the quality and adaptability of cultivated G. *uralensis* in regions characterized by low precipitation or water scarcity (Xie *et al*., 2019) [17].

Zhang *et al*., (2021) [22] in his study stated that drought stress negatively impacts the growth and yield of *Glycyrrhiza uralensis* plants. Bacillus pumilus G5, a beneficial bacterium, has the potential to enhance drought tolerance in these plants. The study also revealed that drought stress inhibits seedling growth and biomass by affecting C- and N-metabolism. However, inoculating the seedlings with Bacillus pumilus G5 effectively reverses these effects. The bacterium regulates metabolic processes, resulting in increased levels of primary metabolites (soluble sugars, proteins, and amino acids) and glycyrrhizic acid, a key secondary metabolite. This improvement is achieved by restoring the expression of enzymes involved in glycyrrhizic acid biosynthesis. Ultimately, Bacillus pumilus G5 enhances drought tolerance in cultivated *Glycyrrhiza uralensis* plants by modulating metabolic pathways and promoting the accumulation of secondary metabolites.

Studies have indicated that the development and quality of Glycyrrhiza plants are influenced by a range of environmental factors, such as the specific climate and soil characteristics of their local surroundings (Alsaadi *et al*.,2020) [1]

Tabrizi *et al*., (2021) [14] in a study found that mycorrhizal fungi had positive effects on the growth and quality of *Glycyrrhiza glabra* L. plants under heavy metal stress. Mycorrhizal plants showed increased root dry weight and higher levels of total antioxidant activity and total phenol content. They also had lower concentrations of heavy metals in shoots but higher concentrations in roots, indicating their ability to sequester metals in the root system. The Ramjerd ecotype performed better than the Baft ecotype and mycorrhizal association improved the growth and yield of both ecotypes. Considering heavy metal accumulation in licorice's belowground organs is crucial for its medicinal use.

Liquorice residues, which are cellulose-rich by-products from the production of Chinese medicine and related products, can be effectively used as organic amendments and soil conditioners. Through microbial fermentation, these residues can be transformed into beneficial organic fertilizers for plants. Utilizing organic fertilizers not only enhances plant productivity by providing slow-release nutrients but also improves overall soil properties. This approach offers a sustainable and environmentally friendly method to increase agricultural yields while avoiding pollution (HE, 2017, Zhang *et al*., 2019, Raza *et al*., 2016) [8, 19, 13].

He *et al*., (2020) study examined how Dark Septate Endophytes (DSE) impact the growth of liquorice plants when exposed to sterilized fermented and unfermented organic residues. The findings revealed that DSE, particularly Acrocalymma vagum, had a positive influence on various aspects of plant development, such as biomass, root growth, and active ingredient content. A. vagum specifically promoted the growth of lateral roots, while *Paraboeremia putaminum* enhanced taproot development. The most favourable outcomes were observed when DSE and fermented residues were combined. These results enhance our understanding of the ecological role of DSE fungi in nutrient-depleted environments and offer valuable insights for optimizing liquorice cultivation practices.

Chunhua *et al*., (2010) [5] study explores the *in vitro* regeneration of *Glycyrrhiza glabra*, specifically targeting the production of glabridin. With the wild G. glabra population declining, somatic embryogenesis becomes crucial for clonal propagation and genetic transformation. The researchers investigated factors influencing callus and embryo induction, maintenance, and multiplication. Hypocotyl explants exhibited the highest frequency of callus formation, and specific medium compositions were identified for optimal embryo induction. Histological analysis confirmed the embryogenesis originating from a single cell. Successful acquisition of green embryo-like cultures and subsequent shoot regeneration was achieved. The study provides valuable insights and a step-by-step protocol for *in vitro* regeneration of G. glabra, opening possibilities for glabridin conservation and sustainable production.

Salt stress is a highly detrimental factor that significantly hampers plant growth, development, and yield (Chunhua *et al*., (2010) [5]. It arises due to various factors such as excessive fertilizer use, irrigation with low-quality water, poor soil drainage, and high soil evaporation. Salinity stress primarily manifests through three major effects: ion toxicity, osmotic stress, and oxidative stress. These effects can have severe consequences on plant health and productivity (Hnilickova *et al*., 2017) (Behdad *et al*., (2021) [2].

(Behdad *et al*., 2021) [2] study investigated the effects of varying levels of NaCl on different parameters in licorice populations. The findings demonstrated that salinity negatively influenced growth, leaf water content, and membrane stability, while thicker rhizomes exhibited better resilience in saline soil. Chlorophyll index, gas exchange, and soluble carbohydrate content were also reduced under salinity stress. Oxidative stress was induced in licorice rhizomes by salinity, resulting in an increase in antioxidant capacity, phenolic compounds, and flavonoid content. Sodium (Na^+), chloride (Cl^-), and calcium (Ca^{2+}) levels were elevated, whereas potassium (K^+) concentration decreased. Different population responses to salinity were observed between the Fars and Khorasan populations. Licorice demonstrated its ability to tolerate salinity up to 600 mM NaCl, making it a potential halophyte plant. The study highlights the importance of osmolytes, nutrient balance, and antioxidant capacity in protecting licorice under osmotic, ionic, and oxidative stress induced by salinity.

Behdad *et al*., (2020) [3] research investigated the impact of various NaCl concentrations on licorice rhizomes, focusing on growth, osmolyte content, oxidative stress markers, antioxidant enzymes, K+/Na+ ratio, glycyrrhizin content, and gene expression associated with glycyrrhizin production. The findings revealed that salt stress hindered growth and increased proline levels. The K+/Na+ ratio declined, indicating ion imbalance. Salt stress induced oxidative stress, but glycyrrhizin content rose at lower NaCl levels. Gene expression patterns varied among populations, with the Fars population exhibiting higher glycyrrhizin content. The study indicates that licorice has the potential to adapt as a halophyte plant.

Lang *et al*., (2020) [12] study revealed that salt stress in G. *uralensis* seedlings can lead to an increase in reactive oxygen species (ROS), resulting in membrane lipid peroxidation and disruption of C and N metabolisms, leading to inhibited plant growth. However, the application of MeJA (methyl jasmonate) shows promise in alleviating the negative effects of salt stress on G. *uralensis*. MeJA enhances the activities of antioxidant enzymes and non-enzymatic antioxidants, while also regulating the activities of C and N metabolizing enzymes in salt-stressed G. *uralensis* seedlings. Interestingly, the effectiveness of MeJA varies depending on its concentration, with concentrations of 15 to 30 µM showing positive effects on most antioxidant enzymes and non-enzymatic antioxidants, while a concentration of 60 µM enhances C and N metabolizing enzymes. Further research is needed to gain a deeper understanding of the mechanisms by which different concentrations of MeJA impact G. *uralensis* plants under salt stress conditions.

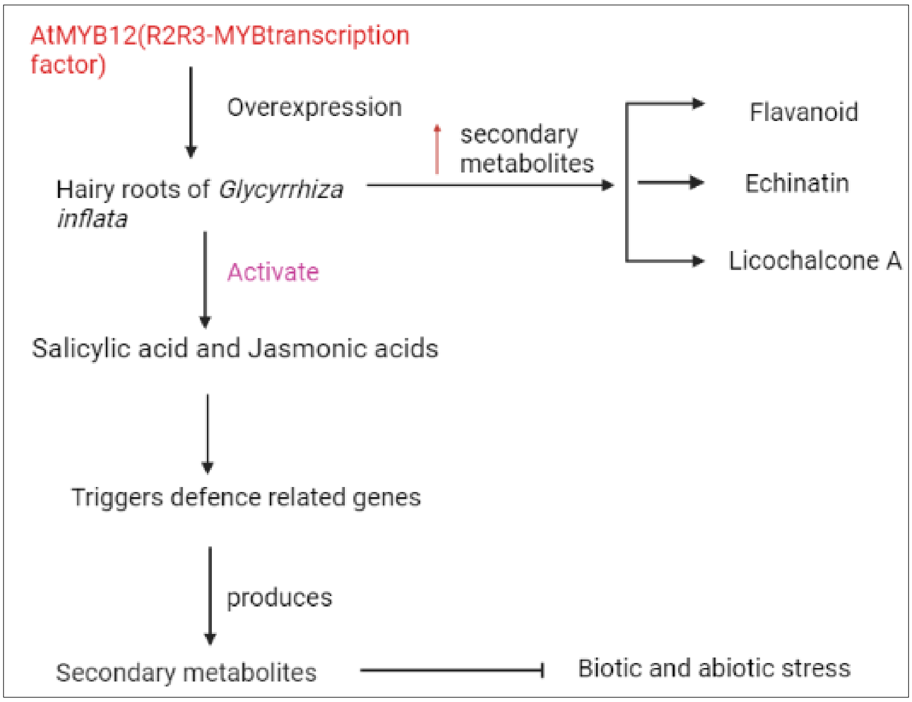

Fig 1: Overexpressing the R2R3-MYB transcription factor, AtMYB12, in the hairy roots of *Glycyrrhiza inflata* leads to the activation of defense-related genes through the induction of salicylic acid and jasmonic acid signaling pathways. This activation subsequently results in the production of secondary metabolites, including flavonoids, echinatin, and licochalcone A (Wu *et al*., (2022) [16].

Future perspective

Identification of stress-tolerant genotypes: Further research should focus on identifying and characterizing Glycyrrhiza genotypes that exhibit enhanced tolerance to drought and salt stress, using advanced molecular techniques such as genomic sequencing and marker-assisted selection.

Elucidation of stress tolerance mechanisms: Understanding the molecular and physiological mechanisms that enable certain Glycyrrhiza genotypes to tolerate drought and salt stress is crucial. This knowledge can be used for genetic engineering or genome editing to enhance stress tolerance in Glycyrrhiza plants.

Beneficial microorganisms: The use of beneficial bacteria and mycorrhizal fungi has shown promise in improving the growth and stress tolerance of Glycyrrhiza plants. Further research should explore the diversity of microbial communities associated with Glycyrrhiza and develop microbial-based biofertilizers or biocontrol agents.

Integration of multi-stress tolerance: Investigate the interactive effects of drought stress, salt stress, and other abiotic stresses on Glycyrrhiza plants to develop strategies for enhancing multi-stress tolerance.

Climate change adaptation: Assess the impact of changing climatic conditions on Glycyrrhiza production, including altered temperature, precipitation patterns, and atmospheric CO_2 levels. Develop climate-smart agricultural practices and predictive models to guide cultivation strategies.

By focusing on these important points, future research can contribute to the development of stress tolerant Glycyrrhiza genotypes, elucidate stress tolerance mechanisms, harness the potential of beneficial microorganisms, integrate multi-stress tolerance, and adapt to climate change. These efforts will ensure sustainable and resilient cultivation practices for Glycyrrhiza plants in challenging environments.

Conclusion

In conclusion, drought stress and salt stress have significant detrimental effects on the growth and development of Glycyrrhiza plants. These stresses disrupt metabolic pathways, particularly carbon and nitrogen metabolism, leading to reduced chlorophyll content, hindered photosynthesis, and altered gene expression and enzyme activities. However, several approaches have been explored to mitigate the negative impacts of these stresses. Beneficial bacteria such as Bacillus pumilus G5 and Dark Septate Endophytes (DSE) have shown potential in enhancing drought tolerance and improving various aspects of plant growth and active ingredient content. Furthermore, mycorrhizal fungi have been found to positively affect the growth and quality of *Glycyrrhiza glabra* L. plants under heavy metal stress. Additionally, the application of methyl jasmonate (MeJA) has demonstrated positive effects in alleviating salt stress by enhancing antioxidant activities and regulating metabolic enzymes. Temperature, precipitation, altitude, and soil characteristics also play important roles in determining the growth and quality of medicinal plants like licorice. Overall, these findings contribute to our understanding of the mechanisms underlying stress tolerance and offer insights into strategies for improving the adaptability and productivity of Glycyrrhiza plants in challenging environments.

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