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Review Article Open Access

Comparison of Yield Stability among Different Legume Species in Zhejiang

Yu Zhan
Zhejiang Green Giant Biotechnology Co., Ltd., Jinhua, 321071, Zhejiang, China
Corresponding author: 3601303@qq.com

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Abstract Legume crops play an important role in ensuring food security, improving soil fertility, and promoting sustainable agricultural development. However, yield instability caused by climatic fluctuations, environmental stresses, and management variations remains a major challenge for legume production in Zhejiang Province. This study aims to systematically compare the yield stability of different legume crops in Zhejiang by integrating long-term yield data, ecological information, and statistical evaluation methods. The research will analyze yield variation characteristics, stability differences, and environmental adaptability among major legume crops, including soybean, broad bean, pea, mung bean, and common bean. Multiple stability assessment approaches, such as coefficient of variation, AMMI model, GGE biplot analysis, and multivariate statistical methods, will be applied to identify crops with high yield stability and broad ecological adaptability. Furthermore, the physiological, ecological, and genetic mechanisms underlying yield stability will be explored, with emphasis on climate adaptation, resource utilization efficiency, and genotype-environment interactions. A case study of typical ecological regions in Zhejiang will be conducted to reveal regional differences in legume production stability and provide targeted optimization strategies. The findings will contribute to the selection of stable-yielding legume varieties, improvement of precision cultivation practices, and development of climate-resilient legume production systems in Zhejiang Province.

Keywords Legume crops; Yield stability; Genotype-environment interaction; Ecological adaptability; Zhejiang Province

1 Introduction

Legume crops are strategically important to agricultural production, human nutrition, and the ecological transformation of cropping systems in Zhejiang. Legumes provide protein-rich grain and diverse food uses, and many species also contribute to fodder, green manure, and functional food development (Jarecki and Migut, 2022). Their wider value lies not only in seed production but also in their role in sustainable intensification, because legumes fix atmospheric nitrogen through symbiosis, improve soil fertility, reduce synthetic fertilizer demand, and enhance rotational performance of subsequent crops. Meta-analytic and review evidence further shows that legumes support self-sufficiency in plant protein, reduce greenhouse-gas emissions relative to non-legume crops, improve biodiversity and soil health, and fit well with conservation-oriented farming systems. In China, food legumes have long been embedded in rotation, intercropping, and mixed-cropping systems, and the country remains a leading producer of pea, faba bean, mung bean, and adzuki bean. At the same time, China's vast territory and complex ecological conditions create strong spatial heterogeneity in legume adaptation, while changing market conditions have reduced the profitability and planting area of some dry grain legumes, increasing the need to identify species and cultivars with better productivity and stability under local conditions. Zhejiang is characterized by humid subtropical monsoon conditions, diverse micro-environments, and intensive multiple-cropping systems, so production risk is shaped not only by average yield level but by the consistency of yield across years, sites, and management situations. For this reason, evaluating yield stability among legume species is more useful for regional crop and variety recommendation than comparing yield in a single environment alone. This focus is also supported by broader agronomic evidence showing that stable performance across environments is a central target in crop improvement, because yield is a complex quantitative trait jointly controlled by genotype, environment, and their interaction (Islam et al., 2021). A genotype or species is considered practically valuable when it combines relatively high mean yield with low fluctuation across environments, thereby reducing production risk and supporting reliable legume expansion in diversified cropping systems (Baraki et al., 2020).

Research in China and abroad has shown that genotype × environment interaction (GEI) is the key scientific issue underlying yield instability and the differential ranking of materials across test sites. GEI reflects the fact that genotypes behave differently under different environmental conditions, and understanding this interaction is essential for predicting adaptation, identifying ideal testing environments, and selecting either broadly adapted or specifically adapted materials. In legumes, this phenomenon has been repeatedly confirmed. In Ethiopian cowpea, environments, genotypes, and GEI all had significant effects on grain yield, and GEI accounted for a larger share of variation than genotype alone, indicating strong crossover responses among test locations. In Ugandan cowpea, environment contributed the largest share of grain-yield variation, and GEI was associated with weather variables such as temperature, rainfall, and humidity as well as yield components (Mbeyagala et al., 2021). In mung bean, significant effects of genotype, environment, and GEI have also been reported, both in drought-tolerance evaluation in Bangladesh and in multi-year testing in northern Ethiopia, confirming that stable high-yielding selection requires trials over years and locations. Comparable conclusions have been reported in faba bean, lentil, Bambara groundnut, and field pea, where multi-environment experiments were necessary to identify stable, high-yielding genotypes and to distinguish widely adapted materials from those suited to specific mega-environments (Ghaffar et al., 2023). Methodologically, AMMI and GGE biplot have become the most widely used tools for this purpose because ANOVA alone can detect significance but cannot adequately resolve the non-additive structure of GEI. AMMI emphasizes additive main effects and multiplicative interaction structure, whereas GGE integrates genotype main effects with GEI and provides intuitive graphical judgment of "which-won-where," mean-versus-stability, and environment representativeness (Mullualem et al., 2024). These methods have been applied successfully not only in legumes such as cowpea, mung bean, lentil, faba bean, Bambara groundnut, and pea, but also in other crops, where they effectively identify ideal genotypes, discriminating test sites, and mega-environment structure. At the same time, recent work suggests that stability evaluation can be strengthened by combining AMMI and GGE with complementary indices such as ASV, GSI, WAASB, WAASBY, or MTSI, especially when high yield must be balanced with multiple traits or adaptation goals. Another important insight is that the long-standing belief that legumes are inherently unreliable is overstated: long-term experiments across northern Europe showed that grain legume yields were as reliable as those of other spring-sown crops when stability was assessed appropriately, although agronomic improvement is still needed to raise both yield level and stability.

Against this background, the objective of this study is to compare the yield stability of different legume species under Zhejiang conditions and to provide an evidence base for regional species selection, variety deployment, and optimized cropping-system arrangement. More specifically, the study aims to determine whether different legume species differ significantly in mean yield and stability, to quantify the relative contributions of species effects, environment effects, and their interaction to yield variation, and to identify species or materials with either broad adaptability or specific adaptation to particular production environments. This objective follows the common logic of multi-environment crop evaluation, in which significant GEI implies the need to evaluate genotypes across diverse sites before recommendation. The technical framework therefore includes multi-environment field trials across representative ecological zones or seasons in Zhejiang, standardized measurement of grain yield and key agronomic traits, and joint statistical analysis combining analysis of variance with AMMI and GGE biplot methods. In this framework, ANOVA is used first to test the significance of species, environment, and interaction effects, while AMMI is used to partition interaction structure and assess general adaptability, and GGE biplot is used to visualize winning species, ideal genotypes, representative test sites, and possible mega-environment differentiation. Where necessary, stability indices can be introduced to integrate mean yield and stability ranking, improving the robustness of recommendation decisions. Because species differ in both production potential and functional traits, the interpretation of results should also consider their broader agronomic roles: soybean often shows high seed and protein yield, faba bean can be productive but more variable across years, white lupin may outperform other lupins in yield, and cowpea and mung bean can maintain useful forage production even under drought-related stress. Ultimately, by integrating productivity, stability, and ecological value, this study is expected to support the selection of legume species that are better suited to Zhejiang's diverse environments and cropping systems, thereby improving farmer resilience, land-use efficiency, and the sustainable development of legume-based agriculture.

2 Production Status and Ecological Characteristics of Legume Crops in Zhejiang Province

2.1 Planting structure and regional distribution of legume crops in Zhejiang Province

Legume crops occupy an important but structurally differentiated position within Chinese cropping systems, and Zhejiang should be understood within this broader pattern. In China, pulses are commonly embedded in rotation, intercropping, and mixed-cropping systems, which gives legumes value not only as food crops but also as flexible components of diversified farmland use. The major food legume groups grown nationally include pea, faba bean, common bean, mung bean, adzuki bean, and cowpea, together with a wider set of locally distributed minor legumes, indicating that species choice is closely tied to local ecological and market conditions. For Zhejiang, this implies a planting structure in which soybean, mung bean, adzuki bean, cowpea, and vegetable-type legumes are more likely to coexist than to form a single dominant dry-grain system, especially under intensive land use and multiple-cropping conditions. At the national scale, soybean planting is also organized by agroecological zones, and the middle-lower Yangtze River double-cropping ecoregion is recognized as one of the major soybean production zones in China, which places Zhejiang within a wider south-eastern soybean adaptation belt (Mei et al., 2024).

From the perspective of spatial distribution, legume planting in Zhejiang is likely to show obvious regional heterogeneity because crop layout in China generally responds to both natural suitability and comparative economic return. Studies of crop planting structure in other Chinese regions show that spatial allocation is shaped by climate, geography, and population-related factors, and that optimizing crop distribution requires attention to county- or regional-scale differences rather than uniform provincial planning. Remote-sensing evidence from major grain regions further shows that higher-benefit crops tend to concentrate in locations with better accessibility and stronger production conditions, while less competitive crops contract or shift spatially when relative profitability declines (Liu and Wang, 2022). Applied to Zhejiang, this suggests that vegetable legumes and fresh-use beans are more likely to cluster in peri-urban plains and developed horticultural belts, whereas dry-grain legumes are more likely to persist in hilly areas, upland fields, or as rotation crops within smaller and more fragmented farming systems. In recent decades, reduced profits have caused decreases in the sowing area of some dry food legumes in China, while vegetable food legumes have expanded because of stronger market demand and shorter growth duration (Figure 1).

2.2 Agro-ecological characteristics

Zhejiang belongs to China's subtropical agricultural zone, and its agro-ecological background is generally characterized by abundant heat and rainfall occurring in the same season, a combination that strongly supports intensive cropping but also increases ecological sensitivity. Research on China's subtropical region shows that this broad zone has synchronous hydrothermal conditions and serves as one of the country's traditional agricultural cores (Chen et al., 2025). Comparable studies from humid subtropical production areas show annual precipitation concentrated in the April-October growing season and high overlap between peak temperature and rainfall. For legume crops in Zhejiang, such conditions are generally favorable for rapid biomass accumulation and multiple cropping, but they also mean that excess moisture, cloudy-rainy periods, and strong seasonal fluctuations can affect sowing dates, flowering, pod setting, and harvesting stability.

At the same time, Zhejiang's ecological suitability for crops is shaped not only by climate but also by topography and soils. A land ecological suitability analysis conducted specifically in Zhejiang selected annual mean temperature, accumulated temperature above 10 °C, extreme low temperature frequency, humidity, slope, aspect, altitude, soil type, and soil texture as key determinants of crop distribution, showing that provincial crop suitability depends on the joint action of climate, terrain, and edaphic factors. The same study demonstrated that GIS-based suitability zoning can effectively reproduce the current distribution of crops in Zhejiang, indicating strong spatial differentiation in ecological adaptation across the province. This is especially relevant for legumes because the province includes plains, river valleys, coastal zones, and hilly or mountainous land, so temperature resources, drainage conditions, and soil physical properties are unlikely to be uniform. In such a setting, differences in ecological niche among legume species are expected to translate directly into differences in regional yield level and yield stability.

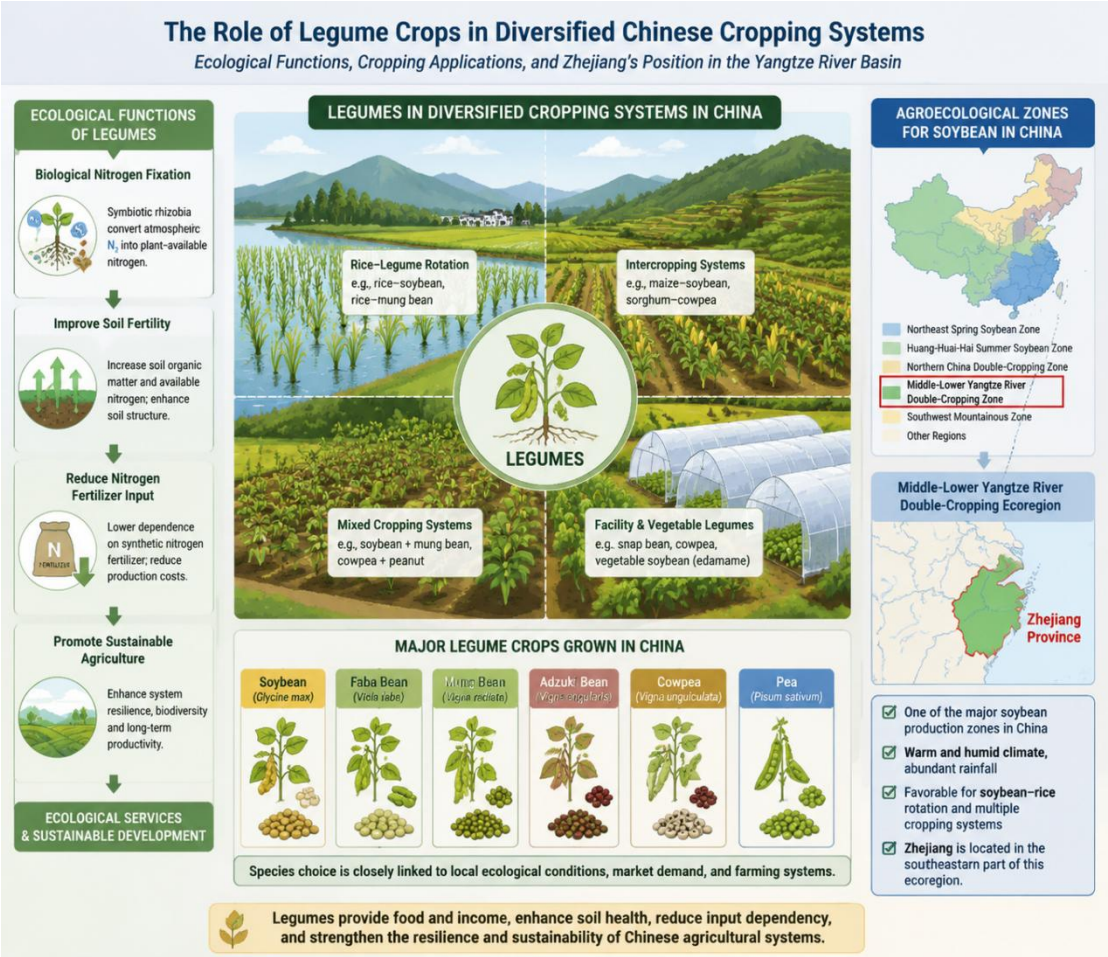

Figure 1 Conceptual framework illustrating the ecological functions of legume crops within Chinese diversified cropping systems and the position of Zhejiang Province within the middle-lower Yangtze River soybean adaptation zone

2.3 Limiting factors

The limiting factors affecting legume production in Zhejiang are multiple and should be understood as the interaction of abiotic, biotic, management, and socio-economic constraints. Comparative work on food-crop systems identifies these four categories as the main contributors to yield gaps, with the specific combination varying by crop and farming system. More general crop-production research similarly shows that low productivity commonly results from drought or excess moisture, soil constraints, diseases and insect pests, lack of improved varieties, weak infrastructure, and insufficient input efficiency. Under Zhejiang conditions, this means that yield instability in legumes is unlikely to arise from a single stress; rather, it is likely to reflect the combined effects of humid subtropical weather, fragmented land use, uneven soil conditions, and differences in management intensity across regions. Because precipitation and temperature are central drivers of yield variation in Chinese cropping systems, climate-related fluctuations remain a basic source of production risk (Gao et al., 2022).

For legume crops specifically, several constraints are especially relevant. Mungbean, one of the representative short-season legumes suited to diversified systems, is constrained by insect pests, diseases, drought, waterlogging, salinity, and heat stress, illustrating the broad stress spectrum that legume crops may face in production. In China, another major bottleneck is continuous cropping: because limited arable land often forces repeated planting of the same legume on the same plot, continuous-cropping obstacles have become common and can seriously impair yield formation through soil degradation and crop-soil interaction problems (Lei et al., 2023). This issue is highly relevant to Zhejiang's intensive farming context, where land scarcity and high cropping intensity may encourage short rotations or repeated use of the same fields for similar legume types. In addition, subtropical production systems can face nutrient surplus, declining nutrient-use efficiency, and soil acidification under intensive management, suggesting that sustainable improvement of legume production in Zhejiang depends not only on

varietal adaptation, but also on better rotation design, soil management, and input regulation. Overall, the production status and ecological characteristics of legume crops in Zhejiang are defined by a diverse planting structure, a humid subtropical resource base, and multiple interacting production constraints. These features make Zhejiang a suitable setting for comparing yield stability among legume species across contrasting ecological and management environments.

3 Data Sources and Methods for Yield Stability Evaluation

3.1 Data sources and research materials

The yield stability evaluation in this study should be based on multi-environment trial data, because this design is the standard framework for judging crop adaptability and stable performance across contrasting production conditions. In legume research, multi-environment trials have been used to compare grain yield across locations, seasons, and years so that genotype or species performance can be evaluated under realistic production variability (Hu et al., 2025). Accordingly, the data source for this study can be defined as yield records collected from replicated field experiments on major legume species grown in representative ecological zones of Zhejiang, with each site-year combination treated as an independent environment. This structure makes it possible to separate average productivity from environmental sensitivity and provides the basic dataset needed for subsequent stability analysis.

The research materials should include several legume species with practical production relevance in Zhejiang, together with replicated observations from multiple environments. Existing legume stability studies typically use a randomized block or lattice design with three replications, which helps reduce field error and improves the reliability of yield comparisons (Habtegebriel and Abebe, 2023). Similar work in soybean has also used multi-location trials with elite lines and check cultivars across several environments, showing that replicated comparative testing is appropriate for identifying widely adapted and stable materials (Abebe et al., 2024). On this basis, the present study may use soybean, mung bean, adzuki bean, cowpea, and other locally important legumes as research materials, arranged in a uniform experimental design across test sites, with grain yield as the core trait and key agronomic characteristics recorded as auxiliary indicators.

3.2 Evaluation indicators

Yield stability should be evaluated from both productivity and response consistency, because high mean yield alone does not guarantee reliable adaptation across heterogeneous environments. Earlier soybean work showed that selection for yield alone often sacrifices some stability, while selection for stability alone may also reduce yield, so combined evaluation is more reasonable. Therefore, this study should first use mean yield as the primary performance index, and then combine it with classical stability statistics such as the coefficient of variation, regression coefficient, deviation from regression, Wricke's ecovalence, and Shukla's stability variance. These indices reflect different concepts of stability and can jointly describe whether a legume species performs consistently across favorable and unfavorable environments.

Recent studies indicate that no single stability parameter is sufficient for all breeding or agronomic decisions, so a composite indicator system is preferable. In lentil, significant positive relationships were reported among many parametric, non-parametric, and AMMI-based statistics, suggesting that several indices can be used together within the dynamic stability framework (Hossain et al., 2023). At the same time, some indicators behave differently because they emphasize distinct aspects of stability, and even the conventional coefficient of variation may require cautious interpretation when variance depends systematically on mean yield. For this reason, the present study can combine mean yield, CV, ASV, WAASB, and yield-stability ranking indices, so that both static consistency and high-yield adaptability are reflected in the final evaluation.

3.3 Data analysis methods

The statistical analysis should begin with combined analysis of variance, because ANOVA is the basic method for testing whether genotype or species effects, environmental effects, and their interactions are significant in multi-environment yield trials. When the interaction term is significant, further stability analysis becomes necessary, since changes in rank across environments mean that simple average yield cannot fully explain

adaptation differences. In practice, the analysis process may include tests of normality, calculation of environment-wise and pooled means, and partitioning of total variation into species, environment, and species-by-environment interaction components. This step provides the statistical basis for deciding whether the comparison of yield stability among legume species in Zhejiang is meaningful.

After ANOVA, the study should combine AMMI and GGE biplot analysis, because these two methods are widely used and complementary in interpreting genotype-by-environment interaction (Zhang et al., 2025). GGE biplot is especially useful for identifying winning genotypes, judging mean-versus-stability performance, and screening discriminating or representative test environments, while AMMI is more effective for decomposing interaction effects and deriving indicators such as ASV. To strengthen the robustness of conclusions, the results can further be compared with regression-based methods such as Eberhart-Russell analysis and with integrated ranking procedures such as WAASB, YSi, or average rank, because different methods may emphasize either responsiveness, static stability, or broad adaptation. In this way, the final methodological framework can achieve a more balanced evaluation of both yield level and yield stability among different legume species in Zhejiang. Overall, the data sources, evaluation indicators, and analytical methods for this study should all serve the same goal: comparing yield stability among different legume species in Zhejiang under realistic multi-environment conditions. A design centered on replicated field trials, multi-index stability evaluation, and combined ANOVA-AMMI-GGE analysis is well aligned with the research question.

4 Comparison of Yield Variation Characteristics among Different Legume Crops

4.1 Differences in long-term average yield among different legume crops

Across legume crops, long-term average yield differs substantially among species, and the basic pattern in the literature is that soybean usually ranks among the highest-yielding grain legumes, while some lupins or minor legumes remain clearly lower-yielding. In a direct species comparison, soybean averaged 3.99 t·ha^{-1} and had the highest seed yield among the tested legumes, whereas narrow-leafed and yellow lupin had the lowest yields. Long-term evidence from northern Europe also showed that narrow-leafed lupin yielded on average 11% more than faba bean and 25% more than pea, while faba bean still averaged 11% higher than pea, indicating that the ranking among non-soybean legumes can change by species and environment. For Zhejiang, this suggests that interspecific yield comparison should not assume a single universal order, but should distinguish between high-yielding species, moderate-yielding species, and species with lower but potentially more specialized adaptation (Figure 2).

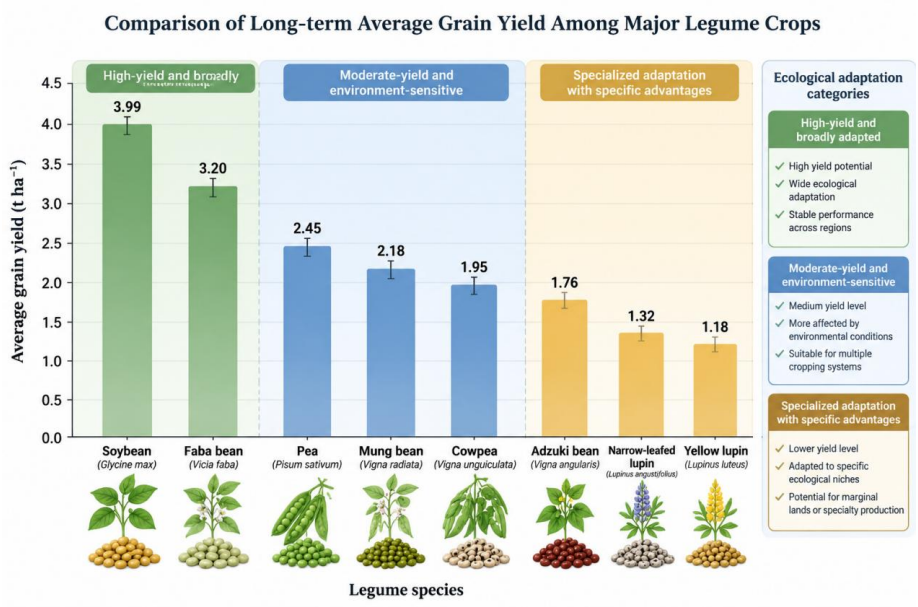

Figure 2 Comparison of long-term average yield performance among major legume crops and their ecological adaptation categories. The figure summarizes differences in yield potential and emphasizes that productivity ranking varies among species and environments

The differences in long-term average yield are also shaped by cropping system and management, rather than species identity alone. In southeast China, a nine-year rotation study found that kidney bean average yield under one rotation system was 7.47% higher than under another when recommended fertilization was used, showing that mean legume yield can shift markedly with system configuration. In another long-term subtropical rotation experiment, rice yield under a rice-faba bean sequence reached 8.73 t·ha^{-1}, 19.1% above the rice-wheat control, indicating that legume inclusion can raise the productivity of the whole system and indirectly alter the comparative value of different legume species within rotations (Yang et al., 2024). Therefore, when comparing average yields among legumes in Zhejiang, it is more appropriate to interpret observed species differences as the joint result of genetic potential, rotation fit, and management compatibility.

4.2 Annual variation trends

Annual yield variation is a core part of stability analysis because mean yield alone can mask large year-to-year fluctuations. A long-term experiment in southeast China showed that crop yield varied greatly over time under the influence of climate, precipitation, and other environmental factors, and that the yield trends of kidney bean and mustard differed significantly between rotation treatments over nine years (Zhang et al., 2022). Evidence from broader crop-yield analysis in China likewise showed that crop yields generally increased over time at the national scale, but with strong provincial differences in annual growth rates, indicating that temporal yield trajectories are rarely uniform across regions or crop types. Applied to Zhejiang legumes, this means annual variation should be evaluated not only by whether yield rises or falls, but also by the rate, direction, and consistency of change under different production environments.

Different legume crops also appear to differ in the magnitude of their interannual fluctuations. In species-comparison research, faba bean maintained relatively high yields but varied strongly among years, and one low-rainfall year caused a clear yield reduction, showing its sensitivity to annual weather anomalies (Jarecki and Migut, 2022). Broader evidence suggests that temporal instability in legumes is not necessarily extreme relative to other spring crops: across long-term European experiments, grain legumes showed 30% temporal instability, only slightly above spring cereals at 27%, and were judged as reliable as other spring-sown crops when assessed with scale-adjusted methods. For Zhejiang, annual yield trend analysis should therefore pay particular attention to whether specific legumes combine acceptable mean yield with limited fluctuation under variable rainfall, temperature, and seasonal light conditions.

4.3 Ecological-region performance

Yield performance across ecological regions differs because legume crops respond strongly to local climate, soil, and resource conditions. A large soybean dataset from China showed grain yield ranging from 1.4 to 4.5 t·ha^{-1} across 146 locations, with the main climatic drivers shifting by region: temperature dominated in cool high-latitude areas, whereas radiation was more important in hot, humid low-latitude regions (Wu et al., 2023). Complementary evidence from explainable AI analysis across nine grain legumes found that different species respond differently to environmental factors, with cowpea showing higher productivity at elevated latitudes, while garden pea and faba bean were especially sensitive to soil moisture, organic matter, and soil type. This supports the expectation that legumes in Zhejiang will show clear regional differentiation between plains, coastal zones, and hilly uplands.

Regional comparison studies further show that legume-related yield outcomes often improve most in areas with stronger resource constraints or favorable hydrothermal conditions. A national meta-analysis found that legume green manure increased grain-crop yields in most regions of China, with the largest increase in Northeast China, and that benefits were greatest where annual precipitation exceeded 600 mm and annual mean temperature exceeded 10 °C (Liang et al., 2022). Similarly, a meta-analysis of soybean/maize intercropping concluded that the Yangtze River Basin was the most suitable region for adoption and that the system was especially beneficial in areas with limited agricultural resources. Since Zhejiang lies within the broader Yangtze River agricultural zone and has humid subtropical conditions, these results suggest that regional differences in yield performance among legume crops in the province are likely to reflect both ecological suitability and the extent to which each species

can exploit diversified, resource-efficient cropping systems. Overall, the available evidence supports comparing Zhejiang legume species through three linked dimensions: average yield level, annual fluctuation, and regional adaptation. This is the most appropriate basis for identifying legume crops that are not only productive, but also stable across Zhejiang's diverse ecological settings.

5 Comprehensive Evaluation of Yield Stability among Different Legume Crops

5.1 Stability comparison based on statistical indicators

Statistical indicators show that yield stability should be judged by combining mean performance with fluctuation measures rather than by yield alone. In soybean, high and stable yield is treated as the desired breeding target, but analyses also show that selecting only for yield can sacrifice stability, whereas selecting only for stability can reduce yield potential. For this reason, classical indicators such as the coefficient of variation, regression coefficient, deviation from regression, cultivar superiority, and rank-sum type indices are useful because they describe different aspects of adaptation and response consistency across environments.

At the same time, different indicators do not always rank materials in the same way, so their interpretation must be cautious. In lentil, several parametric and non-parametric statistics showed significant positive associations with mean yield, supporting the use of multiple indices under the dynamic stability concept (Hossain et al., 2023). By contrast, work on oat found that some commonly used statistics, including standard deviation, deviation from regression, Wricke's ecovalence, Shukla's variance, and AMMI stability value, tended to favor lower-yielding materials, while Pi, Bi, and YSI aligned better with yield improvement (Kebede et al., 2023). In addition, the traditional coefficient of variation can be misleading when variance depends systematically on mean yield, so adjusted forms of CV may provide a more defensible estimate of temporal stability.

5.2 Stability evaluation based on multi-model approaches

A more reliable comprehensive evaluation comes from multi-model analysis, especially the joint use of ANOVA, AMMI, GGE biplot, and integrated stability indices. In recent faba bean work, stability was assessed simultaneously through regression coefficient, deviation from regression, cultivar superiority, AMMI stability value, and genotype selection index, while AMMI and GGE were used to distinguish broadly adapted from specifically adapted genotypes (Wondaferew et al., 2024). Similar soybean studies also combined AMMI, GGE, WAASB, MTSI, and regression-based methods, showing that yield stability analysis is strongest when the same material is examined from several statistical perspectives rather than a single model alone (Habtegebriel and Abebe, 2023).

The value of this approach is that different models emphasize different dimensions of stability and can therefore complement one another. AMMI focuses on decomposing genotype-by-environment interaction and often identifies stable genotypes through low interaction scores, whereas GGE biplot is more useful for visualizing "which-won-where," ideal genotypes, and representative testing environments. Recent comparative analysis also showed that ASV, GGE, and GYT can produce slightly different rankings because one emphasizes interaction magnitude, another genotype-plus-interaction alignment, and another yield-trait balance, which means stability is inherently multidimensional. This interpretation is consistent with legume evidence from common bean, where AMMI, GGE, WAASB, and GSI identified overlapping but not identical stable genotypes and test environments (Estifanos et al., 2025).

5.3 Factors influencing yield stability

The main factors influencing yield stability are the environment, genotype-by-environment interaction, and the specific climatic and soil conditions that shape crop response. Across many legume trials, environment is often the largest source of variation: in field pea, environmental effects explained 59.62% of total variation and GEI explained 30.11%. In faba bean grown across different regions, the environment explained 81-93% of variation for most traits, while genotype contributed much less for many characteristics (Papastylianou et al., 2021). This means that differences in rainfall, temperature, season, and location are likely to dominate yield fluctuation among legume crops in Zhejiang.

More specifically, yield instability arises from the interaction of weather, soil fertility, and agronomic or biological stress. Cowpea evidence showed that GEI was associated with temperature, rainfall, relative humidity, maturity, and yield components, indicating that climatic variation directly alters performance rankings among genotypes. Soil effects can be equally important: in West African cowpea, year was the largest source of annual variation, but differences in soil nitrogen and available phosphorus explained much of the yield contrast among soil types under the same rainfall regime. Other legume studies further identify moisture stress, poor soil fertility, disease, insect pressure, weak management, and lack of adaptable varieties as recurrent constraints on stable production (Eskezia et al., 2025). For Zhejiang, this suggests that the comprehensive evaluation of yield stability should not stop at ranking crops statistically, but should also link those rankings to rainfall variability, soil conditions, cropping management, and species-specific stress tolerance. Overall, the comprehensive evaluation of yield stability among different legume crops in Zhejiang should integrate statistical indices, multi-model validation, and environmental interpretation. This is the most appropriate basis for identifying legume crops that combine relatively high yield with stable performance across Zhejiang's diverse ecological conditions.

6 Mechanisms Underlying Yield Stability Formation in Legume Crops

6.1 Climate adaptability and yield stability mechanisms

Climate adaptability is a primary mechanism underlying yield stability in legume crops because climate change increasingly exposes legumes to overlapping stresses rather than isolated constraints. Concurrent heat and drought stress disrupt growth, development, and yield formation, and these combined stresses are especially damaging during reproductive stages because they shorten the crop life cycle and alter seed number, size, and composition. More general synthesis across crops shows that drought-heat episodes reduce harvest index and intensify yield loss when they occur during flowering and seed filling, which explains why stable-yielding legumes must maintain reproduction under seasonal weather variability. For Zhejiang, where high temperature, heavy rainfall, and intermittent summer drought can alternate within the same season, yield stability is therefore closely tied to the capacity of different legume species to buffer reproductive processes against fluctuating hydrothermal conditions.

This buffering capacity depends on coordinated physiological and molecular stress responses rather than on a single tolerance trait. Legumes exposed to combined heat and drought rely on osmolytes, antioxidants, and stress-responsive genes, while signaling pathways involving Ca^{2+}, reactive oxygen species, and transcriptional regulators help integrate stress perception and adaptive responses (Priya et al., 2025). Drought tolerance also depends on traits such as improved root system architecture, stomatal regulation, antioxidant defense, and solute accumulation, which together reduce water loss and oxidative injury while sustaining carbon assimilation under stress. These findings indicate that climate adaptability contributes to yield stability when legumes can maintain source-sink balance, reproductive success, and resource use efficiency across variable weather conditions rather than only under average years.

6.2 Physiological and ecological characteristics

Stable production in legumes is strongly associated with physiological traits that sustain nitrogen acquisition and biomass formation under variable field conditions. Biological nitrogen fixation is central because it supports plant nutrition while reducing dependence on external nitrogen inputs, and its effectiveness depends on successful nodulation and rhizosphere functioning (Qiao et al., 2024). Long-term diversification experiments showed that legume rhizodeposition can reshape rhizosphere metabolites and microbial functions, thereby enhancing the growth and nitrogen-fixing activity of free-living bacteria and increasing nodulation by symbiotic Bradyrhizobium. This means that yield stability is not only a property of the plant itself, but also of the plant-microbe system that supports nitrogen supply across different soil and management environments.

Ecological interactions further strengthen stable production by improving root conformation, nitrogen transfer, and resource partitioning. In mixed cropping across three ecological zones, legumes showed higher nodulation, better root-system configuration, greater aboveground dry matter, and enhanced nitrogen fixation, while the main drivers of atmospheric nitrogen fixation included cropping pattern, ecological zone, soil nitrogen status, microbial

biomass nitrogen, and nodule number (Luo et al., 2024). Root interaction studies also showed that closer interspecific contact can intensify symbiotic nitrogen fixation, promote nitrogen assimilation and amino-acid export, and ultimately raise nitrogen-use efficiency and yield in intercropped pea. For Zhejiang, these results suggest that stable legume yields are more likely where species possess efficient nodulation, strong root plasticity, and compatibility with diversified cropping systems that improve belowground ecological function.

6.3 Genetic characteristics and genotype-environment interactions

The genetic basis of yield stability in legumes is inseparable from genotype-environment interaction, because the same genotype can perform very differently across locations, years, and management conditions. In faba bean, yield instability is explicitly attributed to GEI, and large interaction effects reduce heritability and weaken the correspondence between phenotypic performance and genetic value, making selection progress slower (Sokolović et al., 2025). Similar evidence from chickpea shows that stability breeding must distinguish between static stability, where performance changes little across environments, and dynamic stability, where genotypes respond predictably while maintaining high productivity (Eskezia et al., 2025). This implies that legume yield stability in Zhejiang should be interpreted not as simple invariance, but as the ability of a species or genotype to maintain relatively high yield under environmental fluctuation without excessive rank reversal.

A second mechanism is the presence of usable genetic diversity for adaptive traits, together with analytical tools that identify broadly or specifically adapted materials. Studies in faba bean show that stable variety development depends on substantial genetic variation, because this diversity provides the basis for selecting cultivars suited to local climate, altitude, and soil conditions. At the same time, crossover interactions can shift genotype rankings across yield environments, so identifying where these crossovers occur helps separate narrowly adapted from broadly adapted materials and avoids yield penalties from poor genotype choice. Breeding for stable legume production therefore requires combining diverse germplasm, multi-environment testing, and methods such as GGE, AMMI, or multi-trait stability indices to match genotypes to the ecological heterogeneity typical of Zhejiang. Overall, yield stability formation in legumes depends on three linked mechanisms: climate adaptation under compound stress, efficient physiological-ecological support for nitrogen and biomass production, and genetic adaptation shaped by genotype-environment interaction. These mechanisms jointly determine whether a legume species can sustain reliable yield across Zhejiang's diverse environments.

7 Case Study: Yield Stability Analysis of Legume Crops in Typical Ecological Regions of Zhejiang Province

7.1 Selection of study regions and research design

The case study regions should be selected to represent the main ecological contrasts that shape legume production in Zhejiang, such as plains, river-valley basins, coastal areas, and hilly uplands. Multi-environment trials are the standard design for evaluating adaptation and stability across heterogeneous conditions, because they allow the same crop materials to be compared under differing temperature, rainfall, soil, and management backgrounds. Experience from Chinese pea trials further shows that broad climatic coverage across locations and years is necessary for identifying both widely adapted and specifically adapted materials, rather than assuming that one genotype or species performs uniformly everywhere.

Accordingly, the research design for Zhejiang can be organized around several representative county-level sites, with each site-year combination treated as an independent environment and each legume species tested under a unified field protocol. Similar legume studies commonly use randomized complete block or lattice designs with three to four replications, which improves the precision of yield comparison under variable field conditions (Haile and Tesfaye, 2024). Case studies from other ecological regions also show that dividing production areas into mega-environments helps identify groups of locations with similar crop responses, making regional interpretation more meaningful than using provincial averages alone (Figure 3).

7.2 Yield stability performance

In the case study regions, the first expectation is that different legume crops will show clear differences in both mean yield and stability because genotype, environment, and their interaction usually all contribute significantly

to grain-yield variation. This pattern has been observed in soybean multi-location trials, where grain yield ranged from 979.8 to 3645 kg·ha^{-1} and significant differences were detected among genotypes, environments, and GEI. Comparable results were reported for common bean, where AMMI analysis confirmed that grain yield changed significantly across environments and supported the identification of both broadly adapted and specifically adapted genotypes (Daemo, 2024).

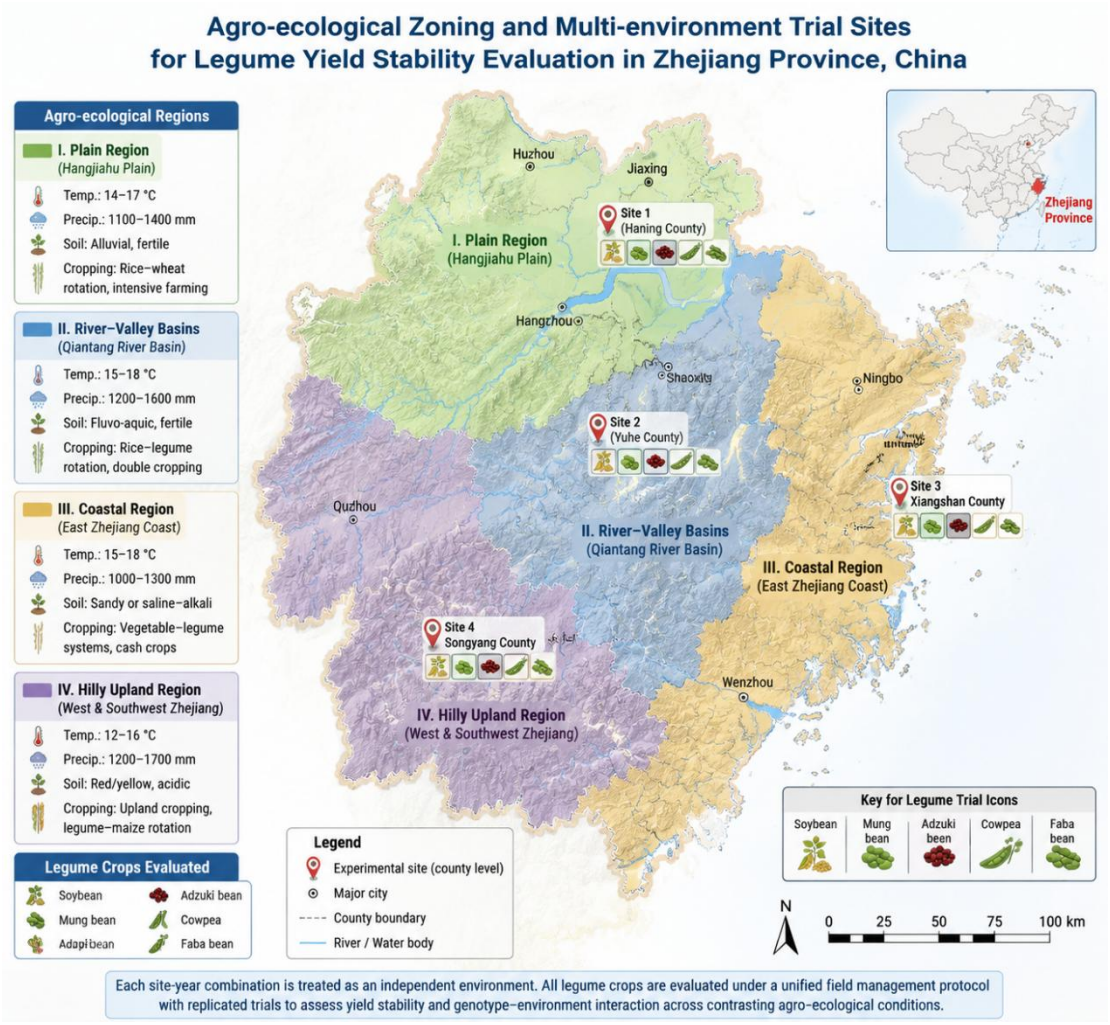

Figure 3 Agro-ecological zoning and representative multi-environment trial locations for evaluating legume yield stability across Zhejiang Province, China. The map highlights contrasting production environments including plains, river valleys, coastal zones, and hilly uplands

A second likely pattern is that some legume crops or varieties will be broadly adaptable across Zhejiang, while others will perform best only in certain ecological zones. In faba bean, GGE biplot analysis identified genotypes with low GEI and broad adaptability, showing that materials less affected by environmental fluctuation are more reliable across diverse sites (Wondaferew et al., 2024). In southern China pea trials, the tested environments were partitioned into two mega-environments, and some genotypes were stable across many sites while others showed clear specific adaptability to particular environments. For Zhejiang, this implies that soybean or mung bean may perform more steadily across multiple regions, whereas adzuki bean, cowpea, or pea may express stronger ecological specialization depending on thermal and moisture conditions.

7.3 Implications and optimization

The main implication of the Zhejiang case study is that production recommendations should be based on region-specific matching of crop type and ecological setting, rather than on a single provincial ranking of legume crops. Work from other Chinese ecological regions shows that the best-performing legume mixtures differed among locations, and the recommended crop combinations were therefore region-specific rather than universal

(Luo et al., 2023). More broadly, identifying representative test environments is valuable because selection in those environments can improve the efficiency of screening and strengthen recommendations for similar agro-ecological conditions.

Production optimization should therefore combine species selection with cropping-system adjustment and soil management. Legume-based rotations tend to maintain yields and production stability by improving soil fertility and reducing dependence on external inputs. Long-term evidence from China also shows that legume inclusion can support more stable subsequent crop production and greater resistance and resilience under changing fertilization conditions, indicating that stability benefits are not limited to a single season (Liu et al., 2023). In practical terms, Zhejiang should prioritize legume species with broad adaptation for large-scale promotion, reserve specifically adapted species for matching ecological niches, and integrate legumes into rotations or intercrops where soil quality, resource-use efficiency, and system-level stability can all be improved. Overall, this case-study framework supports a comparative and regionally differentiated interpretation of yield stability among legume crops in Zhejiang. It is most useful for identifying which legumes are broadly stable, which are locally advantageous, and how production can be optimized through ecological matching and diversified cropping systems.

9 Conclusions and Future Perspectives

This study conducted a systematic comparison of yield stability among major legume species cultivated across Zhejiang Province, drawing on multi-year, multi-location data to capture the full spectrum of environmental variability characteristic of the region. The results reveal that soybean and broad bean exhibit relatively high yield stability, with lower coefficients of variation across years and ecological zones, while cowpea and adzuki bean display greater interannual fluctuation. These differences are attributable to a combination of physiological traits, root system architecture, and sensitivity to temperature and precipitation extremes during critical growth stages. A second key finding is that genotype-environment interaction effects are pronounced for all legume species examined, meaning that a variety performing well in one ecological subregion of Zhejiang may underperform in another. The application of multiple stability parameters-including the coefficient of variation, Shukla's stability variance, and AMMI-based stability scores-demonstrated that no single indicator fully captures the complexity of yield stability, and a composite evaluation framework is essential for robust ranking. Taken together, the findings provide an evidence base for tailoring legume variety selection and cropping system design to specific agro-ecological conditions within the province.

Several limitations should be acknowledged. First, the temporal coverage of the yield data, while spanning multiple years, may not fully capture the increasing frequency of extreme weather events associated with ongoing climate change, potentially limiting the predictive value of historical stability estimates. Second, the analysis relied primarily on regional aggregate statistics and published trial records; farm-level heterogeneity in management practices, soil fertility, and pest pressure could not be fully controlled, introducing unquantified variability into the stability comparisons. Third, the study focused on the most widely planted legume species and did not include minor or underutilized legumes that may hold untapped resilience potential. Future research should prioritize several directions. Long-term, standardized field experiments across representative ecological zones of Zhejiang are needed to generate high-resolution, plot-level yield data under controlled management protocols. Integrating crop growth models with climate projection scenarios would allow researchers to simulate yield stability under future warming and altered precipitation patterns, providing forward-looking guidance rather than retrospective description. Additionally, genomic and molecular tools-including marker-assisted selection and genome-wide association studies-should be leveraged to identify quantitative trait loci associated with stability-related traits, accelerating the breeding of climate-resilient legume varieties. Finally, socio-economic dimensions such as farmer adoption behavior, market price volatility, and the profitability of stable versus high-yielding varieties warrant investigation to ensure that agronomic recommendations are economically viable.

The legume industry in Zhejiang Province holds considerable development potential, driven by growing consumer demand for plant-based protein, the ecological benefits of legumes in crop rotation systems, and policy support for

sustainable agriculture. Strengthening the breeding and extension of stable-yielding, climate-adapted varieties tailored to the province's diverse ecological subregions is a strategic priority. Precision cultivation technologies-including drip irrigation, sensor-based nutrient management, and mechanized planting and harvesting-can further reduce yield variability and improve resource-use efficiency, particularly in the face of labor shortages and rising input costs. Looking ahead, the integration of legume production into broader sustainable cropping systems offers a pathway to enhance soil health, reduce nitrogen fertilizer dependency, and improve the resilience of the entire agricultural landscape. Policy incentives that encourage legume inclusion in rice-based rotations, coupled with the development of regional legume brands and value-added processing chains, could simultaneously raise farmer incomes and strengthen domestic protein supply. Collaborative networks among research institutions, extension services, and producer cooperatives will be essential to translate stability research into practical, scalable improvements across the province's legume sector.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Physiological Responses of Photosynthetic Characteristics and Antioxidant Systems in Wheat Leaves under Drought Stress

Ling Jin
Northwest A&F University, Xianyang, 712100, Shaanxi, China
Corresponding author: 2024012253@nwafu.edu.cn

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Abstract Drought stress is one of the major environmental constraints limiting wheat growth, productivity, and yield stability, particularly under increasing climate variability. This review summarizes the physiological responses of wheat leaves to drought stress, with emphasis on photosynthetic characteristics and antioxidant defense mechanisms. Drought-induced reductions in leaf water status and stomatal conductance restrict CO_2 diffusion and progressively impair photosynthetic carbon assimilation. Meanwhile, disturbances in photosynthetic electron transport enhance the production and accumulation of reactive oxygen species (ROS), resulting in oxidative damage to cellular membranes and photosynthetic structures. Wheat responds to oxidative stress by activating enzymatic antioxidant defenses, including superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase, together with non-enzymatic antioxidants involved in the ascorbate-glutathione cycle. The coordinated regulation of stomatal behavior, chlorophyll fluorescence, photoprotection, ROS scavenging, hormonal signaling, and redox homeostasis contributes to drought adaptation. Case studies further demonstrate differences among drought intensity, duration, and rewatering conditions in determining photosynthetic recovery and antioxidant capacity. Finally, strategies involving irrigation management, nutritional regulation, exogenous substances, drought-tolerant cultivar selection, and precision agriculture are discussed. Integrating physiological, biochemical, and molecular approaches will improve the understanding and practical management of wheat drought tolerance.

Keywords Wheat; Drought stress; Photosynthesis; Antioxidant system; Reactive oxygen species

1 Introduction

Wheat is one of the world's most important staple crops and provides a substantial share of human calories and protein, so its stability under water-limited environments is tightly linked to global food security. Yet drought has become one of the most serious abiotic constraints on wheat production worldwide, and climate change is increasing both the frequency and severity of water scarcity across major wheat-growing regions (Franco-Navarro et al., 2025). Projections suggest that the area exposed to drought in wheat-producing regions could expand markedly during this century, with simultaneous severe water scarcity affecting far more wheat land than at present even under mitigation scenarios. This threat is especially serious because much of global wheat production already occurs in water-stressed breadbaskets; one analysis estimated that 65% of wheat produced across major global breadbaskets faces high water scarcity. At regional scales, winter wheat frequently experiences serious crop water deficit in major production zones such as the North China Plain and northwestern China, especially from greening to jointing and from jointing to anthesis, and these deficits can reduce yield by 60% and 55%, respectively, when water supply is insufficient. Future modeling further indicates that concurrent meteorological and agricultural drought can widen wheat yield gaps more than single-type drought, with projected impacts on wheat often stronger than on maize and strongly shaped by soil moisture constraints. Although global mean production losses may appear moderate in some projections, the burden is highly uneven, with many countries expected to face large drought-related crop losses, reinforcing the need for drought adaptation in wheat systems (Kraklow et al., 2025). Because reproductive and grain-filling stages are particularly sensitive to water deficit, drought not only depresses biomass accumulation and grain formation but also threatens the reliability of wheat production under increasingly unstable climates (Bohra et al., 2024).

The harmful effect of drought on wheat productivity is mediated through a cascade of physiological disturbances, among which impairment of photosynthesis is one of the earliest and most consequential responses (Nyaupane et al., 2024). Under water deficit, wheat leaves rapidly close stomata to limit transpirational water loss, but this protective response also restricts CO_2 diffusion and reduces carbon assimilation, leading to declines in net photosynthetic rate and stomatal conductance. Experimental studies across wheat genotypes consistently show that drought reduces net photosynthesis, stomatal conductance, relative water content, chlorophyll content, and grain yield, although the magnitude of decline differs among cultivars according to drought tolerance capacity. In the early phase of drought, photosynthetic inhibition is often dominated by stomatal limitation, whereas under prolonged or severe stress non-stomatal limitations become increasingly important, including chlorophyll loss, reduced Rubisco-related activity, inhibition of PSII electron transport, and damage to the oxygen-evolving complex and light-harvesting system (Todorova et al., 2022). Chlorophyll fluorescence measurements confirm that drought decreases Fv/Fm, ΦPSII, qP, and electron transport rate, while often increasing energy dissipation pathways, indicating progressive photochemical impairment and photoinhibition risk. This physiological progression is further intensified when drought co-occurs with heat, because high temperature amplifies declines in PSII efficiency, carbon uptake, and water use efficiency, particularly above critical thermal thresholds and in sensitive genotypes. At the same time, drought disturbs photosynthetic electron flow and enhances the incomplete reduction of oxygen, promoting the formation of reactive oxygen species such as superoxide and hydrogen peroxide that damage lipids, proteins, membranes, and the photosynthetic apparatus if not effectively detoxified. Accordingly, antioxidant defense is a central component of drought tolerance in wheat. Water deficit commonly elevates the activities of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, together with osmotic regulators such as proline, soluble sugars, and related metabolites that help preserve membrane integrity and cellular hydration. Tolerant genotypes generally sustain lower hydrogen peroxide and lipid peroxidation, stronger or longer-lasting antioxidant activity, and better recovery after rewatering, indicating that resistance depends not only on limiting water loss but also on maintaining redox homeostasis and protecting photosynthetic tissues.

Against this background, understanding the coordinated responses of photosynthetic characteristics and antioxidant systems in wheat leaves is essential for clarifying how drought injury develops and how tolerance is expressed at the physiological level. Existing research has established that drought affects gas exchange, chlorophyll fluorescence, pigment stability, osmotic adjustment, and antioxidant metabolism, but several important questions remain open, particularly regarding the temporal coupling between photosynthetic decline and oxidative defense, the extent to which stomatal versus non-stomatal limitation predominates under different stress intensities, and which leaf traits best distinguish tolerant from susceptible wheat genotypes. There is also strong practical value in identifying physiological indicators that respond sensitively to drought and recovery, because such traits can support cultivar screening, mechanistic phenotyping, and management strategies aimed at improving water productivity and resilience. Therefore, this study is framed to examine how drought stress alters leaf photosynthetic performance and antioxidant defense in wheat, with emphasis on changes in gas exchange, chlorophyll-related traits, reactive oxygen metabolism, and protective enzyme activities. The central scientific questions are whether drought-induced reductions in photosynthetic capacity are accompanied by coordinated activation of antioxidant enzymes, whether these responses are sufficient to limit oxidative injury in leaves, and which physiological indices can most effectively explain variation in drought tolerance). By integrating these dimensions, the study can help bridge the gap between whole-plant drought outcomes and leaf-level mechanisms, and provide a physiological basis for breeding and management strategies that enhance wheat adaptation to increasingly water-limited environments.

2 Effects of Drought Stress on Leaf Water Status and Physiological Processes in Wheat

2.1 Changes in leaf water status and water relations

Drought stress first disrupts leaf water balance in wheat by lowering leaf water potential and relative water content, thereby weakening turgor maintenance and impairing normal physiological activity. This decline in water status is consistently accompanied by higher leaf or canopy temperature, indicating reduced evaporative cooling and

tighter hydraulic constraints on leaf function. Across genotypes, the magnitude of these changes depends on their capacity to preserve hydration through osmotic regulation, elastic adjustment, and sustained water transport, rather than through simple dehydration avoidance alone. In drought-tolerant wheat, maintenance of higher relative water content and leaf water potential is often associated with better xylem exudation, stronger membrane stability, and improved chlorophyll retention under stress (Akter et al., 2023).

The physiological basis of these water-relation differences lies in the coordination between osmotic adjustment and hydraulic behavior. Resistant wheat genotypes commonly accumulate soluble sugars and other osmolytes that lower osmotic potential, improve soil water uptake, and help preserve cell function under declining soil moisture. By contrast, increasing water deficit causes sharper declines in relative water content, water potential, and membrane stability in susceptible materials, showing that inadequate adjustment accelerates dehydration injury. Leaf structural and anatomical integrity also matters, because maintaining functional leaf hydraulic properties and internal diffusion pathways helps sustain photosynthetic capacity as drought progresses (Yang et al., 2026). For this reason, traits such as relative water content, excised leaf water retention, relative water loss, leaf rolling, and waxiness are widely treated as practical indicators of drought tolerance in wheat.

2.2 Stomatal regulation and transpiration responses

Stomatal regulation is one of the earliest and most decisive responses of wheat leaves to drought stress because it directly controls both carbon dioxide influx and transpirational water loss. As soil dries, stomatal conductance usually declines before many other gas-exchange traits, and this reduction lowers transpiration and leaf cooling while progressively constraining photosynthesis (Pflüger et al., 2024). Experimental evidence further shows that stomatal conductance in wheat is positively linked with leaf water potential and hydraulic conductance, but negatively associated with abscisic acid accumulation and, under severe stress, with altered stomatal traits such as density and pore area. Thus, stomatal behavior reflects not only passive water loss control but also active integration of hydraulic and hormonal signals across stress intensities.

Wheat does not rely on a single stomatal strategy under drought. Under mild stress or early drying, some genotypes maintain relatively high conductance and behave more anisohydrically, allowing continued carbon assimilation despite falling hydraulic conductance. With increasing drought severity, however, stomata shift toward a more conservative, isohydric pattern, reducing transpiration to protect leaf water status and hydraulic safety. Genotypic differences are important in this transition: drought-tolerant lines can delay full stomatal closure during reproductive stress, whereas sensitive lines often close stomata earlier and enter a passive survival mode sooner (Onyemaobi et al., 2021). In parallel, structural stomatal traits contribute to performance, since smaller or more numerous stomata, depending on genetic background, can support faster regulation and improved transpiration efficiency under drought.

2.3 Water use efficiency and drought adaptation

Water use efficiency in wheat under drought is shaped by the balance between conserving water and sustaining assimilation. Moderate restriction of stomatal opening often increases instantaneous or integral water use efficiency because transpiration declines faster than photosynthesis, even though absolute carbon gain may still be reduced. Drought-resistant cultivars tend to achieve this balance more effectively, showing lower lifetime water consumption or transpiration together with better maintenance of productive photosynthesis under stress. This trade-off is central to wheat adaptation, since excessive water saving can protect tissues but penalize biomass accumulation, whereas insufficient control accelerates dehydration and oxidative damage. As a result, drought adaptation depends on optimizing, rather than maximizing, stomatal restriction and tissue hydration (Franco-Navarro et al., 2025).

At the whole-plant level, improved water use efficiency emerges from the integration of stomatal behavior, root water acquisition, osmotic adjustment, and management context. Deficit irrigation imposed at suitable growth stages can raise wheat water use efficiency, especially when stress is moderated and rewatering occurs after sensitive periods such as heading. Management interventions can also enhance efficiency: combining deficit irrigation with soil amendments improved water use efficiency in pot experiments, indicating that agronomic

measures can complement intrinsic physiological tolerance (Irkiso et al., 2025). At the genetic level, wheat lines with tighter stomatal control, improved root development, and lower relative water loss show higher instantaneous water use efficiency and less physiological damage under water limitation. Together, these findings show that drought adaptation in wheat is best understood as coordinated regulation of water loss, water capture, and photosynthetic protection rather than as a single-trait response (Figure 1).

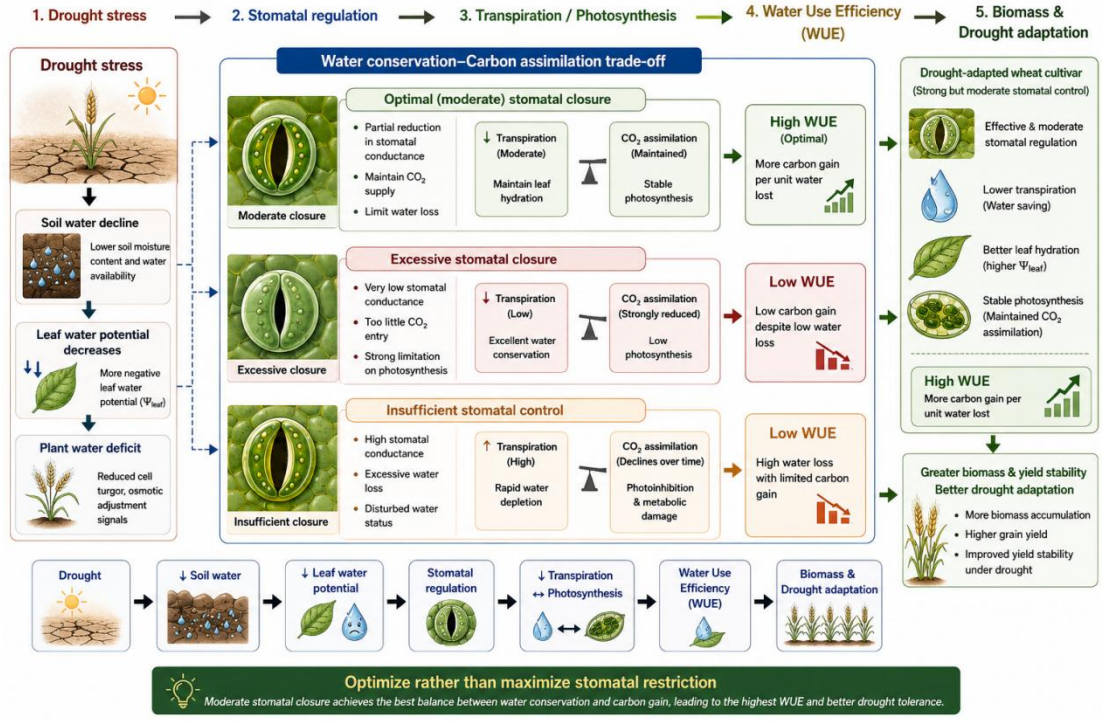

Figure 1 Trade-off between water conservation and carbon assimilation in wheat under drought stress. Moderate stomatal restriction can improve water use efficiency by reducing transpiration while maintaining relatively stable photosynthetic carbon assimilation, whereas excessive stomatal closure can restrict carbon gain and biomass accumulation. Drought-adapted wheat cultivars optimize stomatal regulation and tissue hydration to balance water conservation with productive photosynthesis

3 Changes in Leaf Photosynthetic Characteristics of Wheat under Drought Stress

3.1 Dynamic responses of gas exchange parameters

Drought stress suppresses leaf gas exchange in wheat primarily through progressive declines in net photosynthetic rate, stomatal conductance, and transpiration, and these changes often appear early during soil drying. Across experiments, stomatal conductance is typically the most sensitive gas-exchange trait, and once it falls sufficiently, limitations on CO_2 supply begin to constrain photosynthetic carbon gain more strongly (Pflüger et al., 2024). This early phase is therefore dominated by stomatal limitation rather than irreversible biochemical damage, especially under mild to moderate stress. As drought intensifies, reduced stomatal opening also weakens evaporative cooling and accelerates feedback effects on leaf metabolism, which further depresses assimilation.

With longer or more severe drought, gas-exchange inhibition becomes increasingly shaped by non-stomatal factors, including reductions in carboxylation capacity and electron transport. In wheat, this transition is often reflected by lower chlorophyll content, smaller assimilating leaf area, and rising intercellular constraints that indicate impairment beyond stomatal closure alone (Guizani et al., 2023). Genotypic differences are substantial: tolerant cultivars generally maintain higher photosynthesis and conductance under stress, whereas sensitive genotypes adopt stronger water-conserving closure at the cost of carbon uptake. After rewatering, gas exchange can recover markedly, but recovery depends on how far drought has progressed before relief.

3.2 Chlorophyll fluorescence and photosystem function

Chlorophyll fluorescence shows that drought alters the photochemical functioning of wheat leaves even when visible injury is limited. Sensitive fluorescence traits usually include declines in ΦPSII, qP, and ETR, together

with stronger non-photochemical energy dissipation as the use of absorbed light for photochemistry becomes restricted (Abdullaev et al., 2024). Consistent with this, droughted wheat often exhibits lower Fv/Fm and Fv/F0, linking reduced gas exchange with reduced photochemical efficiency of PSII. These responses indicate that the photosynthetic apparatus is not only receiving less CO_2 because of stomatal closure, but is also redistributing excitation energy toward protective heat dissipation.

However, PSII damage is usually limited during mild drought and becomes pronounced mainly under severe dehydration, when protective mechanisms are no longer sufficient (Sommer et al., 2023). Under stronger stress, wheat shows increases in F0 and decreases in Fm, Fv/Fm, qP, and ETR, while OJIP-based indices such as RC/ABS, PIabs, and PItot also decline, revealing fewer active reaction centers and lower overall PSII performance. Mechanistically, drought reduces PSII electron transport and leaves a larger fraction of absorbed energy to be handled by alternative electron sinks and cyclic electron flow, which appear to contribute to photoprotection. Fluorescence traits therefore capture both injury and acclimation, making them useful indicators for distinguishing tolerant and sensitive wheat genotypes.

3.3 Photosynthetic pigments, carbon assimilation, and photosynthetic product accumulation

Drought commonly reduces photosynthetic pigment content in wheat leaves, including chlorophyll and carotenoids, and this loss contributes directly to lower light capture and weaker carbon assimilation. Reviews and experiments converge on the point that drought-induced chlorophyll decline is associated with chloroplast damage, thylakoid disruption, and reduced activity of Calvin-cycle components such as Rubisco, which together depress atmospheric carbon fixation. At the molecular level, drought also down-regulates many wheat genes linked to PSI, PSII, light-harvesting complexes, cytochrome b6f, and ATP synthase, providing a transcriptional basis for declining pigment function and photochemical capacity (Karami et al., 2025). Because tolerant cultivars usually retain chlorophyll more effectively, pigment stability is often treated as a practical marker of drought resilience.

The reduction in leaf carbon assimilation under drought also changes downstream carbohydrate metabolism and assimilate accumulation. As photosynthesis falls, starch accumulation is often reduced, while soluble sugars and sucrose-related metabolism become more dynamic and can increase as part of osmotic adjustment or stress reallocation (Nyaupane et al., 2024). In developing wheat grains, drought during early grain filling significantly increases soluble sugars and sucrose synthase activity, while sucrose and total starch can first decline and then rebound, indicating a temporal restructuring of carbon partitioning rather than a uniform shutdown. Experimental manipulation also shows that improving stress protection can preserve chlorophyll and moderate drought-induced shifts in sucrose and starch pools, reinforcing the close coupling between leaf photosynthetic performance and whole-plant carbon economy.

4 Drought-Induced Oxidative Stress and Reactive Oxygen Species Metabolism

4.1 Production and accumulation of reactive oxygen species

Drought stress triggers a rapid increase in reactive oxygen species (ROS) production in wheat leaves because stomatal closure restricts CO_2 fixation, causing absorbed light energy and reducing power to exceed the utilization capacity of the Calvin cycle. This excess excitation energy drives electron leakage to molecular oxygen, primarily generating superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) in chloroplasts, peroxisomes, and mitochondria. Under normal conditions, only a small fraction of electrons passing through the electron transport chain incompletely reduce oxygen, but drought shifts this balance toward uncontrolled ROS generation. The resulting overaccumulation of ROS disrupts cellular redox homeostasis and initiates oxidative cascades that damage biomolecules (Panda et al., 2024).

ROS accumulation in wheat is highly dependent on drought severity and duration. Under progressive drought, ROS levels remain stable during early stress phases but increase significantly once drought exceeds a critical threshold, indicating that the ROS generation rate eventually overwhelms the plant's scavenging capacity. Drought-sensitive wheat cultivars accumulate higher ROS levels than tolerant ones, reflecting greater photosynthetic inhibition and a higher potential for oxidative damage. Tolerant cultivars mitigate ROS accumulation through enhanced non-photochemical quenching and sustained antioxidant enzyme activity, which

together prevent the oxidative burst (Moloi et al., 2024). Severe drought further accelerates ROS production by impairing PSII electron transport, leaving a larger fraction of absorbed energy to be processed by alternative electron sinks.

4.2 Membrane lipid peroxidation and cellular oxidative damage

Excessive ROS production during drought causes direct oxidative damage to cellular structures, with membrane lipid peroxidation being one of the most damaging consequences. Malondialdehyde (MDA) content is widely used as a biochemical marker for lipid peroxidation, and drought-stressed wheat consistently shows elevated MDA levels alongside increased hydrogen peroxide and superoxide generation. Drought-sensitive wheat varieties exhibit substantially greater MDA accumulation in both leaves and roots compared to tolerant varieties, indicating more severe membrane damage from uncontrolled ROS spread (Moloi et al., 2024). Tolerant varieties, by contrast, limit MDA increases through stronger enzymatic antioxidant capacity and lower tissue ROS content.

Beyond lipid peroxidation, ROS attack proteins, nucleic acids, and other macromolecules, causing base substitutions, structural protein alterations, and eventual cell death. Electrolyte leakage serves as a direct physiological indicator of this membrane disruption, and drought-stressed wheat shows elevated electrolyte leakage proportional to the severity of oxidative injury (Muslemyar & Kaya, 2025). Drought acclimation can moderate this damage; wheat seedlings exposed to initial mild stress exhibit better membrane stability and lower electrolyte leakage during subsequent severe stress compared to non-acclimated plants. Exogenous applications of antioxidants such as thiourea, selenium, and melatonin further reduce MDA and electrolyte leakage by enhancing ROS-scavenging enzyme activities.

4.3 ROS signaling and drought stress adaptation

At low concentrations, ROS function as signaling molecules that activate acclimatory and defense responses rather than causing damage. Hydrogen peroxide acts as a secondary messenger in signal transduction pathways, triggering stress-defense gene expression and coordinating adaptive responses to water deficit. ROS signaling is tightly linked to abscisic acid (ABA) pathways, calcium fluxes, and sugar sensing, positioning ROS both upstream and downstream of ABA-dependent drought signaling cascades. Maintaining ROS homeostasis is therefore essential for drought tolerance, as balanced ROS levels enable protective signaling without triggering destructive oxidative cascades (Yang et al., 2026).

Wheat exploits this signaling role through transcriptional and enzymatic networks that regulate ROS levels. Mitogen-activated protein kinase cascades, including the TaMYB2-TaMAP3K17 module, enhance drought tolerance by promoting ROS scavenging and reducing malondialdehyde accumulation under water deficit. SnRK2 protein kinases interact with catalase to modulate ROS detoxification, while MAPK6 and antioxidant gene expression respond rapidly to short-term osmotic stress (Bhanbhro et al., 2025). Drought acclimation further refines this signaling: transcription factors such as TaWRKY2 and TaNAC1 are upregulated during severe stress, priming antioxidant defenses for subsequent drought events. Together, these signaling networks allow wheat to integrate ROS signals into coordinated physiological and molecular adaptations that improve survival under recurring drought.

5 Responses of the Wheat Leaf Antioxidant System to Drought Stress

5.1 Enzymatic antioxidant defense system

Drought activates the wheat leaf enzymatic antioxidant system because excess reactive oxygen species must be detoxified before they disrupt membranes, proteins, and the photosynthetic apparatus (Nyaupane et al., 2024). The first defensive step is usually the conversion of superoxide to hydrogen peroxide by SOD, followed by H_2O_2 removal through CAT, POD, and especially APX working in coordinated sequence. This response is not random but reflects a functionally integrated network in which enzymatic antioxidants act as the main biochemical barrier against oxidative injury. In wheat under drought, activities of SOD, CAT, APX, and related enzymes therefore commonly increase as stress intensity rises.

The magnitude and stability of this induction differ strongly among genotypes and stress histories. Drought-tolerant wheat generally shows stronger increases in CAT, POD, APX, and SOD, together with lower

H_2O_2 and lipid peroxidation, whereas susceptible genotypes show weaker antioxidant activation and greater oxidative damage. Some field studies indicate that CAT can decline under severe or late-stage stress even when APX and guaiacol peroxidase remain highly responsive, suggesting that H_2O_2 detoxification shifts toward enzymes with higher substrate affinity in stressed leaves (Dvojković et al., 2023). Short-term osmotic stress also alters the expression of CAT, APX, and related antioxidant genes, showing that drought regulation occurs at both biochemical and transcriptional levels. Repeated drought exposure can further strengthen this system, since acclimated wheat seedlings maintain more coordinated antioxidant induction than non-acclimated plants under later severe stress.

5.2 AsA-gsh cycle and non-enzymatic antioxidants

The AsA-GSH cycle is a central pathway for H_2O_2 scavenging in wheat leaves under drought because it links APX-dependent peroxide removal with continuous regeneration of reduced ascorbate and glutathione pools. In this cycle, APX uses AsA to reduce H_2O_2, while MDHAR, DHAR, and GR restore the reduced state of AsA and GSH and thereby preserve antioxidant capacity across cellular compartments (Laus et al., 2021). Drought commonly stimulates this pathway, and wheat tissues often show increased APX, GR, DHAR, and MDHAR activities as oxidative pressure intensifies. This pattern supports the view that AsA-dependent detoxification is one of the most consistent biochemical features associated with drought defense.

Non-enzymatic antioxidants complement the AsA-GSH cycle by directly quenching radicals and buffering the intracellular redox environment. AsA and GSH are the core soluble antioxidants in this system, but phenolic compounds, carotenoids, tocopherols, proline, and related metabolites also contribute to ROS neutralization and membrane protection (Dvojković et al., 2023). Under drought, wheat often shows depletion of reduced AsA with parallel increases in APX activity, while glutathione turnover and GR activity rise to sustain redox cycling. Genotypic differences are again important: tolerant materials can maintain higher GSH-related buffering capacity or faster AsA recovery, whereas sensitive lines often show sharper depletion of antioxidant pools and greater oxidative injury. In some traditional cultivars, enhanced phenolic accumulation appears to add a further protective layer by limiting lipid peroxidation during early drought response.

5.3 Antioxidant systems and maintenance of redox homeostasis

Drought tolerance in wheat depends less on the absolute abundance of a single antioxidant than on the coordinated maintenance of redox homeostasis across leaf compartments. Enzymatic and non-enzymatic antioxidants work jointly to minimize, buffer, and scavenge excess ROS, thereby preventing a shift from signaling-level oxidation to destructive oxidative stress. This coordination is especially important because ROS have dual roles: at controlled levels they participate in stress signaling, but at high levels they trigger lipid peroxidation, metabolic disruption, and cell death. Accordingly, wheat drought adaptation requires both efficient ROS removal and preservation of the reduced AsA/GSH redox state that stabilizes cellular metabolism. When this balance fails, oxidative damage accumulates rapidly and leaf function declines (Duvnjak et al., 2024).

Evidence from acclimation and signaling studies shows that redox homeostasis is actively regulated rather than passively maintained. Drought-acclimated wheat preserves the ascorbate-glutathione redox pool, restricts H_2O_2 accumulation, and limits membrane damage through coordinated induction of APX and related detoxifying enzymes. ABA and H_2O_2 also interact with the AsA-GSH cycle, and inhibition studies indicate that they regulate different components of this pathway during drought stress. At the signaling level, drought-induced modules such as TaMYB2-TaMAP3K17 enhance tolerance by promoting ROS scavenging and stabilizing ROS homeostasis under water deficit (Yang et al., 2026). Together, these findings indicate that the wheat leaf antioxidant system functions as an integrated redox regulatory network, and its effectiveness is a major determinant of drought tolerance and post-stress recovery.

6 Coordinated Regulation of Photosynthetic and Antioxidant Systems

6.1 Coupling between photosynthetic electron transport and ROS production

Under drought stress, the coordination between photosynthetic and antioxidant systems begins with the tight coupling between restricted carbon assimilation and over-reduction of the photosynthetic electron transport chain.

Stomatal closure lowers CO_2 availability, decreases Calvin-cycle consumption of NADPH, and leaves more electrons without productive sinks, which promotes ROS generation through pathways such as the Mehler reaction (Vijayaraghavareddy et al., 2022). In wheat leaves, this imbalance appears as a mismatch between electron excitation and electron use, so drought-induced declines in assimilation directly increase the formation of superoxide and hydrogen peroxide in chloroplasts. The result is not simply a passive side effect of dehydration, but a mechanistic link between impaired photosynthesis and oxidative stress that intensifies as water deficit progresses.

This coupling also explains why photochemical injury deepens when excess electrons are not safely redistributed. Drought gradually decreases PSII electron transport in wheat, yet a substantial fraction of electron flow is diverted away from carbon assimilation and photorespiration, indicating activation of alternative electron sinks. At the same time, enhanced PSI cyclic electron flow helps prevent over-reduction of the PSI acceptor side and lowers the risk of oxidative stress in chloroplasts. When stress becomes severe, however, ROS accumulation can suppress PSII repair and damage core proteins such as D1, shifting the system from regulated photoprotection toward photoinhibition and structural injury. This is why the balance between electron dissipation, PSI protection, and antioxidant capacity is central to drought tolerance in wheat (Figure 2).

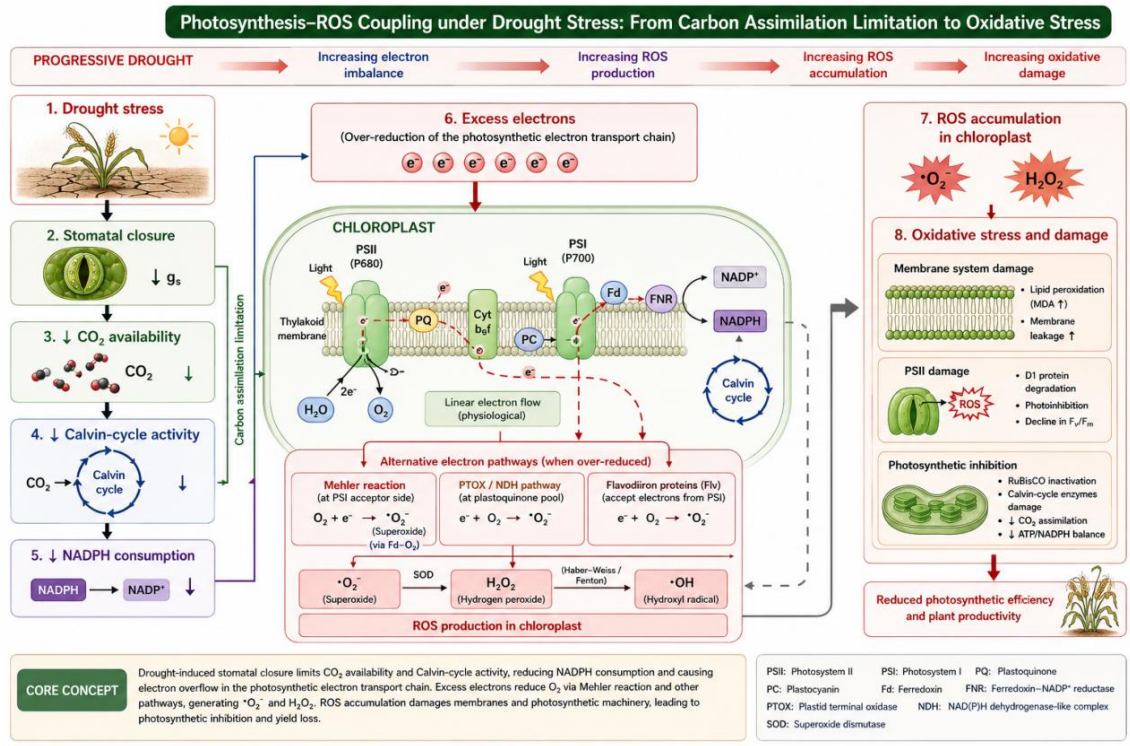

Figure 2 Coupling between drought-induced photosynthetic limitation, electron over-reduction, and reactive oxygen species generation in wheat leaves. Stomatal closure restricts CO_2 availability and decreases Calvin-cycle consumption of NADPH, thereby increasing the probability of electron over-reduction and ROS formation in chloroplasts. Progressive drought stress can consequently establish a mechanistic connection between impaired carbon assimilation and oxidative stress

6.2 Coordination between photosynthetic protection and antioxidant defense

Wheat limits oxidative damage under drought by coordinating photoprotective energy dissipation with biochemical ROS scavenging. Non-photochemical quenching rises under drought as a rapid feedback mechanism that dissipates excess excitation energy as heat and thereby restrains ROS overaccumulation before antioxidants become overwhelmed. In parallel, carotenoids contribute both to excess-energy dissipation and to direct protection of the photosynthetic apparatus, linking pigment-based photoprotection with antioxidant defense at the chloroplast level (Qiao et al., 2024). This coordination is especially important because PSII is highly vulnerable to oxidative damage, and preservation of its function depends on keeping excitation pressure and ROS production below damaging thresholds.

The evidence indicates that tolerant wheat genotypes do not rely on scavenging alone, but combine lower ROS production with stronger detoxification capacity. Higher carotenoid levels in tolerant leaves help dissipate excess excitation energy and likely restrain ROS formation at its source, while soluble sugars appear to support antioxidant capacity and redox buffering during drought. Consistent with this, droughted wheat can maintain lower ROS accumulation when NPQ increases effectively, even without disproportionately larger transcript increases of scavenging enzymes, showing that preventing ROS formation is as important as removing it. Earlier cultivar comparisons also showed that the more sensitive wheat cultivar displayed stronger xanthophyll de-epoxidation and non-radiative dissipation only after larger declines in PSII efficiency, suggesting that photoprotection can be protective yet still insufficient once stress injury has advanced. Together, these responses show that drought tolerance depends on synchronizing light-energy management with antioxidant metabolism rather than maximizing either system in isolation.

6.3 Molecular regulation, hormones, and signaling networks

At the molecular level, this coordination is governed by signaling networks that connect chloroplast redox status with hormone-mediated stress responses. Chloroplast-derived ROS act not only as toxic by-products but also as retrograde signals that alter nuclear transcription and induce antioxidant and stress-response genes. ABA is a major integrating signal in this network, because drought-responsive regulators can simultaneously enhance ABA biosynthesis or sensitivity and increase ROS-scavenging capacity, as shown by TaFDL2-1A overexpression, which increased endogenous ABA content, stomatal hypersensitivity, and SOD and GPX activities under drought. This coupling allows wheat to coordinate water-saving stomatal responses with protection of chloroplast metabolism.

Downstream of ABA perception, wheat uses interconnected signaling modules to regulate osmotic adjustment, stomatal movement, and ROS detoxification in a coordinated way. The TaPYL9/TaPP2C6/TaSnRK2.8/TabZIP1 pathway links ABA signaling to activation of TaP5CS2, TaSLAC1-1, and TaCAT2, thereby integrating proline biosynthesis, stomatal control, and ROS scavenging. Transcriptomic evidence also shows coordinated up-regulation of PP2C, SnRK2, and MAPKKK genes under drought, together with repression of LHCB1-related light-harvesting genes, which likely reduces excess light capture while strengthening antioxidant defense and stomatal regulation (Wu et al., 2026). Other regulators, including TaPPR13, further connect chloroplastic ROS homeostasis, chloroplast structural integrity, ABA signaling, and photosynthetic efficiency under water deficit. Overall, the molecular network that coordinates photosynthetic protection and antioxidant defense in wheat under drought is a redox-sensitive, hormone-linked system that optimizes survival, recovery, and productivity under limited water supply.

7 Case Studies: Photosynthetic and Antioxidant Responses of Wheat Leaves under Different Drought Conditions

7.1 Case study 1: photosynthetic responses to progressive drought stress

Progressive drought stress in wheat leaves is characterized by an early decline in stomatal conductance, followed by reductions in transpiration and net photosynthetic rate as soil water becomes increasingly limiting (Pflüger et al., 2024). In controlled progressive-drying experiments, stomatal closure responded faster than the decline in photosynthesis, indicating that the first limitation is usually diffusive rather than fully biochemical. As drought deepens, chlorophyll content and photochemical efficiency also decrease, and these changes become more evident when water deficit is prolonged or combined with additional atmospheric demand. Genotypic variation is substantial, but across cultivars the overall pattern remains consistent: declining water availability progressively constrains CO_2 uptake, leaf cooling, and carbon assimilation.

The later phase of progressive drought involves stronger impairment of the photosynthetic apparatus itself. A month-long progressive drought reduced the quantum yield of PSII photochemistry while increasing the fraction of absorbed energy released as fluorescence, showing a shift away from productive electron use as stress intensified (Jia et al., 2023). Field-capacity gradients from mild to severe drought similarly reduced net photosynthesis, stomatal conductance, relative water content, and chlorophyll retention across diverse wheat

genotypes, although tolerant materials maintained higher function under severe stress. These case studies indicate that progressive drought in wheat begins with stomatal restriction but eventually extends to pigment loss, altered PSII energy partitioning, and broader metabolic inhibition. They also show that evaluating drought response against available soil water, rather than stress duration alone, gives a clearer view of physiological thresholds.

7.2 Case study 2: antioxidant system responses to drought stress

A second case-study pattern is the strong activation of the wheat leaf antioxidant system under drought-induced oxidative stress. Water deficit increases hydrogen peroxide, malondialdehyde, and proline while stimulating the main enzymatic defenses, including SOD, CAT, and APX, and these responses become stronger as stress severity increases (Nasirzadeh et al., 2020). In a separate greenhouse study across growth stages, drought likewise increased SOD, CAT, POD, and APX activities in leaves, supporting the view that enzymatic detoxification is a core component of wheat drought defense. This antioxidant activation reflects the need to detoxify ROS generated when drought restricts carbon assimilation and disturbs cellular redox balance. At the same time, the response is not uniform across all genotypes or all enzymes, which makes antioxidant profiling useful for distinguishing different drought-response strategies.

Case comparisons further show that drought tolerance depends on the coordination, rather than the mere presence, of antioxidant defenses. Drought-resistant varieties often rely more heavily on enhanced ROS-detoxifying enzyme activity, whereas drought-sensitive genotypes can depend more on non-enzymatic antioxidant components that become particularly important during recovery. Repeated stress exposure can also prime the antioxidant machinery: in acclimated wheat seedlings, severe subsequent drought caused less H_2O_2 accumulation and membrane damage because APX activity and the ascorbate-glutathione redox pool were maintained more effectively. These findings suggest that the most informative antioxidant case studies are those that integrate ROS levels, membrane injury, osmolyte accumulation, and enzyme coordination across contrasting genotypes or stress histories. They also underline that antioxidant capacity contributes directly to preserving leaf function under drought, not merely to damage repair after stress has already occurred.

7.3 Case study 3: photosynthetic recovery and antioxidant regulation during drought-rewatering cycles

Drought-rewatering case studies show that many wheat leaf responses are reversible, but recovery depends strongly on prior stress severity and genotype. After rewatering, photosynthetic traits such as net assimilation, stomatal conductance, transpiration, and water use efficiency can show full or even over-compensatory recovery following drought, indicating substantial physiological resilience when stress has not exceeded critical thresholds (Todorova et al., 2022). This reversibility is not unlimited, however, because moderate stress tends to permit near-complete restoration of photosynthetic processes, whereas severe drought often leaves persistent impairment in membranes, ROS balance, and final productivity. Recovery therefore reflects both the extent of prior injury and the plant's ability to reactivate gas exchange, re-establish water relations, and restore redox control. In this context, fast recovery of photosynthesis is a meaningful indicator of drought tolerance rather than a simple return to pre-stress values.

Antioxidant regulation during rewatering is equally dynamic and can persist even after visible stress relief. In winter wheat, most pigment, photosynthetic, and antioxidant traits recovered effectively after stress relief within a certain range, but after longer combined stress, antioxidant activities remained elevated while oxidative damage was still not fully mitigated. Repeated drought-rewatering cycles can even enhance post-stress performance: intermittent drought increased the maximum photosynthetic rate after rewatering and strengthened stress-response indicators relative to non-primed plants, with ABA responding particularly rapidly. Lower-ranked but important recovery studies extend this picture by showing that after atmospheric-soil drought, stomatal conductance and photosynthetic rate recovered relatively quickly, whereas leaf hydraulic conductance recovered more slowly, suggesting different repair kinetics within the same leaf system (Wang et al., 2025). Taken together, these case studies show that rewatering success in wheat depends on coordinated restoration of photosynthesis, antioxidant defenses, and hydraulic function, with moderate prior stress often producing the strongest recovery capacity.

8 Agricultural Applications and Comprehensive Evaluation of Drought-Resistance Regulation in Wheat

8.1 Water management and irrigation regulation

Water management in drought-prone wheat production aims to avoid severe inhibition of photosynthesis while improving water-use efficiency through controlled deficit rather than continuous full irrigation. Across irrigation-gradient studies, mild water stress generally maintained a relatively high photosynthetic rate and sometimes even improved biomass accumulation and 1 000-grain weight compared with fully irrigated controls, indicating that moderate restriction can enhance water productivity without a major physiological penalty (Zhao et al., 2020). A similar conclusion emerged from stage-based field-capacity experiments, in which mild stress around 65%-70% field capacity improved canopy photosynthesis, assimilate allocation to grain, yield, and water-use efficiency, whereas moderate and severe stress increasingly constrained these responses. These results suggest that irrigation scheduling should target the threshold at which stomatal regulation conserves water but does not yet trigger major non-stomatal damage to the photosynthetic system.

The timing and delivery mode of irrigation are as important as total water supply. Growth-stage-based deficit irrigation sustained a high photosynthetic rate when soil relative water content was maintained at 60%-75%, and the treatment irrigated from jointing to filling achieved the best balance between water saving and acceptable yield loss in Northwest China. Under drip irrigation, mild deficit imposed at tillering or jointing increased antioxidant enzyme activity, reduced ROS and lipid peroxidation, delayed flag-leaf senescence, and improved yield relative to stronger deficit treatments, with the tillering-stage mild deficit giving the clearest agronomic advantage (Che et al., 2025). Complementary field management can strengthen this effect: ridge-furrow mulching with moderate deficit irrigation improved soil water status, raised flag-leaf photosynthesis, enhanced SOD, POD, CAT, and APX activities, and reduced oxidative damage, making it a practical water-saving strategy in semi-arid systems.

8.2 Nutritional regulation and application of exogenous substances

Adequate mineral nutrition can buffer the drought-induced decline in photosynthetic and antioxidant function in wheat leaves. Nitrogen supply is especially important because drought-stressed plants receiving moderate or high N maintain higher photosynthesis and N-metabolism activity than low-N plants, while also showing stronger SOD, POD, CAT, GR, and APX responses and better osmotic adjustment (Ru et al., 2022). At vegetative stages, higher N also helped wheat sustain leaf water potential, chlorophyll and Rubisco content, and lower lipid peroxidation under drought, indicating that nutritional regulation supports both carbon assimilation and ROS detoxification. Thus, fertilizer management should be viewed as part of drought regulation rather than a separate yield-input issue.

Exogenous substances provide an additional tool for protecting wheat leaves when drought cannot be avoided. Root-applied strigolactones enhanced the expression of antioxidant-defense, chlorophyll-biogenesis, and light-harvesting genes, and this was accompanied by higher SOD, POD, and CAT activities, increased chlorophyll, and a higher photosynthetic rate under drought (Song et al., 2023). Foliar salicylic acid, zinc, and glycine betaine reduced H_2O_2, $O_2^{\bullet -}$, MDA, and lipid oxidation while promoting antioxidant enzymes, osmolyte accumulation, and grain yield under reproductive-stage drought, with glycine betaine showing the strongest overall effect in that study. Other regulators act through related mechanisms: Zn increased SPAD, Fv/Fm, ascorbate, glutathione, phenolics, and flavonoids while reducing H_2O_2 and lipid peroxidation, and GABA increased phenolic acids and antioxidant enzyme activity, further supporting the use of exogenous compounds to reinforce redox protection under water deficit.

8.3 Drought-tolerant cultivar screening and precision agriculture applications

Drought-tolerant cultivar screening is most effective when it integrates photosynthetic, antioxidant, water-status, and yield-related traits rather than relying on any single indicator. Field evaluation in the North China Plain reduced 24 conventional traits to a smaller set of practical drought indicators, identifying canopy temperature, leaf water content, photosynthetic rate, intercellular CO_2 concentration, POD, MDA, and ABA among the most

informative variables for cultivar discrimination. Controlled screening studies similarly showed that tolerant genotypes maintain higher chlorophyll content, relative water content, membrane stability, gas exchange, and antioxidant activity than sensitive lines, confirming that drought tolerance in wheat is a composite physiological syndrome rather than a single trait (Ahmad et al., 2022). This supports selection frameworks that combine carbon-assimilation traits with oxidative-stress markers.

Precision agriculture tools now make this integrated screening scalable at breeding and field levels. Canopy temperature is already a low-cost, large-scale indicator with a negative linear relationship to grain yield during grain filling under drought. Spectral approaches further improve throughput: NDWI and the red-edge chlorophyll index closely tracked drought-tolerance indices in field trials and can serve as inexpensive alternatives for identifying tolerant genotypes, while multi-environment phenotyping showed that NDVI and MTSI are effective tools for identifying stable drought-tolerant germplasm across locations. UAV-based crop-health indices also correlated with yield in sodic rain-fed environments and successfully separated tolerant from sensitive genotypes, and sensor-based irrigation-gradient platforms validated NDVI, PRI, and fluorescence-related indices as non-destructive selection tools for breeding programs (Soares et al., 2025). Overall, agricultural regulation of wheat drought resistance is most effective when mild, stage-specific water deficit, targeted nutritional or exogenous support, and integrated phenotyping-based cultivar selection are used together. In the context of wheat leaves, these approaches converge on the same physiological objective: preserving photosynthetic capacity while strengthening antioxidant protection under limited water supply.

9 Conclusions and Future Perspectives

Drought stress imposes a coordinated physiological challenge on wheat leaves, simultaneously disrupting carbon assimilation and accelerating reactive oxygen species (ROS) accumulation. The initial response is predominantly stomatal: as leaf water potential declines, stomata close to conserve water, which restricts CO_2 influx and immediately reduces net photosynthetic rate. This stomatal limitation is the earliest diffusive barrier to photosynthesis, but it rapidly triggers downstream metabolic constraints. With restricted CO_2 fixation, the Calvin cycle slows, NADP+ regeneration fails to keep pace, and the photosynthetic electron transport chain becomes over-reduced. This excess excitation energy drives electron leakage to molecular oxygen, generating superoxide and hydrogen peroxide primarily in the chloroplasts, peroxisomes, and mitochondria. The resulting oxidative stress damages membranes through lipid peroxidation, impairs PSII repair cycles, and degrades photosynthetic pigments, creating a destructive feedback loop where impaired photosynthesis generates more ROS, and ROS further inhibit photosynthesis. Tolerant wheat genotypes break this cycle through the coordinated activation of photoprotective and antioxidant mechanisms. Non-photochemical quenching dissipates excess light energy as heat, while enzymatic antioxidants-superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase-detoxify ROS in a compartment-specific manner. The ascorbate-glutathione cycle plays a central role in maintaining redox homeostasis by continuously regenerating reduced antioxidant pools. Non-enzymatic antioxidants, including ascorbate, glutathione, carotenoids, and proline, provide additional buffering capacity. Crucially, ROS at controlled concentrations also function as signaling molecules, activating ABA-dependent and ABA-independent pathways that upregulate stress-responsive genes. This dual role of ROS-as toxic byproducts and essential signals-means that drought tolerance is not achieved by maximizing ROS elimination but by maintaining redox balance within a signaling-compatible range. The integration of photosynthetic protection, antioxidant defense, and hormone-mediated signaling networks ultimately determines whether a wheat leaf survives, acclimates, or suffers irreversible damage under drought.

Despite extensive documentation of drought-induced physiological changes in wheat, several critical gaps remain in understanding the temporal and spatial coordination of photosynthetic and antioxidant responses. Most studies measure antioxidant enzyme activities and photosynthetic parameters at discrete time points, providing only a snapshot of dynamic processes that unfold over hours to weeks. The real-time kinetics of ROS generation, antioxidant activation, and photosynthetic decline within specific subcellular compartments remain poorly resolved. Furthermore, the threshold at which ROS shift from signaling molecules to destructive agents is not clearly defined for wheat leaves under field-relevant drought conditions. This knowledge gap limits the ability to

identify precise intervention points for enhancing drought tolerance through targeted genetic or chemical strategies. A second major gap concerns the translation of controlled-environment findings to field conditions where drought interacts with heat, high vapor pressure deficit, and variable soil nutrient availability. Most mechanistic studies are conducted in growth chambers or greenhouses with uniform stress application, yet field drought is inherently heterogeneous in timing, severity, and duration. The interactive effects of drought with other abiotic stresses on photosynthetic electron transport and antioxidant redox signaling are insufficiently characterized in wheat. Additionally, the genetic architecture underlying the coordination of photosynthetic efficiency and antioxidant capacity is not fully resolved. While individual transcription factors and signaling modules have been identified, the regulatory networks that integrate chloroplast-to-nucleus retrograde signaling, ABA pathways, and antioxidant gene expression under recurrent drought cycles remain largely uncharacterized. These gaps raise key questions about how priming and acclimation memory are molecularly encoded and whether they can be reliably induced in diverse wheat genetic backgrounds.

Future research should prioritize dynamic, non-destructive monitoring of photosynthetic and antioxidant responses in wheat leaves under realistic field drought scenarios. Advances in chlorophyll fluorescence imaging, thermography, and hyperspectral reflectance offer the potential to track spatial and temporal heterogeneity in photosynthetic performance and canopy temperature at high resolution. Coupling these phenotyping tools with real-time ROS detection probes and redox-sensitive reporters would enable researchers to map the kinetics of oxidative stress and antioxidant activation at the tissue and subcellular levels. Such approaches could reveal the critical thresholds at which protective mechanisms fail and identify the earliest markers of irreversible photosynthetic damage. Integrating these physiological measurements with unmanned aerial vehicle-based multispectral imaging would further bridge the gap between leaf-level mechanisms and canopy-level drought responses in breeding nurseries and production fields. A second priority is the dissection of regulatory networks that coordinate photosynthetic protection with antioxidant defense through multi-omics integration. Combining transcriptomics, proteomics, metabolomics, and redox proteomics across time-series drought and rewatering cycles could identify the key nodes where ABA, ROS, and retrograde signaling converge to regulate both systems. Genome-wide association studies and CRISPR-based functional validation should target genes controlling the balance between ROS signaling and oxidative damage, particularly those governing the AsA-GSH cycle and PSII repair. Finally, research should explore how exogenous substances-such as strigolactones, melatonin, and glycine betaine-can be deployed under field conditions to prime antioxidant capacity and sustain photosynthesis during critical reproductive stages. Translating these findings into precision agriculture frameworks, where irrigation timing, nutrient management, and cultivar selection are optimized together, will be essential for maintaining wheat productivity under increasingly unpredictable drought regimes.

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The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of Different Pinching Treatments on Branch Formation and Flower Yield of *Chrysanthemum morifolium*

Chengbin Jiang
Tongxiang Lukang Chrysanthemum Industry Co., Ltd., Tongxiang, 314501, Zheiiang, China
Corresponding author: 331297460@qq.com

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Abstract Topping is an important cultivation practice for regulating plant architecture, branching development, and flower production in chrysanthemum. However, the effects of different topping methods on branch formation and flower yield in Hangbaiju (*Chrysanthemum morifolium* Ramat.) remain insufficiently understood. This review systematically examines the effects of topping timing, intensity, and frequency on vegetative growth, axillary bud development, canopy structure, photosynthetic performance, flower bud differentiation, and yield formation in Hangbaiju. Topping can alleviate apical dominance and stimulate axillary bud outgrowth, thereby increasing the number and complexity of primary and secondary branches. Appropriate topping may improve canopy architecture, enhance light interception and photosynthetic productivity, and promote assimilate accumulation and allocation toward reproductive organs. In contrast, excessive or improperly timed topping may delay plant development, reduce photosynthetic source capacity, and adversely affect flower bud formation and final yield. A representative case study framework is proposed to evaluate different topping treatments based on plant morphology, branching characteristics, flowering traits, and yield components. Integrating physiological responses with yield-related indicators can facilitate the identification of optimal topping strategies. Overall, moderate and appropriately timed topping is expected to provide an effective approach for coordinating branching, canopy development, and flower production, offering a theoretical basis for precision cultivation and standardized production of Hangbaiju.

Keywords Hangbaiju; Topping methods; Branch formation; Flower yield; Plant architecture

1 Introduction

Hangbaiju, a cultivated form of *Chrysanthemum morifolium*, has production value that extends beyond ornamental use because it is widely cultivated and economically important in China, while even its leaf residues contain recoverable polyphenols with antioxidant potential and value-added processing prospects (Yuan et al., 2025). More broadly, chrysanthemum has become an important commercial floricultural crop because of its uses as a pot plant, loose flower, cut flower, and landscape species, and this multifunctionality has supported rapid expansion of its production and trade in diverse regions. The commercial importance of chrysanthemum also means that cultivation is judged not only by whether plants flower, but by whether they produce the branching pattern, flower number, flower size, and harvest timing required by different markets. In practical production, this creates a need for cultivation strategies that can coordinate vegetative architecture with reproductive output, especially in forms grown for numerous harvestable capitula rather than a few large blooms (Kumar et al., 2024). For Hangbaiju, whose harvested flower heads determine much of its direct economic return, management practices that increase productive shoots and stabilize inflorescence yield are therefore closely tied to industrial development and grower profitability.

Among such practices, topping or pinching is especially important because it directly alters apical dominance and redistributes growth toward lateral buds. Chrysanthemum studies consistently show that unpinched plants tend to be taller and earlier flowering, whereas pinched plants usually become shorter, wider, and more branched (Kathad et al., 2024). The basic mechanism has been described similarly across experiments: removal of the terminal growing point suppresses apical dominance, promotes axillary bud outgrowth, and redirects assimilates toward side shoots, thereby increasing branch number and canopy spread. This architectural shift is important because flower number in chrysanthemum is strongly linked to the number of productive lateral shoots, so treatments that

enhance branching often improve flower production per plant. Quantitative studies support that pattern: pinching at 30 days after transplanting increased branches and flower yield in annual chrysanthemum, while double or repeated pinching often produced even more branches and flowers than no pinching. At the same time, the response is not one-dimensional. No pinching can advance bud initiation and flowering and often gives larger individual flowers, whereas more intensive pinching tends to delay flowering and reduce single-flower size even as total flower number rises (Mohanty et al., 2025). Some studies likewise show that double pinching maximizes flower count but not necessarily flower yield by weight or economic return, because single pinching at the right stage can outperform double pinching for total harvested mass and benefit-cost ratio). This means the agronomic value of topping depends not only on whether pinching is applied, but on how many times it is done and when it is imposed.

That variability is exactly why research on different topping methods remains necessary for Hangbaiju. Existing chrysanthemum evidence shows that the optimal pinching regime differs with genotype, production target, and associated cultivation conditions. In *Chrysanthemum morifolium* cv. Ratlam Selection, pinching at 30 days after transplanting promoted branch number and plant spread, indicating that early topping can strongly improve branch formation in the same species targeted here. In other chrysanthemum types, however, double pinching produced the highest numbers of flowers or branches, while single pinching sometimes gave the best balance among yield, flower quality, and plant form. Studies that combined pinching with growth regulators or nitrogen further indicate that topping effects interact with crop management background, affecting not only branching but also leaf area, stem thickness, flowering span, and total yield. This broader evidence suggests that a topping treatment cannot be assumed optimal across all production systems, and recommendations developed for annual chrysanthemum, spray chrysanthemum, or pot types may not transfer directly to Hangbaiju grown for capitulum harvest. A focused study on different topping methods in Hangbaiju is therefore significant for both theory and practice: theoretically, it helps clarify how manipulation of apical control shapes branch formation and yield allocation in *C. morifolium*; practically, it can identify a topping regime that improves flower-head production while balancing flowering time and plant architecture for commercial cultivation. On that basis, the present study examines the effects of different pinching treatments on branch formation and flower yield of *Chrysanthemum morifolium* to provide a more targeted foundation for efficient Hangbaiju production.

2 Biological Basis of Growth and Branch Formation in Hangbaiju

2.1 Characteristics of vegetative and reproductive growth in Hangbaiju

Hangbaiju is a perennial short-day chrysanthemum in which vegetative growth and reproductive transition are tightly connected to production performance. In chrysanthemum, substantial variation exists in plant height, spread, branching, and flowering behavior among genotypes, indicating that branch architecture is a biologically variable trait rather than a fixed cultivar feature (Bala and Kaur, 2026). In Hangbaiju specifically, flowers are borne at the tops of branches, so shoot architecture directly determines the capacity for reproductive output and final harvestable flower number.

The transition from vegetative to reproductive growth also changes the pattern of meristem activity along the shoot. In one chrysanthemum genotype that underwent floral transition, the apex shifted from vegetative growth at the early stage to floral transition and then stopped initiating new axillary buds, showing that reproductive development restructures further branch initiation potential. Photoperiod studies support the same trade-off, because conditions favoring stronger vegetative growth increased branch and leaf formation, whereas greater flower induction tended to coincide with reduced branch or leaf production as resources and developmental priority shifted toward reproduction (Yang et al., 2024).

2.2 Apical dominance and axillary bud development

The biological basis of branch formation in chrysanthemum begins with apical dominance, whereby the active shoot tip suppresses outgrowth of axillary buds below it. During vegetative growth, chrysanthemum plants show short inhibited buds near the apex, and this pattern is a direct expression of apical control over lateral development. More generally, axillary branch development proceeds through bud formation, bud release or dormancy, and bud

elongation, so the visible branch number observed after pinching reflects regulation at multiple developmental stages rather than only a single bud-break event (Figure 1).

This control system is mediated by interacting hormonal and carbohydrate signals. In chrysanthemum, release from apical dominance after floral transition coincided with decreased auxin and increased cytokinin levels in subapical buds, together with altered expression of branching regulators such as CmBRC1, CmDRM1, and CmMAX1. Broader apical-dominance research shows that auxin does not act alone, because sugars can trigger rapid cytokinin increases after decapitation, strigolactones mainly constrain later sustained growth rather than the earliest release step, and branching emerges from a network involving auxin, cytokinin, strigolactones, sugars, and gibberellins (Cao et al., 2023).

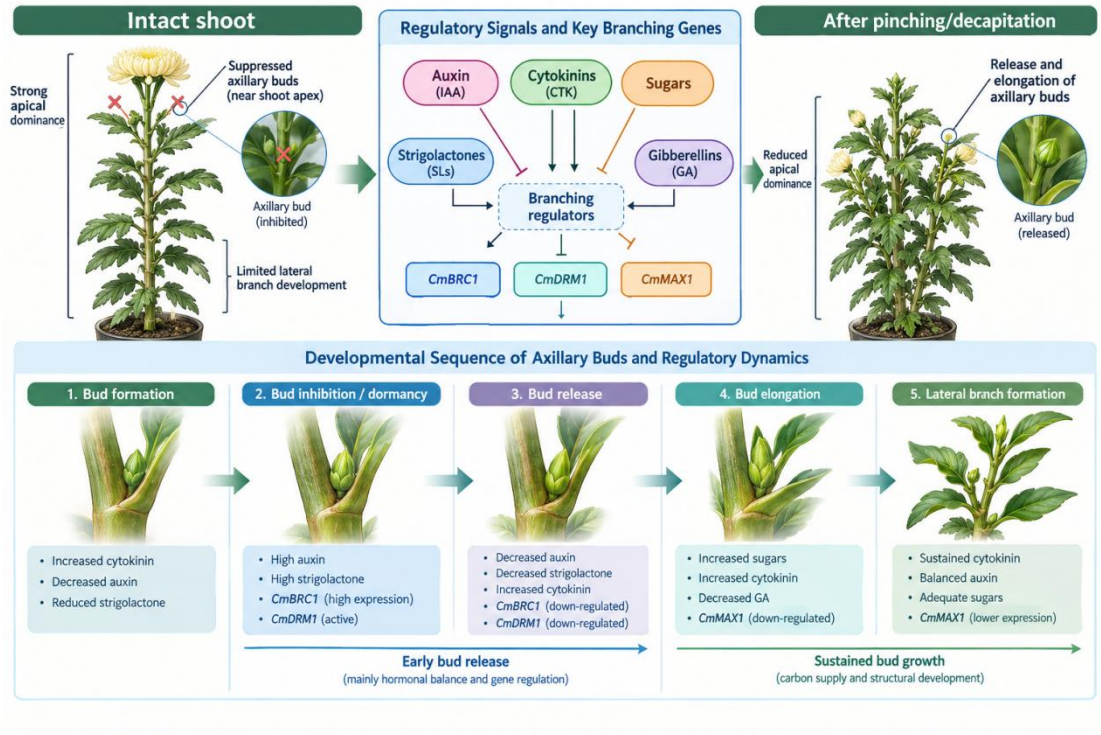

Figure 1 Biological basis of apical dominance release and axillary branch formation in chrysanthemum. The schematic illustrates the transition from shoot-apex-mediated inhibition to axillary bud release and subsequent lateral shoot elongation following pinching or decapitation, with coordinated regulation by auxin, cytokinins, sugars, strigolactones, gibberellins, and chrysanthemum branching-related genes

2.3 Relationship between branch number and inflorescence formation

The number of branches is closely tied to inflorescence formation because chrysanthemum flowers are produced terminally on shoots, making each successful lateral branch a potential flowering unit. In Hangbaiju, the importance of this link is explicit: the number of shoot branches is considered crucial for achieving high flower yield, which is why manual decapitation has traditionally been used to stimulate branching despite its labor cost. Across chrysanthemum production studies, treatments that increase branch number commonly increase flower number per plant as well, confirming that branch proliferation usually expands the population of reproductive sites (Jena et al., 2021).

The relationship is positive but not unlimited, because branch promotion also changes flowering time and the allocation of assimilates among floral units. Pinching studies show that double or repeated pinching increases primary branching and raises flower number and flower yield, but it often delays flowering and can reduce individual flower size compared with unpinched plants (Das and Patra, 2025). Nutrient and light-environment studies add that inflorescence productivity depends on how well branch formation is supported by whole-plant growth conditions, since balanced fertilization can enhance branching and flowering traits, and side lighting can markedly increase branch number while simultaneously promoting flowering and morphophysiological

performance. Overall, the biological basis of branch formation in Hangbaiju is a coordinated system in which vegetative vigor, release from apical dominance, and conversion of lateral shoots into flower-bearing branches jointly determine yield. This is why different pinching treatments can produce large differences in flower production: they intervene directly in the developmental network that links shoot architecture to reproductive output.

3 Cultivation and Regulatory Mechanisms of Different Topping Methods

3.1 Effects of single topping on plant architecture

Single topping primarily alters plant architecture by removing the terminal bud, weakening apical dominance, and redirecting growth toward lateral shoots. In potted chrysanthemum, one terminal-bud pinching reduced final plant height relative to the unpinched control while increasing leaf number, branch number, and flowers per plant, showing that a single intervention can already shift plants from vertical growth to a more compact and productive canopy. A similar architectural response was recorded in *Chrysanthemum morifolium* cv. Ratlam Selection, where pinching at 30 days after transplanting produced the maximum number of branches and the greatest plant spread among the tested pinching times.

Single topping also affects reproductive rhythm by trading earlier flowering for improved lateral development. In annual chrysanthemum, no pinching gave the largest flowers and earliest bud appearance, whereas single pinching improved canopy spread and still maintained favorable flower characters compared with more intensive pinching. Another factorial study found that single pinching at 15 days after transplanting gave the highest flower weight and earliest 50% flowering, while single pinching at 30 days ranked next and produced broader plant spread, indicating that one well-timed topping can balance architecture and yield without the stronger flowering delay associated with repeated pinching (Mohanty et al., 2025).

3.2 Effects of multiple topping and hierarchical pruning

Multiple topping strengthens the branching response by repeatedly interrupting apical dominance, thereby increasing the number of active lateral growing points and expanding canopy spread. In *Chrysanthemum indicum*, three pinching events produced the highest branch number, leaf number, plant spread, and flower number, while unpinched plants remained the tallest and least branched (Ehsanullah et al., 2021). In annual chrysanthemum, double pinching likewise produced the shortest plants but the greatest numbers of leaves, primary branches, and flowers per plant, supporting the general rule that repeated topping shifts assimilate use away from main-stem elongation and toward branch proliferation.

Hierarchical pruning effects depend not only on pinching frequency but also on which node positions are retained after each cut. In garland chrysanthemum, new-shoot emergence after the first pinching was higher than after the second, and lateral-shoot development varied systematically with node position (Matsunaga and Sasaki, 2026). Shoots arising from the first and second nodes of the main stem showed stronger subsequent regeneration, whereas regrowth from lower nodes on upper first-order laterals was weaker, indicating that hierarchical pruning should retain node positions with higher regenerative capacity rather than treating all lateral shoots as equivalent.

3.3 Interactive effects of topping intensity and timing

The effect of topping intensity depends strongly on timing, because earlier and later pinching modify different developmental phases of the crop. In garland chrysanthemum, pinching in the nursery at 20 days after sowing produced the highest branch number, plant spread, and stem girth, whereas non-pinched plants stayed consistently taller, showing that early topping can be more effective for branch induction than delayed intervention. In *C. morifolium* cv. Ratlam Selection, pinching at 30 days after transplanting outperformed pinching at 45 days for branch number and plant spread, while no pinching still led to earlier bud initiation and 50% flower opening, which highlights the trade-off between structural improvement and earliness.

Increasing topping intensity can further raise flower number, but delayed or repeated pinching often postpones flowering and may reduce individual-flower traits. In annual chrysanthemum, double pinching at 15 and 30 days gave the highest flower number, but it also significantly delayed flowering and reduced individual flower size.

Tea-applied chrysanthemum showed a related pattern at the production scale: two-time pinching produced the highest crown width, flower number, fresh mass, yield per plant, and yield per unit area, while postponing pinching reduced plant height, and a separate factorial study found that the statistical interaction between planting time and pinching was non-significant for measured flowering and yield traits, suggesting that pinching timing matters greatly but does not always interact strongly with calendar planting date (Thumar et al., 2020). Overall, different topping methods regulate Hangbaiju through a common developmental mechanism: removal of terminal dominance promotes lateral shoot growth, but the agronomic outcome depends on how many times, how early, and at which hierarchical positions the plant is pinched. For practical flower production, single topping tends to provide a balanced canopy response, whereas repeated or hierarchically optimized topping is more suitable when maximizing branch number and total flower yield is the primary goal.

4 Effects of Topping Treatments on Branch Formation and Canopy Structure of Hangbaiju

4.1 Changes in plant height, stem diameter, and internode characteristics

Topping consistently suppresses vertical elongation in chrysanthemum by removing the active apex and weakening apical dominance, so unpinched plants generally remain the tallest while topped plants become shorter and more compact. In annual chrysanthemum, no pinching produced the greatest plant height, whereas pinching at 30 or 40 days after transplanting significantly reduced height, showing that even a single topping event can redirect growth away from the main axis (Nagdeve et al., 2021). More intensive topping strengthens that effect, as thrice-pinched plants were much shorter than unpinched controls in field-grown chrysanthemum. A similar pattern appears in treatment combinations with growth regulators, where double pinching at 25 and 40 days gave the shortest plants, again indicating that repeated apex removal limits internode extension.

Stem thickening and shortening of the canopy framework often accompany reduced plant height after topping. Pinching at 40 days increased stem girth compared with no pinching in annual chrysanthemum, suggesting that reduced longitudinal growth is associated with stronger radial development. A newer study likewise found that pinching increased stem diameter while no pinching maintained the greatest height, reinforcing the trade-off between elongation and structural robustness (Mangulal et al., 2025). Internode behavior follows the same trend, because the longest internodal distance was recorded in unpinched plants, while stronger pinching shifted plants toward shorter, denser shoot architecture. Nitrogen-interaction results support this interpretation: maximum stem diameter, branch number, plant spread, and leaf area occurred under pinching with adequate nitrogen rather than under the tallest no-pinching treatment.

4.2 Formation of primary, secondary, and higher-order branches

The most direct architectural consequence of topping is the release of axillary buds, which increases the number of primary branches and creates the structural basis for secondary and tertiary branching. In *Chrysanthemum morifolium* cv. Ratlam Selection, pinching at 30 days after transplanting produced the highest branch number throughout growth, consistent with the view that early apex removal stimulates lateral bud outgrowth. In another chrysanthemum study, pinching increased primary and secondary branch numbers together with stem diameter and plant spread, showing that lateral activation is not confined to one branch order. Hormone evidence supports the same mechanism: cytokinin treatment increased chrysanthemum branch number and lateral bud diameter, whereas auxin and strigolactone treatments inhibited branching (Wang et al., 2024).

As topping intensity rises, branch hierarchy becomes progressively more complex, but the strongest increase in branch order does not always coincide with the best overall flower yield. Double pinching maximized primary branches and flower number in annual chrysanthemum, indicating that two sequential releases from apical control can amplify reproductive shoot formation. In spray-type chrysanthemum, pinching three times at 30, 40, and 50 days produced the highest primary, secondary, and tertiary branch numbers, demonstrating a clear cumulative effect of repeated pruning on branch hierarchy. Genetic and architectural analyses further show that upper primary branch number and total lateral bud production are highly variable chrysanthemum traits and are sensitive to environment, which helps explain why topping responses differ among cultivars and production systems (Sun et al., 2022). Even so, branch proliferation has practical limits, because larger individual flowers are often retained in

unpinched controls while more heavily pinched plants distribute assimilates across a greater number of flowering shoots.

4.3 Canopy structure and light energy utilization

Once topping increases lateral branching, its importance extends beyond branch number to the three-dimensional structure of the canopy. In flower crops, canopy architecture regulates microclimate, light interception, and resource-use efficiency, and traits such as plant height, branching pattern, and foliage distribution determine how light is distributed within the canopy (Nateshkumar et al., 2025). This is directly relevant to Hangbaiju because topping changes exactly those structural traits, especially canopy width and branch arrangement. Studies in chrysanthemum repeatedly show that pinching increases plant spread, with early pinching often producing the broadest canopy and repeated pinching producing the most expanded crown.

Broader canopy-physics studies clarify why these structural changes matter for light-energy utilization. Light interception and biomass production depend not only on total leaf area but on how radiation penetrates the canopy, with smaller or more open canopies often showing better penetration per unit leaf area, while excessive mutual shading reduces efficiency. Branch angle, internode spacing, and pruning pattern also alter light capture, and simulated canopies show that pruning and reshaping can improve PAR distribution and photosynthetic performance by reducing self-shading (Ran, 2025). Crop-level evidence reaches the same conclusion: optimizing canopy structure improves photosynthesis and yield by improving light-interception distribution within the stand rather than merely increasing vegetative mass. Overall, Hangbaiju topping treatments influence yield formation through a linked sequence of effects: reduced main-stem elongation, enhanced multi-order branching, and reorganized canopy light capture. The most favorable treatment is therefore unlikely to be the one that produces either the tallest plants or the greatest branch number alone, but the one that builds a canopy with efficient branch distribution and effective internal light use.

5 Effects of Topping Methods on Physiological Characteristics and Flower Bud Formation in Hangbaiju

5.1 Leaf photosynthetic characteristics and assimilate accumulation

Different topping methods influence leaf physiological status mainly by changing source–sink balance and canopy development. Pinching generally increases leaf number, and repeated pinching often produces the largest leaf display, indicating stronger assimilatory surface development than in unpinched plants (Ehsanullah et al., 2021). In combination treatments, double pinching also increased leaf area, leaf area index, and leaf area duration, showing that topping can extend the functional photosynthetic surface available for assimilate production.

These structural changes are accompanied by physiological adjustments associated with assimilate accumulation. Single pinching produced the highest leaf area in one annual chrysanthemum study, whereas double pinching maximized branch proliferation, suggesting that moderate topping can favor leaf-level assimilatory capacity while stronger topping favors redistribution into new sinks. Leaf chlorophyll intensity also increased under repeated pinching, which supports the view that topping can enhance the photosynthetic competence of remaining foliage even while delaying reproductive advance.

5.2 Carbon and nitrogen metabolism and dry matter allocation

The effects of topping on carbon and nitrogen metabolism are best understood through changes in assimilate redistribution after the shoot apex is removed. Pinching redirects carbohydrates toward axillary buds and developing lateral shoots, which explains the common increase in branching and canopy expansion after treatment. A related flowering-yield study similarly noted that in pinched plants, energy was shared among developing side branches rather than being concentrated mainly in the main stem, indicating a basic shift in assimilate allocation pattern.

Chrysanthemum dry-matter studies show why this redistribution matters for flower production. During normal growth, dry-matter accumulation continues through inflorescence development, and the developing inflorescence becomes a strong assimilate sink, while nitrogen accumulation peaks earlier and later demand is increasingly met

by remobilization from vegetative tissues. Nitrogen-management research further shows that better photosynthetic function and higher light availability can improve nitrogen-use efficiency, water-use efficiency, and allocation of biomass toward flowers, while predictive modeling confirms that aboveground dry matter and nitrogen accumulation are tightly linked across the chrysanthemum growth period (Liu et al., 2025).

5.3 Flower bud differentiation and flowering progression

Topping affects flower bud differentiation primarily by postponing the reproductive transition while increasing the number of potential flowering sites. Across chrysanthemum studies, no pinching consistently resulted in the earliest flower bud appearance, whereas repeated pinching delayed bud initiation because plants re-entered or prolonged vegetative development after apex removal. This delay can become substantial under stronger treatments, with twice or thrice pinched plants showing longer periods to inflorescence bud formation and later 50% flowering than unpinched controls.

Even though bud initiation is delayed, the later flowering phase often becomes more productive and more extended. Double pinching increased flower number and total yield in annual chrysanthemum, indicating that delayed bud differentiation is compensated by the formation of more reproductive shoots. Other studies show the same developmental trade-off: double pinching delayed flowering progression but increased branch number, flowers per stem, flowering span, and total flower yield, whereas single pinching more often balanced flowering quality and display uniformity (Kaur and Singh, 2024). Overall, different topping methods influence Hangbaiju not only through visible architectural change but through coordinated shifts in leaf function, assimilate partitioning, and timing of reproductive development. In practice, lighter topping tends to preserve earlier flowering and stronger individual-organ performance, whereas repeated topping tends to favor cumulative branch formation and total flower production.

6 Case Study: Effects of Different Topping Methods on Branch Formation and Flower Yield of Hangbaiju

6.1 Case study design and experimental treatments

A case study on different topping methods in Hangbaiju should be framed as a comparative experiment in which branch formation and flower yield are evaluated under clearly defined pinching schedules. Existing chrysanthemum studies commonly use randomized designs with replicated treatment combinations, such as factorial randomized block designs with three replications and treatments contrasting no pinching with one or more topping times after transplanting. This structure is appropriate for Hangbaiju because flower production depends strongly on branch number, and Hangbaiju cultivation has traditionally relied on repeated manual decapitation to increase shoot branching (Zhang et al., 2023).

The treatment system should include at least a control without topping, a single early topping treatment, a single later topping treatment, and one repeated topping treatment, because these contrasts recur across the chrysanthemum literature and capture the main agronomic decision points. For example, studies have compared no pinching with pinching at 30 or 40 days after transplanting, while others have extended the range to twice or thrice pinching treatments to test increasing intensity (Ehsanullah et al., 2021). Similar design logic has also been used in tea-applied chrysanthemum, where different pinching schemes were evaluated together with planting-date effects in a split-split plot field experiment, showing that topping pattern itself can be treated as a main analytical factor (Figure 2).

6.2 Responses of branch formation and flower yield

The expected response pattern in Hangbaiju is that topping reduces apical dominance, increases branch production, and then raises the number of flower-bearing shoots, although the strongest treatment is not always the one that produces the highest marketable yield. In *Chrysanthemum morifolium* cv. Ratlam Selection, pinching at 30 days after transplanting increased branch number and plant spread relative to no pinching, showing the value of timely apex removal for canopy expansion. In tea-applied chrysanthemum, two-time pinching increased crown width, flower number, fresh mass, yield per plant, and yield per unit area over no pinching, while also markedly reducing plant height.

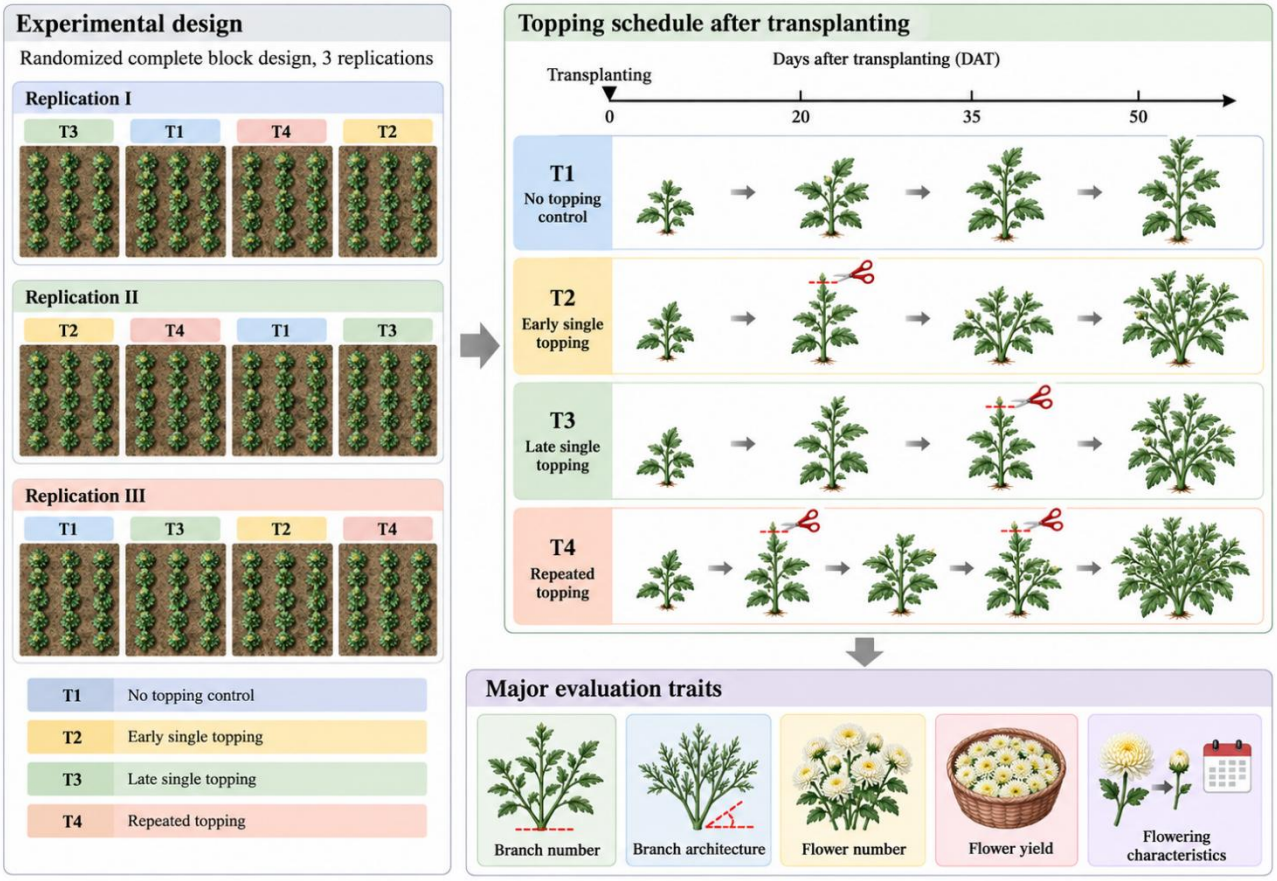

Figure 2 Experimental framework for evaluating different topping methods in Hangbaiju chrysanthemum. The schematic illustrates a replicated randomized field experiment comparing no topping, early single topping, late single topping, and repeated topping, followed by evaluation of branch formation, flowering characteristics, and flower yield

More intensive topping can further increase branch hierarchy and flower number, but it often delays flowering and may not optimize all yield components simultaneously. In annual chrysanthemum, double pinching produced the maximum primary branches and flower yield and was recommended for commercial yield maximization. By contrast, in spray chrysanthemum cv. Shyamal, thrice pinching produced the best vegetative branching traits, but the highest flower number and yield by weight were achieved with pinching twice at 30 and 40 days after transplanting (Das and Patra, 2025).

6.3 Correlations among key traits and selection of the optimal topping method

Trait relationships in a Hangbaiju case study should be interpreted around one central biological link: more effective branch formation usually increases the number of terminal flowering sites, but this advantage must be balanced against flowering delay and changes in single-flower quality. In Hangbaiju itself, shoot branch number is described as crucial for achieving high flower yield because flowers are borne at the tops of branches. Supporting evidence from annual chrysanthemum shows that pinching-induced increases in flower number are associated with vegetative traits such as increased branch number, indicating a positive correlation between branching capacity and reproductive output.

Selection of the optimal topping method should therefore prioritize the treatment that gives the best integrated balance among branch number, canopy spread, flower number, and harvestable yield rather than the tallest plants or the earliest flowering alone. Some systems favor relatively early single pinching, as shown by the Punjab study where pinching at 40 days combined with GA3 produced the highest branch number, leaf number, flower number, and yield per hectare among the tested combinations. Other systems favor moderate repeated topping, and even non-chrysanthemum evidence points in the same direction, with marigold showing maximum flower number and

branching under later pinching rather than the unpinched control (Chandio et al., 2023). For Hangbaiju, the most reasonable conclusion is that the optimal topping method is the one that maximizes productive branching without imposing excessive delay or over-fragmenting assimilates among too many shoots. A case study built on these comparisons would therefore be well positioned to identify whether single early topping or two-time topping gives the best balance for branch formation and flower yield in *Chrysanthemum morifolium*.

7 Integrated Mechanisms Underlying the Effects of Different Topping Methods on Flower Yield

7.1 Release of apical dominance and branching compensation

Different topping methods first act by removing the active shoot apex, thereby weakening apical dominance and releasing suppressed axillary buds. In chrysanthemum, bud outgrowth is strongly governed by apical control, and removal of the shoot apex or floral transition releases lateral buds from inhibition. The same mechanism is supported by decapitation studies showing that intact plants maintain repressed lateral buds because the apex sustains height growth and suppresses bud activity through weak sugar absorption and limited auxin export from buds. After decapitation, those buds break dormancy by exporting more auxin and importing more sugar, which explains why topping rapidly initiates compensatory branching rather than merely stopping stem elongation (Sun et al., 2021).

This compensatory response is regulated by a coordinated hormone–sugar network rather than by auxin alone. In chrysanthemum, release from apical dominance coincides with decreased auxin and increased cytokinin in subapical buds, together with reduced expression of branching repressors such as CmBRC1 and CmMAX1. More broadly, rapid cytokinin accumulation after tip removal appears to be associated with enhanced sugars during the early bud-release phase, while gibberellin contributes more strongly to sustained post-release growth (Cao et al., 2023). This staged view helps explain why stronger or repeated topping usually increases branch number: each removal event reopens dormant buds and renews competitive branch establishment across the canopy.

7.2 Canopy restructuring and photosynthetic productivity

Once buds are released, topping reshapes the canopy by reducing vertical dominance and expanding lateral spread, which changes light interception and the distribution of assimilatory surface. Field studies in chrysanthemum show that no pinching generally maintains the greatest plant height, whereas pinching at 30-40 days after transplanting increases branch number, stem girth, or plant spread depending on treatment timing. Repeated pinching intensifies this structural shift, producing shorter but wider and more highly branched canopies than the control (Ehsanullah et al., 2021). Because canopy architecture governs internal light distribution and resource-use efficiency, these morphological changes are not only geometric but functional for biomass production and flower formation.

The physiological benefit of canopy restructuring depends on whether added branches improve productive light capture more than they increase self-shading. Moderate canopy closure in edible chrysanthemum improved photosynthesis-related traits and increased flower yield, showing that a partially moderated light environment can enhance rather than reduce productivity. In Hangbaiju, branch-promoting regulation by 6-BA plus Pro-Ca increased leaf chlorophyll content and raised flower yield, indicating that architectural control and leaf physiological status can improve together during the floral growth phase. Topping therefore raises yield most effectively when it creates a canopy with enough lateral leaf area and branch spread to intercept light efficiently without imposing excessive mutual shading or an overly long vegetative delay.

7.3 Coupling mechanisms of branch formation, flower bud differentiation, and yield formation

The yield effect of topping ultimately depends on how newly formed branches are converted into reproductive shoots. In Hangbaiju, flower yield depends strongly on shoot branch number because flowers are borne terminally, so each additional successful branch can become an added inflorescence-bearing unit. Across chrysanthemum genotypes, branching and flowering traits vary together, and highly branching material can also show high flower number per plant, confirming that reproductive output is closely tied to shoot architecture rather than to stem elongation alone (Bala and Kaur, 2026). At the developmental level, axillary meristem formation is itself a

decisive step in branching capacity, and KNOX genes appear to promote bud formation partly by enhancing the cytokinin pathway while suppressing auxin and gibberellin pathways.

The key trade-off is that topping usually increases the number of reproductive sites while delaying floral transition and redistributing assimilates among more sinks. Double or repeated pinching often increases flowers per plant and total yield, but unpinched plants typically initiate flower buds earlier and flower sooner. This developmental coupling is consistent with chrysanthemum flowering regulators: CmTFL1c delays flowering, promotes axillary bud formation, and alters inflorescence architecture, showing that branching and reproductive timing are mechanistically linked rather than independent traits. Under controlled photoperiods, the same balance reappears agronomically, with no pinch favoring earlier bud initiation and double pinch favoring more branches and flowers, so the best topping method is the one that optimizes branch-derived flowering sites without delaying harvest beyond the production goal. Taken together, different topping methods affect flower yield through an integrated sequence of bud release, canopy reconstruction, and reproductive conversion of lateral shoots. For Hangbaiju, the most productive treatment is therefore not simply the one with the strongest branching response, but the one that best coordinates branching compensation with efficient canopy function and timely flower-bud formation.

8 Production Applications and Optimized Cultivation Strategies

8.1 Selection of topping methods for different production objectives

For production systems targeting early flowering and larger individual flowers, lighter topping or no topping is generally more suitable than repeated pinching. In annual chrysanthemum, no pinching gave the earliest flower bud appearance and the largest flower diameter, even though branch number and total flower number were lower than under stronger pinching treatments. A similar trade-off was reported in Dendranthema grandiflorum cv. Pusa Shwet, where no pinching produced the longest plants, the longest internodes, and the earliest bud appearance, while double pinching increased flowers per stem (Kaur and Singh, 2024). For balanced commercial performance, single pinching often remains a practical compromise, because single pinching at 30 days after transplanting gave satisfactory flower characters in one study, whereas single pinching at 40 days improved shelf life in spray chrysanthemum.

When the objective is to maximize branch number, flower number, and loose-flower yield, repeated pinching is usually superior. Double pinching increased flower number and yield in several annual chrysanthemum studies, and was explicitly recommended for commercial cultivation where total production is the main target. In spray chrysanthemum, however, the agronomic optimum was not always the most severe treatment: thrice pinching produced the strongest vegetative branching response, but pinching twice at 30 and 40 days gave the highest flower number and yield by weight. For *Chrysanthemum morifolium* cv. Ratlam Selection, pinching at 30 days after transplanting also improved branch number and plant spread, supporting the use of an earlier, moderate topping regime when canopy expansion is prioritized without excessive flowering delay.

8.2 Synergistic regulation of topping, planting density, and water and fertilizer management

Pinching responses are strongly shaped by planting density, so topping should be selected together with spacing rather than as an isolated treatment. In annual chrysanthemum, wider spacing increased stem girth and branching, whereas the highest flower yield per hectare was obtained at 45 × 30 cm combined with pinching at 30 days after transplanting. In *C. morifolium* cv. Ratlam Selection, wider spacing plus pinching at 30 days increased branches and plant spread, while closer spacing accelerated bud initiation and 50% flowering, indicating that density modifies whether the crop favors vegetative expansion or earlier reproductive progression. Planting date also changes the economic optimum, because the highest B:C ratio in one staggered-planting study was obtained with first-week-of-October planting and pinching at 30 days, even though the highest branch number occurred under double pinching.

Fertilizer and growth-regulator management further determine how effectively topped plants convert extra branches into yield. Standard field protocols in chrysanthemum commonly split nitrogen, applying part basally and the remainder around 30 days after transplanting, which aligns nutrient availability with the active regrowth phase after pinching. Nitrogen-response data show that pinching at 20 days combined with 150 kg·N·ha^{-1}

maximized stem diameter, branching, leaf area, and flower yield, indicating that topped plants benefit from stronger nutrient support once apical dominance is removed (Thakare et al., 2020). Comparable synergy appears with growth regulators: double pinching with triacontanol improved vegetative and floral parameters in cv. Pusa Kesari, while GA_3 at 300 ppm combined with pinching at 40 days improved leaves, flowers, and yield per hectare in Punjab conditions.

8.3 Simplified and precision pruning technologies for Hangbaiju

For Hangbaiju, the main production challenge is that branch promotion has traditionally depended on repeated manual decapitation, which is labor-intensive, costly, and associated with higher disease risk. This makes simplified pruning a practical necessity rather than only a refinement of crop management. Because Hangbaiju flowers are borne at branch termini, any simplified pruning method must still preserve high branch numbers if yield is to be maintained. A useful production direction is therefore to replace some manual topping operations with growth-regulating strategies that mimic the branching effect of repeated decapitation while reducing labor inputs.

Current evidence suggests that precision branch regulation in Hangbaiju can be achieved by combining fewer physical interventions with targeted chemical control. In pot-grown Hangbaiju, foliar application of 6-BA combined with Pro-Ca increased flower yield, and the highest yield was recorded in the 6-BA5 + Ca100 treatment in both experimental years. This approach is attractive for precision cultivation because the untreated control still relied on three manual decapitations, whereas the regulator-based system was explicitly tested as an alternative branch-management technology. Beyond yield, Hangbaiju stems are themselves a usable by-product rich in phenolic compounds, so pruning systems that standardize shoot removal could also improve residue collection and downstream valorization (Zhu et al., 2024). Overall, optimized Hangbaiju production depends on choosing the topping method that matches the production objective, then integrating it with spacing, nutrient scheduling, and labor-saving branch regulation. For most commercial loose-flower systems, moderate or repeated topping appears most effective for maximizing productive branching, but simplified precision approaches are likely to be the most sustainable long-term strategy for Hangbaiju.

9 Conclusions and Perspectives

Different topping methods consistently reshaped Hangbaiju architecture by weakening apical dominance and promoting axillary bud outgrowth. Single topping usually produced a moderate increase in branch number and canopy spread, while repeated topping generated more primary and secondary branches and a denser shoot system. The overall pattern shows that topping intensity controls how strongly vegetative growth is redirected from the main stem to lateral shoots. The branching response also depended on topping timing. Early topping tended to trigger earlier and stronger lateral bud release, whereas later or repeated topping often produced a more compact but more highly branched plant type. These differences indicate that branch formation is not determined by topping alone, but by the interaction between pruning intensity, developmental stage, and cultivar response.

Topping influenced flower yield mainly by changing the number of flowering branches and the balance between vegetative growth and reproductive development. Moderate topping often improved total flower number and yield because it increased the number of terminal flowering sites without excessively delaying flowering. In contrast, no topping favored earlier bud initiation and larger individual flowers, but generally produced fewer flowers per plant. Repeated topping usually increased total yield more strongly than single topping when the production goal was loose-flower output. However, too many topping events could prolong the vegetative phase and redistribute assimilates across too many shoots, which may reduce uniformity or delay harvest. The best treatment therefore depends on whether the target is early flowering, large flowers, or maximum total flower yield.

Current evidence still has several limitations. Most studies focus on a limited number of topping schedules, cultivars, and environments, so results cannot be generalized to all Hangbaiju production systems. Many reports also emphasize morphology and yield, while fewer examine hormone regulation, carbon allocation, canopy photosynthesis, and long-term field performance in an integrated way. Future research should combine topping with plant density, fertilization, irrigation, and growth-regulator management to define optimal production

packages. More work is also needed on simplified and precision pruning methods that reduce labor while preserving yield. At the mechanistic level, studies integrating transcriptomics, hormone profiling, and canopy physiology would clarify how topping regulates branch development and floral formation.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Computational Analysis of Growth Characteristics and Active Compound Accumulation in Zhejiang Medicinal Plants

Weiduo Liu [1,2]
1 Zhejiang zhongxin Medical Plant Seed Co., Ltd, Hangzhou, 311402, Zhejiang, China
2 Zhejiang Agronomist College, Hangzhou, 310021, Zhejiang, China
Corresponding author: 454006383@qq.com
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Abstract Zhejiang Province is recognized as one of the important regions for medicinal plant diversity in China, with abundant resources characterized by unique ecological adaptability and rich accumulation of bioactive compounds. However, the growth performance and medicinal quality of these plants are strongly influenced by complex interactions among genetic background, environmental conditions, cultivation practices, and metabolic regulation processes. Recent advances in computational biology, artificial intelligence, and multi-omics technologies provide new opportunities for systematically investigating plant growth dynamics and active compound accumulation. This review summarizes the biological characteristics and developmental patterns of Zhejiang medicinal plants and discusses computational frameworks integrating phenotypic data, environmental parameters, metabolomics, transcriptomics, and machine learning approaches. Particular emphasis is placed on computational modeling strategies for predicting growth traits, identifying key regulatory factors, and elucidating metabolic pathways associated with bioactive compound biosynthesis.Furthermore, advanced technologies, including remote sensing-based digital phenotyping, network biology, and deep learning models, are evaluated for their potential applications in precision cultivation and medicinal quality optimization. A case study framework is presented to demonstrate how machine learning models can integrate environmental variables, growth indicators, and metabolite profiles to predict plant productivity and medicinal compound accumulation.Despite significant progress, challenges remain in data standardization, model interpretability, and the integration of computational predictions with experimental validation.Future development of artificial intelligence-driven platforms, digital twins, and multi-scale biological models will facilitate sustainable cultivation, resource conservation, and quality improvement of Zhejiang medicinal plants.This computational perspective provides a theoretical and technological foundation for advancing intelligent medicinal plant research and precision herbal medicine production.

Keywords Zhejiang medicinal plants; Computational biology; Machine learning prediction; Active compound accumulation; Multi-omics integration

1 Introduction

Medicinal plants form the material foundation of traditional Chinese medicine and remain increasingly important for health care, industry, and regional bioeconomies. At the same time, sustainable development of this resource base is constrained by declining wild populations, uneven cultivation practices, and persistent variation in the quality of cultivated materials. These challenges are especially relevant in provinces such as Zhejiang, where medicinal-plant richness, industrial potential, and geographic heterogeneity coexist, yet development of planting systems, markets, and enterprise distribution remains imperfectly aligned with resource endowment (Shan et al., 2021). Environmental variation further complicates production because soil, climate, and terrain strongly influence plant growth and the accumulation of active ingredients, often causing substantial differences within the same species across habitats and medicinal parts. For Zhejiang medicinal plants, this means that evaluating growth vigor alone is insufficient; robust assessment must also address phytochemical quality, environmental responsiveness, and the interaction between agronomic performance and medicinal value.

Recent empirical work has reinforced the importance of linking growth traits with bioactive compound accumulation through quantitative analysis. In *Polygonatum cyrtonema*, a medicinal and edible species of high economic relevance in southern China, multi-site provenance testing in Zhejiang showed highly significant

differences among provenances, sites, and provenance-by-site interactions for all measured growth and medicinal traits. The same study reported broad phenotypic and genotypic variation, high repeatability for many traits, and positive associations between growth characteristics and key medicinal components, indicating strong potential for selection and breeding. Geo-climatic analyses further suggested that metabolite accumulation is not random: saponin content increased with altitude and temperature, whereas flavonoid accumulation was promoted under drier conditions (Cheng et al., 2026). Such findings underscore a central problem for medicinal plant research in Zhejiang: growth traits, environmental adaptation, and active compound accumulation are tightly coupled, but their relationships are multidimensional and difficult to resolve using conventional descriptive methods alone.

Advances in computational approaches now provide a practical framework for addressing this complexity. Reviews of medicinal plant informatics show that computational research has expanded from molecular docking and molecular dynamics simulation to artificial intelligence, including artificial neural networks, deep neural networks, and related machine-learning tools that reduce the time and cost of conventional screening. More recent work emphasizes that machine learning is particularly valuable when medicinal plant quality must be inferred from multi-source data, because single analytical techniques often fail to capture the multi-component nature of medicinal materials and their environmental responsiveness.Parallel advances in sequencing and analytical chemistry have also widened the computational toolbox: RNA-seq and related technologies can identify genes involved in biosynthetic pathways, while spectroscopy and chromatography generate the metabolite-level data needed for integrated modeling (Singh et al., 2022). At a higher systems level, emerging approaches such as AI-driven multi-omics integration and digital twin modeling seek to connect real-time or high-dimensional data with predictive models of growth dynamics and metabolite yield, although challenges remain in causal inference, interpretability, and cross-species generalization (Chen et al., 2026).

Against this background, this review aims to synthesize current knowledge on how computational analysis can be used to characterize growth characteristics and active compound accumulation in Zhejiang medicinal plants, with particular attention to the integration of ecological, phenotypic, phytochemical, and multi-omics information. The conceptual framework adopted here treats medicinal plant quality as an emergent property of interacting biological and environmental layers, rather than as a single trait measured in isolation (Chen et al., 2026). Accordingly, the review is organized around three connected objectives: first, to summarize the sources of variation in growth performance and secondary metabolite accumulation in Zhejiang-relevant medicinal plants; second, to examine computational methods capable of modeling these variations across scales, from trait statistics to network and AI-based prediction; and third, to identify how such methods can support germplasm evaluation, cultivation optimization, quality control, and sustainable resource use (Cheng et al., 2026). By combining regional cultivation evidence with advances in computational biology, this review positions Zhejiang medicinal plants as a model system for data-driven modernization of medicinal plant research and industry.

2 Biological Characteristics and Growth Dynamics of Zhejiang Medicinal Plants

2.1 Diversity, distribution, and habitat adaptation

Zhejiang medicinal plants should be understood within the broader biogeographic pattern of Chinese medicinal flora, in which richness is concentrated in central and southern China and shaped by fine-scale environmental heterogeneity. High-resolution national mapping showed that a small fraction of grid cells contains most medicinal plant diversity, while endemic medicinal plant richness is positively associated with contemporary precipitation and altitudinal range, both of which are highly relevant to Zhejiang's humid subtropical and topographically varied landscapes. This pattern suggests that Zhejiang's mountainous terrain, dissected watersheds, and strong local climatic gradients likely promote species coexistence, ecological specialization, and habitat partitioning among medicinal taxa rather than supporting a uniform regional flora.

Habitat adaptation in Zhejiang also depends on how populations respond to fragmentation, geographic isolation, and local environmental filters. A Zhejiang-focused population genomic study of *Coptis chinensis* var.*brevisepala* identified Zhejiang as the core distribution area of the species and found strong population differentiation largely driven by geographic isolation, with ultraviolet radiation and low temperature contributing to fine-scale

divergence. Complementary evidence from subtropical mountain forests in nearby Fujian showed clear elevational replacement of medicinal plant communities and stronger environmental effects on shrub and herb β-diversity than on tree-layer diversity, indicating that medicinal plant habitat adaptation in southeastern China is strongly stratified across elevation and vegetation layers.

2. 2 Growth phenology and developmental characteristics

The growth dynamics of Zhejiang medicinal plants are inherently seasonal, and their developmental trajectories are best interpreted through standardized phenological frameworks. Broad phenological theory shows that plants time leafing, flowering, fruiting, and dormancy in ways that reflect adaptation to local environments, while long-term phenology linked with molecular and climate data can improve prediction of future shifts.For medicinal plant research, this matters because the timing and duration of developmental stages determine not only biomass accumulation but also the windows during which organs with pharmacological value are formed, mature, and harvested.

Standardized BBCH-based studies provide practical models for describing these developmental sequences in medicinal species. In *Astragalus membranaceus* var. *mongholicus*, phenological development was organized into distinct vegetative, reproductive, and senescence stages, and the chronology of these stages was linked to accumulated thermal time through growing degree days. Mechanistic developmental evidence from first-year *Panax ginseng* further showed that storage-root thickening follows a stage-specific program in which early growth is associated with auxin, gibberellin, and nitrate signaling, middle growth with cell division and wall biogenesis, and late growth with jasmonic-acid-associated preparation for dormancy.

2.3 Environmental regulation of growth performance

Environmental regulation of medicinal plant growth in Zhejiang is likely to be multidimensional, because temperature, light, water, soil conditions, and nutrient supply act together on both growth and phytochemical traits.Reviews across medicinal species show that environmental optimization can substantially improve plant growth and metabolite synthesis, while ecologically limiting factors such as temperature, lighting, soil water, salinity, and fertility alter both physiological performance and secondary metabolism (Pant et al., 2021). For Zhejiang production systems, this means that growth performance cannot be evaluated independently of environmental management, especially where high humidity, seasonal rainfall, and mountainous microclimates create sharp local differences in resource availability and stress exposure.

More recent cultivation studies indicate that environmental effects are not only strong but also highly context dependent. Controlled-environment systems can improve the consistency, concentration, and yield of bioactive phytochemicals by precisely regulating light, carbon dioxide, temperature, humidity, nutrients, and airflow, whereas climate-related stresses can reshape plant morphology, physiology, and secondary metabolite production in less predictable ways under field conditions. Experimental evidence also shows that microclimate manipulation can shift growth-quality tradeoffs: in medicinal crops grown under dynamic agrivoltaics, heavy shade reduced biomass in most species but increased essential oil yield and sharply lowered evapotranspiration, highlighting the need for computational models that optimize both growth performance and active compound accumulation rather than maximizing yield alone.

3 Computational Frameworks for Growth Trait Analysis in Medicinal Plants

3.1 Data acquisition and multi-source dataset integration

Computational analysis of growth traits in Zhejiang medicinal plants depends first on building datasets that combine field observations with remote sensing and chemical measurements.In medicinal plants, growth and quality vary strongly with environment and across medicinal parts, so single-source measurements often fail to capture the biological basis of active-compound accumulation. Reviews of medicinal-plant monitoring therefore emphasize integrated satellite-, UAV-, and ground-based sensing as the main route toward dynamic growth retrieval, stress monitoring, and non-destructive quality evaluation.At the operational level, multi-source acquisition increasingly joins spectral imagery with synchronous phenotypic sampling.In *Glycyrrhiza uralensis*, UAV multispectral images were collected together with plant height, tiller number, SPAD, and nitrogen content,

showing how field and aerial observations can be aligned into one growth-monitoring framework (Zhang et al., 2025). More broadly, multimodal remote sensing improves crop monitoring because different sensing modalities complement one another and provide a more comprehensive representation of plant growth than single devices alone.

For Zhejiang medicinal plants, dataset integration should also be spatially explicit and cultivation-oriented.Machine learning combined with GIS can organize soil, subregion, and medicinal-plant information to identify suitable cultivation zones and support targeted management strategies (Roopashree et al., 2024). At the same time, UAV-based high-resolution imaging enables plant-level monitoring across the growing season, making it possible to estimate maturity and detect growth problems early enough for intervention (Vigneault et al., 2023). A further development direction is extending data integration beyond morphology into biochemical and omics layers.AI-driven multi-omics workflows are designed to integrate heterogeneous biological datasets so that fragmented information can be assembled into metabolic networks relevant to compound biosynthesis (Figure 1) (Chen et al., 2026). This is especially important in medicinal plants because multi-source data fusion has been shown to improve holistic quality evaluation and prediction compared with single analytical methods.

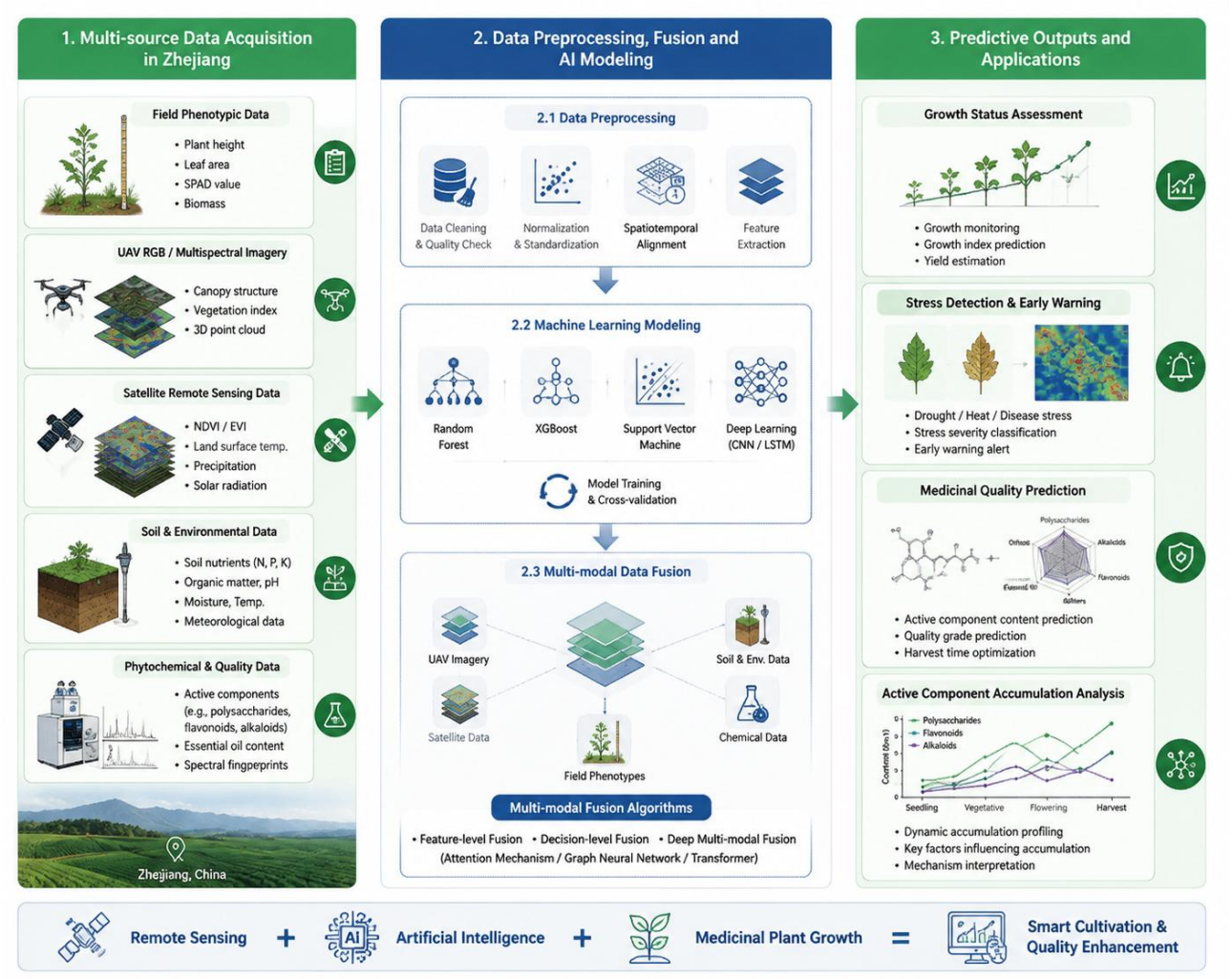

Figure 1 Multi-source data integration framework for computational analysis of growth traits and quality evaluation in Zhejiang medicinal plants

3.2 Machine learning and statistical modeling of growth characteristics

Once multi-source datasets are established, machine learning and statistical models become the core tools for extracting growth patterns and trait relationships.In medicinal plants, these methods help analyze the joint effects

of genetics, environment, and cultivation conditions, and can support decisions on harvesting time, irrigation, fertilization, and genotype selection (Roopashree et al., 2024). More generally, plant phenotyping research shows that machine learning can reduce the bottleneck created by large high-throughput datasets by linking extracted features to measurable phenotypes through classification, regression, and prediction.Model choice depends strongly on sample size, task complexity, and the need for interpretability. Traditional approaches such as PLSR, SVM, and decision-tree methods remain useful when training data are limited because they are interpretable and computationally modest, although they rely on manual feature engineering and capture nonlinear structure less effectively. By contrast, neural-network-based models and related modern phenomics frameworks have improved the precision of long-term forecasting and spatiotemporal pattern recognition in plant-growth prediction tasks (Debbagh et al., 2025).

Recent empirical studies confirm that nonlinear models can accurately estimate medicinal-plant growth indicators. In *Glycyrrhiza uralensis*, BP, SVM, and RF models were used to predict phenotypic indicators and yield, and integrated modeling with multiple indicators substantially improved performance (Zhang et al., 2025). Similar advantages appear in broader crop phenotyping, where deep learning models trained on time-series imagery have predicted future root and shoot growth with performance close to expert annotation and adaptability across plant species.Statistical and machine-learning models are also increasingly used to connect growth traits with metabolite accumulation.ANN-based and neurofuzzy approaches have been proposed as effective tools for modeling multifactorial processes that influence phenolic-compound production, while improving interpretability through rule-based simplification (García-Pérez et al., 2020). Reviews of genotype-to-phenotype prediction likewise argue that machine learning can outperform conventional statistical tools in high-dimensional settings because it can extract features from genetic, environmental, and image data more flexibly.

3.3 Artificial intelligence-based prediction and decision support systems

Artificial-intelligence-based prediction systems extend trait analysis into practical decision support by converting monitoring results into cultivation recommendations.In medicinal-plant production, this is especially valuable because AI can optimize not only biomass growth but also the accumulation of pharmacologically important secondary metabolites across variable conditions (Chen et al., 2026). Recent reviews of medicinal herb breeding therefore frame AI as a platform technology spanning multi-omics integration, trait optimization, intelligent monitoring, and genotype-environment-management interaction. At the farm-management level, decision support is moving from empirical adjustment toward real-time, demand-oriented control. UAV-guided monitoring in *Glycyrrhiza uralensis* was used to support variable irrigation and nitrogen topdressing, illustrating how integrated phenotyping can inform immediate water-nutrient management decisions (Zhang et al., 2025). Comparable work in plant-scale UAV monitoring has shown that logistic growth curves and dashboard-based reporting can project maturity before harvest and guide field interventions under heterogeneous conditions (Vigneault et al., 2023).

A second major application is predictive optimization of active-compound production.AI-based metabolite research shows that ANN models can learn nonlinear relationships among growth regulators, nutrient composition, light, elicitors, and metabolite accumulation, allowing prediction of optimal culture conditions without exhaustive experiments. This shift is important because conventional in vitro metabolite production is often irreproducible due to genetic variability, environmental fluctuation, and pathway regulation, all of which favor data-driven optimization (Srivastava and Bharadvaja, 2026). Future intelligent systems for Zhejiang medicinal plants will likely combine explainable prediction with broader biological and agronomic knowledge. Reviews of AI in medicinal-plant research emphasize not only phytochemical profiling and predictive modeling, but also the integration of AI with traditional ethnobotanical expertise to improve agricultural output and conservation. At the same time, future phenomics frameworks are expected to benefit from tighter integration of domain knowledge with data-driven methods and from more comprehensive standardized datasets, which should improve robustness in real cultivation settings (Debbagh et al., 2025).

4 Computational Analysis of Active Compound Accumulation Mechanisms

4.1 Biosynthetic pathways and regulatory networks of bioactive compounds

Computational analysis of active compound accumulation in Zhejiang medicinal plants begins with reconstructing biosynthetic pathways and the regulatory networks that control them.Recent reviews show that omics platforms now enable comprehensive mapping of biosynthetic pathways, regulatory circuits, and spatial chemical distributions, which has made pathway discovery in medicinal plants far more systematic than earlier gene-by-gene approaches (Latif and Nawaz, 2025). This is important because medicinal metabolites are produced through complex pathways whose full elucidation is necessary for predicting metabolic switches and building sustainable production strategies (Chen et al., 2026). A central advance has been the move from simple correlation to network-based inference of regulatory control. Multi-omics studies can link transcripts, metabolites, proteins, and chromatin features into layered networks that identify co-regulated genes and likely regulatory nodes, while newer AI-enabled approaches aim to infer missing links and pathway bottlenecks more directly. Evidence from medicinal plants supports this framework: transcriptomic analysis in *Cibotium barometz* identified tissue-specific key enzymes and transcription-factor correlations for lignin and flavonoid pathways, while integrated profiling in *Trichosanthes kirilowii* highlighted Tk_ERF4 and related regulators as coordinators of tissue- and stage-specific bioactive compound biosynthesis (Zhang et al., 2025; Gao et al., 2026).

4.2 Metabolomics and multi-omics integration for compound prediction

For compound prediction, metabolomics serves as the most direct layer because metabolites are the executors of gene function and the final products of pathway activity. Metabolomics combined with other omics can reveal metabolite-gene associations at the whole-genome level, identify enzymes that control production or transformation, and provide a systems-level basis for predicting which compounds accumulate in specific tissues or developmental stages. In medicinal plants, this integrated strategy is increasingly viewed as the most effective route to uncover complex specialized-metabolite pathways that cannot be resolved by single-omics data alone.

Prediction becomes more powerful when integration is spatiotemporal rather than static.Transcriptomics and metabolomics provide complementary information by associating temporal and spatial gene-expression patterns with metabolite abundance, and time-series regulatory analysis is especially useful for resolving dynamic pathway components (Singh et al., 2022). Recent medicinal-plant work confirms this value: integrated multiomics in *Trichosanthes kirilowii* resolved tissue-specific accumulation of cucurbitacin B, terpenoids, and flavonoids and identified the color-changing fruit stage as a critical window for secondary metabolism, while spatial multi-omics in other systems is expected to clarify synthesis, transport, and accumulation at cell-level resolution (Wu et al., 2025; Gao et al., 2026).

4.3 Environmental and agronomic regulation of active compound accumulation

Active compound accumulation is also shaped by environmental and agronomic regulation, so computational models must account for external drivers rather than treating metabolism as genetically fixed. Broad reviews show that secondary-metabolite accumulation responds strongly to light, temperature, soil water, fertility, salinity, and other environmental factors, and even a change in one factor can alter metabolite content under otherwise similar conditions (Pant et al., 2021). This sensitivity explains why prediction models for Zhejiang medicinal plants should incorporate field microclimate, soil status, and developmental context when estimating active compound accumulation.

Mechanistically, environmental regulation acts through stress and signaling networks that alter biosynthetic gene expression and metabolite flux. Stress conditions elevate reactive oxygen species, activate MAPK cascades, and trigger hormonal pathways involving salicylic acid, jasmonic acid, ethylene, and abscisic acid, which then regulate transcription factors and biosynthetic modules linked to specialized-metabolite accumulation. Light is one of the clearest examples: different light qualities, intensities, and photoperiods modulate pathway activity through photoreceptor-mediated signaling and circadian regulation, making light management a plausible lever for directed enhancement of medicinal compounds under controlled or precision cultivation systems (Wu et al., 2025).

5 Advanced Computational Technologies for Zhejiang Medicinal Plant Research

5.1 Remote sensing and digital phenotyping technologies

Remote sensing and digital phenotyping are becoming core technologies for medicinal plant research because they replace labor-intensive, subjective field inspection with scalable and dynamic observation.Reviews of medicinal plant cultivation show that remote sensing addresses major limits of conventional methods, while broader assessments indicate that integrated satellite-, UAV-, and ground-based sensing can already support distribution mapping, growth retrieval, stress monitoring, and non-destructive quality evaluation across many medicinal species. For Zhejiang medicinal plants, this is especially valuable because cultivation systems require repeated measurements of canopy condition, habitat variation, and developmental stage over large and heterogeneous production areas. At the field scale, UAV-based phenotyping has shown that medicinal plant growth can be monitored with high precision when spectral indices are combined with direct phenotypic measurements.In *Glycyrrhiza uralensis*, high-resolution full-phenology UAV data supported BP, SVM, and RF models for yield forecasting, and combining multiple indicators improved accuracy beyond single-parameter prediction (Zhang et al., 2025). Similar progress appears in wild-resource surveys, where UAV imagery plus Mask R-CNN enabled automated identification, counting, yield prediction, and spatial mapping of individual *Lamiophlomis rotata* plants in complex high-altitude environments (Ding et al., 2023).

Digital phenotyping is also advancing from simple imaging toward environmentally informed monitoring. Remote sensing can characterize growth-regulating variables such as soil moisture and soil-available silicon, and long-term time-series observation can track medicinal-plant phenology together with climate responses. This matters because medicinal plant phenotypes emerge from genotype-environment interaction, so Zhejiang-focused digital phenotyping platforms should link plant traits with water, nutrient, soil, and weather data rather than treating canopy images as isolated inputs. A further technological shift is the move toward chemical-sensitive phenotyping rather than morphology alone. Hyperspectral imaging captures continuous spectral information related to chemical composition and has supported both traditional machine learning and deep learning for component inversion, including simultaneous prediction of multiple ginsenosides through attention-based temporal convolutional models. Because medicinal plant quality depends on the joint distribution of multiple active constituents, these methods are likely to be especially important for Zhejiang geo-authentic herbs where growth monitoring and quality monitoring must be integrated within the same sensing framework.

5.2 Network biology and systems-level modeling

Network biology provides the systems framework needed to connect growth characteristics with active compound accumulation.Plant metabolic network modeling has emerged as a major tool for integrating and predicting the spatial and temporal distribution of metabolic flows, and recent reviews emphasize that mathematical models are essential for investigating growth, development, metabolic regulation, and secondary metabolism across plant stages (Rao and Liu, 2025). For Zhejiang medicinal plants, this means that systems-level models can serve as bridges between observable traits in the field and less visible pathway-level changes that determine compound accumulation.These models are particularly valuable because medicinal plant metabolism is not a single-pathway process but a coordinated network with competing fluxes, feedback regulation, and environmental sensitivity.Network modeling is now used to quantify pathway fluxes and guide strategies for directing metabolism in plant natural product systems, while broader systems-biology perspectives argue that advances in bioinformatics are shifting medicinal plant research away from reductionist explanation toward network-based interpretation (Noor et al., 2022; Rao and Liu, 2025). This transition is important for Zhejiang studies seeking to explain why the same species shows different growth quality or active-compound profiles under different ecological and agronomic settings.

Another key application is linking chemical constituents to targets, pathways, and biological functions through network pharmacology.This approach typically starts by identifying active compounds and predicting associated genes or targets from chemical representations, providing a structured route from phytochemical data to multi-target functional hypotheses (Noor et al., 2022). In traditional Chinese medicine research more broadly, network pharmacology is used to build multilayer relationships among compounds, targets, and pathways, and it

supports the identification of efficacy-related marker components that can improve quality evaluation of complex herbal materials.Systems-level modeling is also becoming more predictive as it incorporates multi-omics and machine learning. Reviews of medicinal plant metabolic networks note that AI methods can extract key biological features from high-dimensional datasets and support the construction of digital-twin-like surrogate systems for optimizing elicitation, cultivation, and metabolic regulation (Chen et al., 2026). For Zhejiang medicinal plants, this suggests a future workflow in which transcriptomic, metabolomic, and phenotypic data are embedded in network models that can forecast how cultivation decisions alter both plant growth and secondary metabolite output (Chen et al., 2026).

5.3 Deep learning and predictive analytics in medicinal plant science

Deep learning and predictive analytics are extending medicinal plant research from description to high-dimensional inference.Recent reviews show that deep learning is particularly effective for analyzing complex data in bioactivity prediction and drug discovery, while medicinal-plant-specific assessments emphasize that deep models perform well on nonlinear multi-omics datasets and can reveal regulatory modules associated with secondary metabolite synthesis (Prajapati et al., 2025). This makes deep learning especially relevant for Zhejiang medicinal plants, where trait development and active-compound accumulation are shaped by interacting genetic, chemical, and environmental factors. One practical advantage of predictive analytics is automated feature discovery from heterogeneous data.Machine learning has been used to identify crucial metabolite features affecting phenolic synthesis, and in medicinal plant hyperspectral applications it can select characteristic wavelengths for accurate prediction of compounds such as tanshinones and for origin classification (Chen et al., 2026). Beyond spectroscopy, automated machine learning has also been used to predict precursors of specialized metabolites, with regularized linear classifiers outperforming prior methods while remaining interpretable, which is useful for accelerating pathway discovery in plant secondary metabolism.

Deep learning is also reshaping phenotyping and resource assessment through image-based prediction.In medicinal plant surveys, UAV imagery combined with deep learning has enabled high-accuracy instance segmentation of individual plants and improved yield estimation in complex field environments (Ding et al., 2023). In cultivation-oriented work, integrated UAV phenotyping has further supported real-time water-nutrient decision making, showing that predictive models can move beyond passive monitoring toward operational management support (Zhang et al., 2025). Despite these advances, predictive analytics in medicinal plant science still faces limits in interpretability, transferability, and data standardization. Deep learning models often act as black boxes and may not provide biologically verifiable mechanisms, while broader reviews of natural-compound modeling identify the need for benchmark datasets, interpretability tools, and stronger experimental validation (Prajapati et al., 2025). For Zhejiang medicinal plant research, the most promising direction is therefore not deep learning alone, but explainable predictive systems that combine remote sensing, multi-omics, and network knowledge into robust decision-support tools for cultivation and quality control (Prajapati et al., 2025; Chen et al., 2026).

6 Case Study: Computational Analysis of Growth Characteristics and Active Compound Accumulation in Zhejiang Medicinal Plants

6.1 Case selection and experimental dataset construction

A Zhejiang case study is best designed around representative medicinal species with clear economic value, measurable growth traits, and chemically defined active constituents. Case selection should also reflect the fact that medicinal plant quality depends on multi-component composition and varies across medicinal parts and environmental conditions, so dataset construction must capture both growth and compound heterogeneity rather than treating plants as uniform samples. This justifies choosing species for which phenotypic, ecological, and phytochemical data can be collected simultaneously, because multi-source data provide a more complete basis for quality evaluation than single analytical streams.

The experimental dataset should combine field observations, imaging, and chemical profiling in a unified sampling framework. A practical template comes from medicinal plant phenotyping studies that pair UAV or

hyperspectral measurements with synchronous ground traits such as biomass, SPAD, or morphological indicators, and that improve performance further by fusing spectral, texture, and phenotypic variables (Wu et al., 2025). Spatial layers should also be included, because machine learning linked with GIS and soil information can map suitable cultivation zones and rapidly organize medicinal plant growth conditions at regional scale, which is directly relevant for Zhejiang's heterogeneous production environments (Roopashree et al., 2024).

6.2 Machine learning-based prediction of growth performance

For growth-performance prediction, the case study should model biomass, vigor, and relative growth rate using supervised learning algorithms trained on integrated temporal and environmental features. Evidence across plant systems shows that machine learning handles complex functional associations in biomass prediction well, especially when datasets include weather, soil, and agronomic variables rather than image features alone. Ensemble methods are often strong performers in such settings, and agriculturally important predictors repeatedly include temperature, precipitation, slope, elevation, and other environmental drivers that can be measured or inferred for medicinal plant fields (Figure 2) (Cacho et al., 2023).

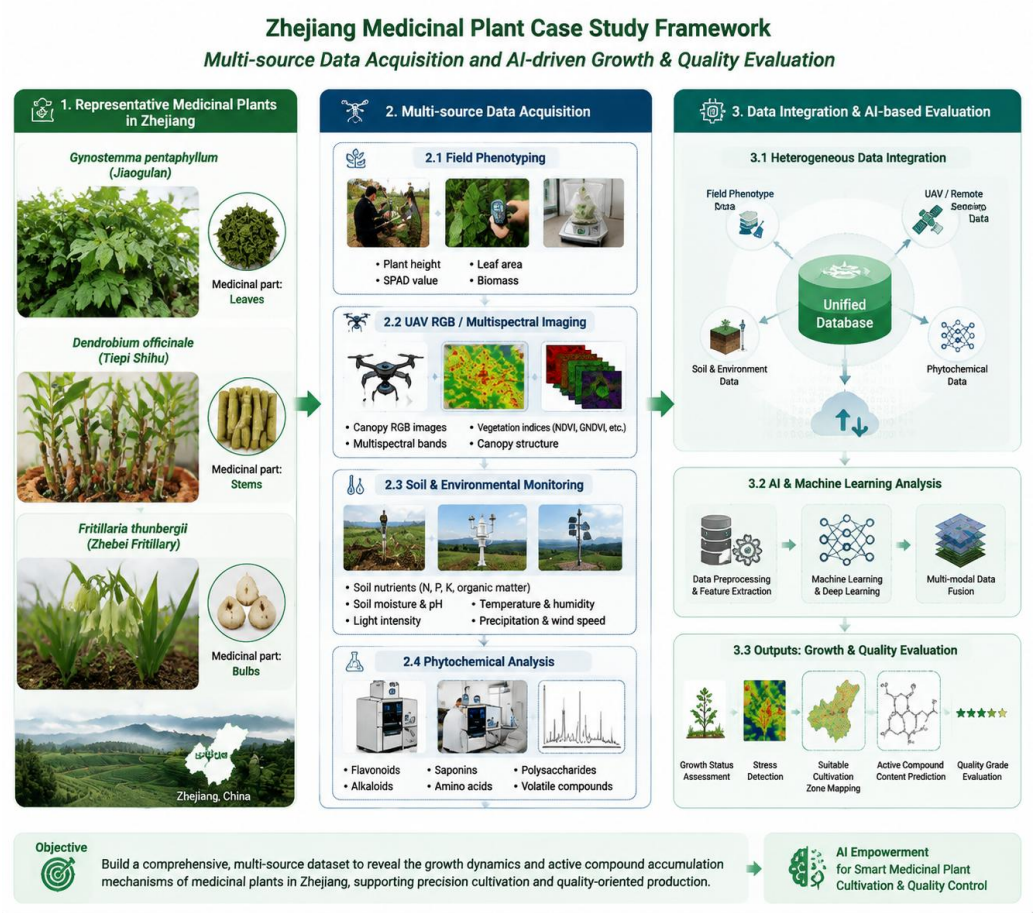

Figure 2 Representative medicinal plant selection and multi-source data acquisition framework for Zhejiang case studies

At the same time, medicinal-plant-specific evidence supports non-destructive and early prediction workflows.Hyperspectral and deep learning approaches have estimated SPAD and biomass in *Lamiophlomis rotata* with good accuracy, while feature fusion improved prediction over single feature types, indicating that Zhejiang medicinal plants would benefit from models that merge spectral and phenotypic descriptors (Wu et al., 2025). Time-resolved prediction is also valuable because early models can identify low-yield batches well before harvest and enable corrective intervention, as shown by cultivation studies where final biomass was predicted by day 8 and process adjustment increased yield by 54.1%.

6.3 Prediction of active compound accumulation and quality optimization

Prediction of active compound accumulation should be based on integrated chemical, biological, and

environmental descriptors rather than concentration values alone. Multi-omics and AI studies show that large heterogeneous datasets can be fused to identify key biosynthetic components, model dynamic metabolic behavior, and support optimization from cultivation conditions to extraction parameters (Chen et al., 2026). This is especially appropriate for Zhejiang medicinal plants because modern omics platforms can map biosynthetic pathways, regulatory networks, and spatial chemical distributions, making compound prediction more mechanistic and less empirical (Latif and Nawaz, 2025).

For quality optimization, the most effective framework is likely to combine spectrum-effect modeling with predictive control of compound content.AI-driven quality-control research in traditional Chinese medicine has shown that fused chromatographic fingerprints and learning models such as PLSR, BP-ANN, CNN, and sequence-attention networks can identify active compounds and then predict their contents non-destructively from near-infrared data (Gao et al., 2026). Complementary optimization studies also show that nonlinear and piecewise linear models can predict total polyphenols and antioxidant activity with high determination coefficients, indicating that Zhejiang case studies can use comparable regression frameworks to link cultivation and processing conditions with stable medicinal quality.

7 Challenges and Future Perspectives

7.1 Current limitations of computational modeling in medicinal plant studies

A central limitation of computational modeling in medicinal plant studies is the heterogeneity of input data.Multi-omics datasets are generated across different platforms and often carry batch effects, scale differences, and technical noise, which complicates integration and weakens downstream model robustness (Chen et al., 2026). This problem is amplified by the broader underuse and fragmented interpretation of omics datasets in plant pathway research, where large volumes of potentially informative data remain insufficiently exploited for biosynthetic inference (Reinhardt et al., 2025). A second limitation is the shortage of biological and computational foundations needed for reliable prediction.Many medicinal plants still lack high-quality reference genomes, and limited sample sizes further reduce model generalizability across species, environments, and developmental stages (Chen et al., 2026). These data constraints align with a broader challenge in AI-based natural product modeling, where prediction quality depends strongly on whether test data fall within the applicability domain of the training set and on whether high-quality integrated datasets are available at all (Xue et al., 2022).

Capturing the dynamic biology of medicinal plants remains another major obstacle.Computational models often struggle to represent nonlinear temporal relationships, especially when transcriptional responses precede measurable metabolite accumulation or when long growth cycles prevent dense time-series sampling across full developmental stages (Chen et al., 2026). More generally, plant biology still underuses mechanistic modeling even though such models are needed to relate hidden mechanisms to measurable traits and to identify emergent relationships that pattern-finding methods alone can miss. Practical adoption is also limited by human and institutional barriers.Many plant biologists still face difficulty selecting appropriate modeling strategies, especially mechanistic approaches, and often lack access to collaborators with quantitative expertise (Dale et al., 2021). In medicinal plant systems specifically, AI performance still varies with species diversity, plant condition, and the scarcity of large, diverse, publicly accessible datasets, which constrains transferability beyond narrowly defined training scenarios.

7.2 Integration of Artificial Intelligence with Experimental Biology

Future progress depends on tighter AI-experiment coupling rather than treating computation and biology as separate workflows. Current reviews emphasize a persistent prediction-validation gap and argue that medicinal plant research needs iterative computation-experiment cycles in which model outputs are repeatedly tested and refined through targeted experiments. This direction is consistent with integrated omics frameworks that combine molecular networking, reaction-pair analysis, and gene-expression patterns to improve pathway discovery while also streamlining downstream validation (Reinhardt et al., 2025). The biological value of this integration is that AI can prioritize the most informative elements for experimental follow-up.Large-scale multi-omics data now provide opportunities to discover enzymes, optimize pathway components, and improve the low abundance or

unstable quality of medicinally important compounds, but these opportunities create a parallel challenge of extracting meaningful patterns from massive datasets (Zhang et al., 2025). AI is well suited to this role because it can identify key biological components in biosynthetic systems and model metabolic behavior over time, thereby guiding experiments toward the most plausible regulatory bottlenecks (Chen et al., 2026).

The next stage is to embed AI into broader biotechnology pipelines rather than restricting it to post hoc analysis.In medicinal herb breeding, AI is already being positioned across multi-omics integration, synthetic biology, precision gene editing, trait optimization, and intelligent monitoring systems, suggesting a unified research architecture rather than isolated analytical tools. In parallel, AI and multi-omics are accelerating plant phenotype analysis, precision breeding, metabolite discovery, and quality control, which makes them increasingly relevant for medicinal plant experiments that seek simultaneous gains in growth performance and active compound output. Even so, integration with experimental biology will require stronger standards for interpretability and interdisciplinary design. Explainable AI is increasingly treated as necessary in natural product research, and meaningful feature design still depends on close collaboration among computational scientists, biologists, and medicinal chemistry specialists (Xue et al., 2022). Earlier computational reviews already suggested that AI shortens the time required for classical experimental strategies, but current evidence indicates that its real value lies in complementing rather than replacing wet-lab validation.

7.3 Future directions toward precision medicinal plant agriculture

The clearest future direction is toward precision medicinal plant agriculture, where sensing, automation, and data science are integrated to optimize both yield and phytochemical quality.Precision agriculture is defined as the coordinated use of sensors, machinery, information systems, and informed management to improve productivity under sustainable conditions, and medicinal plant systems are increasingly being drawn into this broader transition. For medicinal crops specifically, smart farming technologies already appear to improve yield, quality, and sustainability while supporting more consistent production under strict quality requirements (Khan et al., 2026). A major opportunity lies in connecting environmental control directly to biological mechanisms of compound formation. Precision irrigation, AI-driven nutrient management, and controlled-environment cultivation influence photosynthesis, nutrient uptake, stress response, and secondary metabolite biosynthesis, making them relevant not only for growth management but also for active compound optimization (Khan et al., 2026). More broadly, digital twin concepts suggest that real-time data, computational models, and simulation could eventually support continuous prediction of both plant growth and metabolite yield in medicinal plant production systems.

Another priority is the creation of multiscale breeding and management platforms. Conventional breeding struggles with polygenic regulation and the simultaneous optimization of multiple pharmacologically relevant traits across variable environments, whereas AI-based frameworks are beginning to address these combined constraints. A promising response is the proposed genotype-environment-management interactive platform, which explicitly targets data integration, model generalization, and environmental adaptation as linked problems rather than separate technical issues. For Zhejiang medicinal plants, future development will likely depend on building transparent, multiscale systems that connect cultivation, omics, and quality standardization.Recent reviews argue that stronger computational-experimental synergy and more transparent models are essential if AI is to mature into a reproducible discipline in medicinal plant science (Chen et al., 2026). At the same time, high-throughput omics integration is expected to strengthen understanding of environmental adaptation and chemical diversity, which is critical for region-specific precision cultivation and the discovery of improved medicinal plant resources (Zhang et al., 2025).

8 Conclusions

Computational approaches have made medicinal plant research more predictive by linking large, heterogeneous datasets to biologically meaningful outputs such as biosynthetic pathway reconstruction, regulator identification, and metabolic optimization). They have also broadened the analytical scope of the field beyond static screening, as molecular docking, dynamic simulation, and artificial intelligence now jointly support faster interpretation of phytochemical function and bioactivity than traditional experimental workflows alone.A second major

contribution is the integration of multi-source evidence across chemistry, plant organs, and environmental context, which improves medicinal plant quality evaluation and better reflects the ecological regulation of active compounds. At the molecular level, newer sequencing and transcriptome-based approaches have further enabled identification of genes, miRNAs, and biosynthetic pathways underlying medicinal compound formation, giving computational medicinal botany a stronger mechanistic basi. Computational methods have also contributed directly to phytochemical discovery and optimization. Machine learning combined with in vitro culture has been used to identify the major factors controlling phenolic compound biosynthesis and extraction, showing that computational models can guide bioactive compound production rather than only describe it.In parallel, AI-assisted metabolomics has expanded quality assessment, metabolite variation analysis, and early detection tasks, indicating that computational tools are becoming central to the full analytical pipeline of phytochemical research.

These contributions are not limited to medicinal plant cultivation but extend to drug discovery translation.In silico network analysis, screening, and pharmacokinetic prediction can prioritize active phytochemicals and clarify likely mechanisms before wet-lab validation, which reduces experimental burden and accelerates downstream pharmacological studies.More broadly, computational approaches are now treated as an initial key step in natural-product-based drug discovery, especially when integrated with experimental validation and modern AI methods. For sustainable cultivation, computational modeling enables site-specific decision making by connecting medicinal plant performance to soil, climate, and ecological conditions.Machine learning with GIS and soil analysis can rapidly map suitable planting areas and optimize cultivation practices, which is particularly useful for vulnerable or region-specific medicinal herbs. Spatial prediction studies further show that environmental variables can be ranked by their influence on secondary metabolite yield, allowing cultivation zones to be selected not only for survival or biomass, but also for high-value compound accumulation (Dastres et al., 2025). This has direct implications for resource conservation and regional quality management.In geo-authentic medicinal materials, ecological conditions strongly affect active ingredient accumulation, and machine learning models can forecast the geographic distribution of high-quality compounds more systematically than scattered field sampling alone. Cultivation verification studies confirm that combining field sampling, predictive simulation, and regional validation can identify optimal production areas while supporting sustainable utilization and industrial development.

Computational methods also strengthen quality control by shifting evaluation from single indicators toward integrated chemical and biological signatures. AI can correlate chemical fingerprints with documented efficacy, helping build a data-driven framework for standardization, clarification of material basis, and analysis of synergistic effects in complex medicinal systems. This is important because medicinal plant safety and efficacy depend on multi-component quality, while current quality evaluation still often overlooks variation across different medicinal parts. Sustainability also depends on the ability to adapt cultivation systems to climate change and anthropogenic disturbance.Ensemble modeling has shown that future suitable habitats for medicinal species can shift substantially under different emission scenarios, and species-specific barcoding can complement these predictions by improving germplasm identification for cultivation planning. At the production-system level, smart hydroponic and automated monitoring approaches suggest that data-driven cultivation can improve performance while enhancing sustainability, offering useful models for controlled medicinal plant production. The next stage of computational medicinal botany will likely be defined by multiscale integration.Systems biology frameworks increasingly combine co-expression analysis, regulatory network inference, graph-based learning, and explainable AI to generate interpretable predictions for complex traits and environmental responses. This trajectory is especially important for medicinal plants because future progress depends on bridging molecular networks with field-scale phenotypes across species and environments rather than analyzing each data layer in isolation.

A parallel frontier is the development of digital twin frameworks for medicinal plant agriculture. Digital twins can create real-time virtual replicas of crops or farms to support prediction and decision-making, with clear potential for optimizing irrigation, fertilization, pest management, and individualized cultivation strategies. Early agricultural digital twin work already shows that interactive, data-linked platforms can integrate multi-scale

information and move toward closed-loop management, although automated recommendation remains an important next step. Future systems will also depend on continuous monitoring and adaptive control.Reinforcement-learning-based digital twins are emerging as promising tools for optimization, automation, and resource management in agriculture, particularly where virtual environment representations can support policy learning for dynamic decision tasks.More broadly, digital farming and twin-based systems are expected to enhance productivity and efficiency through real-time synchronization between physical farms and virtual models, creating a practical foundation for precision medicinal plant agriculture. The most credible long-term outlook is therefore human-centered and trustworthy AI rather than fully autonomous black-box prediction.Agricultural AI must integrate multimodal information, remain robust to small disturbances, and explain its outputs to domain experts if it is to be trusted in biologically and economically sensitive systems. For Zhejiang medicinal plants, next-generation computational medicinal botany will be strongest when explainable AI, multi-omics, environmental sensing, and experimental validation are combined into transparent decision-support systems for sustainable cultivation and reliable quality control.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Feature Review Open Access

Effects of Grafting on Growth and Yield of Eggplant

Wenjing Xu [1,2]
1 Jiande Riyue Agricultural Development Co., Ltd., Jiande, 311600, Zhejiang, China
2 Zhejiang Agronomist College, Hangzhou, 310021, Zhejiang, China
Corresponding author: 189962720@qq.com
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Abstract Eggplant (*Solanum melongena* L.) is an economically important vegetable crop widely cultivated worldwide, but its productivity is often constrained by soil-borne diseases, continuous cropping problems, and environmental stresses. Grafting has emerged as an effective and sustainable horticultural technique to enhance plant growth, improve stress tolerance, and maintain stable yield under intensive production systems. This review summarizes the effects of grafting on eggplant growth and yield formation, with emphasis on the physiological mechanisms and practical applications of different grafting strategies. The influence of grafting on vegetative growth, including plant morphology, root system development, photosynthetic performance, and biomass accumulation, is discussed in detail. Grafted eggplants generally exhibit stronger root activity, improved nutrient uptake capacity, and enhanced resource utilization efficiency due to the functional advantages of selected rootstocks. In addition, grafting contributes to yield improvement by promoting flowering, fruit setting, fruit development, and maintaining favorable fruit quality characteristics. The role of grafting in enhancing resistance against soil-borne pathogens and abiotic stresses, including drought, salinity, and temperature fluctuations, is also highlighted. A case study of grafted eggplant production under protected cultivation demonstrates that appropriate rootstock selection and integrated management practices can significantly improve plant growth performance, yield stability, and production sustainability. Despite these advantages, further research is required to optimize rootstock-scion combinations, clarify graft-induced physiological and molecular regulation mechanisms, and integrate grafting technology with precision agriculture systems. Overall, grafting represents a valuable approach for sustainable eggplant production by improving plant adaptability, productivity, and resilience under diverse cultivation environments.
Keywords Eggplant; Grafting technology; Rootstock; Growth performance; Yield improvement

1 Introduction

Eggplant (*Solanum melongena* L.) is one of the most important solanaceous vegetables grown worldwide and has substantial nutritional, economic, and agronomic value in both tropical and subtropical production systems. Global eggplant production reached about 55 million tons in 2020, reflecting its wide adaptation and strong market demand, while its continued importance in Asia and the Mediterranean has made it a major target for both productivity improvement and sustainable intensification efforts (Kappel et al., 2024). Despite this importance, eggplant productivity remains uneven across regions because production is constrained by a combination of biotic, abiotic, and management-related factors. Low yields are linked to pest pressure, soil-borne and foliar diseases, temperature and water stress, and inadequate agronomic practices, while many cultivated genotypes still lack sufficient resistance to these stresses under field conditions. These constraints are especially serious in intensive and continuous cultivation systems, where repeated cropping increases inoculum pressure and accelerates soil fatigue. In such settings, bacterial wilt and fungal diseases are among the most damaging constraints, with fungal pathogens alone causing severe economic losses and bacterial wilt frequently producing devastating stand losses in long-term production areas. Conventional approaches based on chemicals, biological agents, or host resistance have often provided incomplete control, particularly for persistent soil-borne pathogens. This production context creates a strong need for management strategies that stabilize yield, reduce crop vulnerability, and remain feasible under commercial field and protected-cropping conditions (Sivasankarreddy et al., 2024).

Within this context, grafting has become an important sustainable technology in vegetable production because it combines a desirable scion with a rootstock selected for stress tolerance, disease resistance, and vigorous root

performance. It is now widely regarded as one of the most valuable tools available against soil-borne diseases and adverse environments, and its expansion accelerated after restrictions on environmentally harmful soil fumigants increased the need for non-chemical alternatives in intensive vegetable systems (Tsaballa et al., 2021). The agronomic value of grafting extends beyond disease control. Reviews across vegetable crops show that grafting can increase yield, improve tolerance to drought, salinity, temperature extremes, waterlogging, nematodes, and other biotic or abiotic stresses, while also supporting longer production cycles and lower dependence on agrochemicals (Kappel et al., 2024). In eggplant, these benefits are particularly relevant because the crop is frequently cultivated in soils affected by wilt complexes and in environments where water scarcity or climatic instability limits productivity. Rootstocks can improve water and nutrient uptake, strengthen plant vigor, and buffer the scion against stress through changes in root architecture, xylem transport, antioxidant activity, and hormonal signaling (Musa et al., 2020). Grafting is therefore not simply a rescue technique for diseased fields; it is increasingly viewed as part of a broader low-input and climate-resilient production strategy. At the same time, its practical value depends on rootstock-scion compatibility, nursery skill, and the ability to offset higher transplant cost through gains in yield, quality, and crop reliability (Awazade and Verma, 2024).

Research on eggplant grafting has expanded from simple compatibility testing to a broader analysis of how rootstocks influence growth, physiology, yield, fruit quality, and adaptation to stress. Early and ongoing work has shown that eggplant benefits substantially from grafting and that a wide range of rootstocks, including *Solanum torvum*, *S. macrocarpon*, *S. aethiopicum*, allied species, and interspecific hybrids, can improve vigor and productivity while offering alternatives where rootstock rotation or local adaptation is needed. Experimental studies consistently report higher total or marketable yield in grafted plants than in self-rooted controls, although the magnitude of improvement depends strongly on the specific graft combination. For example, grafting onto wild relative rootstocks increased vigor and marketable yield in multiple scion backgrounds, and some combinations based on *S. torvum* or *S. aethiopicum* increased marketable yield by about 20%~31% relative to non-grafted plants (Musa et al., 2020; Consentino et al., 2022). More recent work has also clarified some of the mechanisms underlying these responses. Rootstocks with stronger root systems and favorable anatomical traits, such as wider xylem or greater root volume, are associated with better water and nutrient acquisition, improved photosynthetic performance, and higher fruit yield, indicating that grafting effects on production are mediated through whole-plant physiological integration rather than simple disease escape alone (Kappel et al., 2024). Even so, the literature also shows that responses in fruit quality traits are less uniform, with some traits improving, some remaining unchanged, and others varying by environment, harvest maturity, and rootstock-scion interaction.

Current eggplant grafting research increasingly emphasizes performance under specific stress scenarios and integrated production systems, which makes the subject especially relevant for a study focused on growth and yield. Under deficit irrigation, grafted eggplant has shown clear advantages over non-grafted plants through stronger canopy vigor, greater root proliferation, improved water and nutrient uptake, and better maintenance of fruit yield and water productivity; in one recent field study, grafting improved fruit yield by 12.7%-24.5% and reduced yield losses under water deficit while maintaining key fruit-quality traits (Wakchaure et al., 2025). Likewise, under semi-arid conditions, drought-tolerant wild rootstocks such as S. sisymbriifolium and *S. torvum* outperformed non-grafted plants under both full and deficit irrigation, indicating that rootstock choice can materially alter growth and yield stability when water is limiting (Khapte et al., 2025). Beyond water stress, grafting can also reshape the rhizosphere environment, as grafted eggplants have shown increased microbial biomass, greater enzyme activity, lower bacterial wilt incidence, and higher yield than non-grafted controls, suggesting that soil biological effects may contribute to plant performance. Against this background, the objective of the present paper is to examine the effects of grafting on the growth and yield of eggplant, with particular attention to how grafting modifies vegetative vigor, physiological performance, and productivity through rootstock-mediated mechanisms under practical production conditions (Tsaballa et al., 2021).

2 Principles and Mechanisms of Eggplant Grafting

2.1 Graft compatibility and formation of vascular connections

Graft compatibility in eggplant depends on whether the scion and rootstock can heal into a single mechanically stable plant with continuous nonvascular and vascular connections, rather than merely surviving after grafting. Within Solanaceae, compatibility is often strongest in closely related combinations, and tomato-eggplant unions are a clear example of successful intrageneric heterografts in which substantial vascular reconnection supports long-term stability. The graft union forms through a sequence of wound healing events that includes tissue adhesion, vigorous callus proliferation, and later differentiation of new xylem and phloem across the interface (Habibi et al., 2022). During this process, phloem typically reconnects before xylem, and cell-to-cell communication is established through newly formed plasmodesmata, allowing the two partners to coordinate regeneration before the vascular system is fully restored (Tsaballa et al., 2021).

Compatibility is also expressed anatomically at the graft junction. In eggplant-related systems, a successful union is associated with degradation of necrotic layers, close linkage of callus cells, and strong integration between the callus bridge and the vascular network, whereas persistent mismatch at the junction signals functional imbalance (Kappel et al., 2024). Practical assessments therefore often use affinity indices based on the relative diameters of the graft partners, because a balanced junction is more likely to support effective transport of water and nutrients through the developing vascular system (Argento et al., 2023). Incompatibility can be immediate or delayed, and delayed incompatibility is especially important because plants may survive for weeks or longer before mechanical weakness and transport failure become obvious. Molecular work indicates that incompatible unions show disrupted vascular strand reconnection and perturbed expression of key vasculature-related genes, while successful union formation depends on regulators such as SlWOX4 and cell-wall remodeling genes including XTH family genes that sustain callus growth and xylem bridge formation.

2.2 Rootstock characteristics and their functional roles

Rootstock choice is central to eggplant grafting because the rootstock largely determines the belowground capacity for water capture, nutrient uptake, stress tolerance, and resistance to soil-borne pathogens (Musa et al., 2020). In practice, wild *Solanum rootstocks* and selected tomato hybrids are widely used because they can confer higher vigor, stronger root systems, and greater resilience than self-rooted plants. Anatomical traits of the rootstock are one major basis of these functional effects. In grafted eggplant, rootstocks with larger xylem widths and higher cortex cell numbers were associated with higher yield, while *Solanum rootstocks* also showed larger root volume or xylem area than self-rooted plants or some commercial tomato rootstocks (Kappel et al., 2024). These findings indicate that rootstock-mediated differences in sap flow and tissue organization help regulate the later performance of the scion by shaping transport efficiency from the seedling stage onward.

The functional role of the rootstock becomes especially clear under stress. Under deficit irrigation, grafted eggplants developed greater canopy vigor, root proliferation, water and nutrient uptake, and photosynthetic capacity than non-grafted controls, which translated into lower yield loss and higher water productivity (Wakchaure et al., 2025). This supports the broader view that rootstocks do not simply anchor the plant, but actively regulate scion growth, flowering, fruit set, and stress resistance through whole-plant integration (Liu et al., 2025). Rootstocks also contribute indirectly by modifying the rhizosphere environment and disease pressure. In eggplant, grafting onto superior rootstocks reduced bacterial wilt incidence sharply and increased microbial biomass, enzyme activity, and microbial diversity in the rhizosphere, indicating that rootstock effects extend beyond plant anatomy into soil ecological function (Du et al., 2024). This is agronomically important because eggplant is highly vulnerable to persistent soil-borne pathogens, and resistant rootstocks such as *Solanum torvum* are already recognized as effective tools for wilt management where other control methods are unreliable (Sivasankarreddy et al., 2024).

2.3 Physiological and molecular responses induced by grafting

Grafting induces broad physiological changes in eggplant by altering water relations, mineral acquisition, chlorophyll status, and overall vigor through the interaction of the scion with a distinct root system (Musa et al.,

2020). In experimental eggplant systems, grafted plants often show increased stem diameter, higher chlorophyll values, and higher yield than self-rooted plants, indicating that the physiological response is expressed in both vegetative growth and reproductive performance (Du et al., 2024). These physiological changes are coordinated by long-distance signaling between the graft partners. Evidence across grafted vegetables shows that hormones, minerals, mRNAs, non-coding RNAs, and proteins move through the vascular system and contribute to the regulation of scion phenotype after grafting. Hormonal control is especially important at the union, where auxin, cytokinin, ethylene, gibberellin, and jasmonic acid help coordinate wound healing, vascular differentiation, and rootstock-scion communication (Habibi et al., 2022).

At the molecular level, grafting triggers substantial transcriptional reprogramming, and compatible versus incompatible unions can differ sharply in gene expression patterns. Heterografts tend to show stronger activation of oxidative stress and stress-response genes, whereas successful unions more effectively upregulate genes involved in cell-wall synthesis, wound responses, hormone signaling, and vascular regeneration. This helps explain why graft healing is not only an anatomical process but also a genetically regulated response shaped by recognition, metabolic balance, and signaling across the junction. Eggplant studies further suggest that some graft-induced responses involve epigenetic regulation. Heterografting-associated vigor in eggplant has been linked to genome-wide CHH hypomethylation and altered scion gene expression, supporting the idea that rootstocks can reshape scion phenotype partly through methylation-dependent regulation. More broadly, grafting can induce DNA methylation changes, small-RNA-mediated signaling, and transcriptional reprogramming across the union, making epigenetic control a plausible mechanism by which grafting influences growth, stress responses, and yield-related traits in eggplant.

3 Effects of Grafting on Eggplant Vegetative Growth

3.1 Influence on plant morphological development

Grafting generally enhances vegetative vigor in eggplant, especially when vigorous or wild rootstocks are used. Field evidence showed significant differences between grafted and non-grafted plants in plant height, total leaf area, stem diameter, and chlorophyll index, with the Pala/Köksal F1 combination reaching about 1.0 m in height, 5645.04 cm^2/plant leaf area, and a SPAD value of 47.48 (Ulaş, 2021). Greenhouse results similarly found that grafted plants had thicker stems and higher chlorophyll content than self-rooted controls, indicating that grafting promotes stronger shoot development early in crop establishment (Du et al., 2024). The magnitude of morphological improvement depends on the rootstock-scion combination. Grafting onto *Solanum torvum* or *S. aethiopicum* increased plant height at 50 days after transplanting by 11.6% and 9%, respectively, relative to non-grafted plants, while combinations involving wild relatives in open-field trials also showed superior overall growth performance across years (Consentino et al., 2022). Not all vigorous combinations are equally desirable, however, because some highly stimulating rootstocks can induce excessive vegetative growth and disorder, as seen in the Beaufort/Black Bell combination, where vegetative traits were highest but compatibility was weaker and growth became imbalanced (Argento et al., 2023).

3.2 Effects on root system development and nutrient uptake

One of the clearest effects of grafting on vegetative growth is the strengthening of the root system. In grafted eggplant, rootstocks significantly increased shoot and root fresh and dry biomass, with Topan/Köksal F1 reaching 66.29 g/plant root fresh weight and 11.05 g/plant root dry weight, well above the non-grafted control (Ulaş, 2021). Similar evidence from low-cost polyhouse production showed that all grafted plants had superior rooting, and the greatest root number, root length, root fresh weight, and root dry weight were recorded with *S. torvum*, followed by *S. khasianum*.

These root improvements translate into stronger nutrient acquisition and greater tolerance to low-input or stressful conditions. In a soilless system, plants grafted onto *S. torvum* had greater plant dry weight and yield than self-grafted controls, and this vigor was directly linked to major root development and higher accumulation of most analyzed mineral ions. More recent work under deficit irrigation likewise found that grafted plants developed greater root proliferation and improved water and nutrient uptake, which helped reduce yield losses under water stress and supported more stable vegetative performance (Wakchaure et al., 2025).

3.3 Regulation of photosynthetic performance and biomass accumulation

Grafting also regulates vegetative growth by sustaining leaf physiology and photosynthetic function. Under salt stress, grafted plants maintained higher photosynthetic pigment levels than non-grafted plants, and both *S. grandifolium* × *S. melongena* and *S. torvum* rootstocks improved chlorophyll fluorescence parameters and reduced Na^+ accumulation in the scion, which supported better growth under stress (Mozafarian et al., 2023). Under drought-related stress, grafting combined with vermicompost was positively associated with SPAD, leaf area, relative water content, shoot dry weight, and root fresh weight, indicating coordinated improvement in leaf function and biomass retention (Kıran et al., 2026).

Biomass accumulation in grafted eggplant appears to be driven by improved whole-plant assimilation and allocation rather than by leaf greenness alone. Deficit-irrigation experiments showed that enhanced canopy vigor, photosynthesis, and resource uptake in grafted plants increased fruit yield by 12.7%-24.5% while also supporting shoot biomass under stress (Wakchaure et al., 2025). Anatomical and comparative growth studies further indicate that larger root volume, greater crown diameter, and higher SPAD values are linked with earlier and faster growth in grafted eggplant, while broader grafting research using eggplant rootstocks has associated higher relative growth rate, leaf area ratio, and net assimilation rate with superior grafted-plant performance (Figure 1) (Kappel et al., 2024).

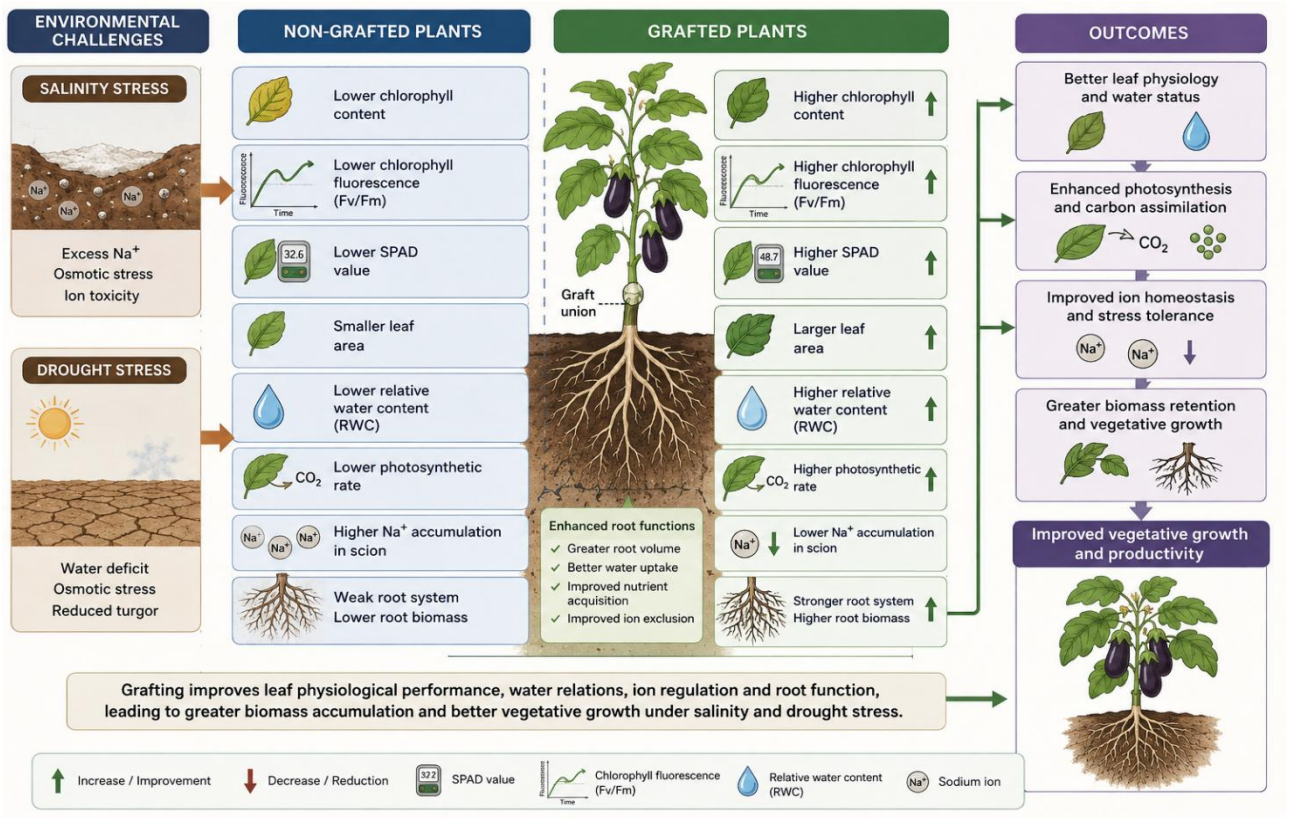

Figure 1 Conceptual framework illustrating the effects of grafting on leaf physiology, photosynthetic performance, ion regulation, and vegetative growth of eggplant under salinity and drought stress

4 Effects of Grafting on Eggplant Yield Formation

4.1 Improvement of flowering and fruit setting characteristics

Grafting can improve reproductive development in eggplant by increasing precocity and supporting earlier transition to effective fruit production when vigorous and compatible rootstocks are used (Musa et al., 2020). In interspecific rootstock systems, the strongest combinations produced greater fruit earliness together with higher early yield, indicating that reproductive advantage begins before final harvest and is closely linked to rootstock vigor and compatibility. This pattern suggests that grafting can shift yield formation forward in time, especially where rapid establishment and strong sink support favor early flowering and fruit retention (Kappel et al., 2024).

The effect on flowering itself is not universally positive, and some studies show that reproductive timing remains highly genotype-dependent. In open-field comparisons, non-grafted CE showed the earliest first flower formation even though several grafted combinations later performed better for overall growth and yield traits (Musa et al., 2020). Reproductive expression after grafting also includes changes in seed set and fruit-setting characteristics, as shown by protected-cultivation work where certain combinations had the highest seed number, confirming that rootstock choice can modify reproductive allocation rather than simply increase vegetative vigor (Mozafarian et al., 2023). Therefore, grafting tends to improve effective fruit setting more consistently than it accelerates first flowering across all scions and environments.

4.2 Effects on fruit yield and yield components

The most consistent effect of grafting on eggplant yield formation is an increase in total and marketable fruit yield, especially when scions are combined with vigorous wild or allied rootstocks. Wild relative rootstocks increased total and marketable yield, fruit number per plant, and average fruit weight relative to non-grafted and self-grafted controls (Musa et al., 2020). Similar results were reported for commercial combinations grafted onto *Solanum torvum* or *S. aethiopicum*, where marketable yield increased by 31.4% and 20.0%, respectively, confirming that yield improvement is often expressed through both heavier fruits and more fruits per plant (Consentino et al., 2022).

Yield gains also appear under stress and under protected cultivation, showing that grafting stabilizes productivity rather than acting only in optimal conditions. Under deficit irrigation, grafted plants improved fruit yield by 12.7%-24.5% and reduced yield losses by 12%-44%, while under salinity, grafting onto SH increased total fruit yield mainly through higher average fruit weight (Wakchaure et al., 2025). Greenhouse and field studies likewise found large practical gains, including marketable-yield increases of 53.0% on *S. torvum* in greenhouse production and total fruit yield of 4 711.89 g/plant in the Pala/Köksal F1 combination in the field (Consentino et al., 2022). These responses are commonly attributed to improved water and nutrient uptake from stronger root systems, which increase reproductive support and raise the efficiency of fruit filling (Mauro et al., 2022).

4.3 Effects on fruit quality and nutritional characteristics

Fruit-quality responses to grafting are more variable than yield responses, but favorable combinations often improve commercial and nutritional traits. In one metabolomic and transcriptomic study, the Sm64R rootstock increased fruit size, yield, total soluble solids, phenolic acids, total amino acids, total sugar, and vitamin C, with associated changes in phenylpropanoid, phospholipid, and nucleotide metabolism (Yan et al., 2023). Other studies similarly found that grafting can increase ascorbic acid, chlorogenic acid, protein, potassium, and zinc, while lowering glycoalkaloids, indicating that some rootstocks enhance both nutritive value and functional quality (Consentino et al., 2022; Sabatino et al., 2022).

At the same time, quality outcomes are not uniform across cultivars, seasons, or rootstocks. Reviews and experiments agree that traits such as soluble solids, peel color, firmness, phenolics, and sensory properties can increase, decrease, or remain unchanged depending on the graft combination and environment. For example, grafting onto *S. torvum* sometimes reduced total phenolics or altered fruit color negatively, whereas other combinations increased total phenols, calcium, boron, zinc, and overall nutraceutical composition under more targeted nutrient management (Mauro et al., 2022). Overall, grafting improves eggplant yield formation most reliably through higher fruit number, fruit weight, and marketable yield, while fruit quality enhancement depends on selecting rootstocks that match the scion, environment, and production objective.

5 Role of Grafting in Enhancing Stress Resistance of Eggplant

5.1 Resistance to soil-borne diseases and pathogens

Grafting has become a central strategy for protecting eggplant against soil-borne pathogens because susceptible scions can be combined with rootstocks that express broader resistance than cultivated eggplant alone (Musa et al., 2020). This role is especially important in intensive production systems, where continuous cropping increases inoculum pressure and severe yield losses from bacterial wilt, Fusarium wilt, Verticillium wilt, nematodes, and

related root diseases can accumulate rapidly (Akanksha et al., 2025). In practical terms, grafting is now used not only to reduce infection in a single crop cycle but also to limit disease development and suppress inoculum build-up in the soil, which improves the health of subsequent crops (Thies, 2021). Experimental evidence in eggplant shows that this protection can be large. In greenhouse production, grafting onto 'Huimei Zhenba' reduced bacterial wilt incidence to 3.33% compared with 55.56% in non-grafted controls, while also increasing yield substantially. Under artificial pathogen pressure, grafted plants also showed strong resistance to Verticillium and Fusarium wilts: Hawk and KingKong F_1 were completely resistant to Verticillium, and several rootstocks showed complete resistance to Fusarium, with Hawk increasing marketable yield by 68.28% over the non-grafted control under Fusarium stress. Rootstock screening further supports the durability of this approach, with *S. torvum* showing the strongest multi-pathogen resistance among tested germplasm for bacterial wilt, southern blight, and Fusarium wilt (Akanksha et al., 2025).

Disease suppression by grafting is not explained by host resistance alone, because the rootstock also reshapes the rhizosphere environment in ways that favor plant health. Grafted eggplants had higher microbial biomass carbon, nitrogen, and phosphorus, together with higher phosphatase and β-glucosidase activity, indicating a more active and nutrient-cycling rhizosphere than self-rooted plants. These shifts likely matter biologically because soil microbial communities, biomass, and enzyme activity are recognized determinants of nutrient cycling, soil fertility, and the severity of soil-borne disease expression around the root zone (Du et al., 2024). Even with these clear benefits, resistance remains rootstock-dependent rather than universal. Reviews emphasize that disease-resistant rootstocks are a sustainable replacement for heavily chemical disease management, but they also note the need to expand the rootstock pool because pathogen populations evolve and currently favored resistance sources may not remain sufficient indefinitely (Thies, 2021). Accordingly, future improvement in pathogen resistance will depend on broader rootstock breeding, especially for combinations that retain high compatibility while combining resistance to bacterial wilt, Fusarium, Verticillium, nematodes, and other persistent soil pests (Akanksha et al., 2025).

5.2 Tolerance to abiotic stress conditions

Grafting also improves eggplant tolerance to abiotic stress, particularly drought and salinity, by allowing sensitive commercial scions to exploit the root systems of more tolerant wild or interspecific rootstocks. This is agronomically important because eggplant is drought-sensitive, and water restriction reduces both yield and fruit quality, while salinity creates ionic and osmotic stress that constrains growth and productivity. Across vegetable systems, grafting is therefore treated as a rapid, non-chemical alternative to breeding for resistance to drought, salinity, temperature stress, and related environmental constraints. Under drought, grafted eggplants consistently retain higher productivity than non-grafted plants. In a two-year field study, grafting onto wild rootstocks improved fruit yield by 12.7%-24.5%, reduced yield losses by 12%-44%, and increased water productivity under deficit irrigation through stronger canopy vigor, root proliferation, water uptake, and photosynthesis (Wakchaure et al., 2025). A second semi-arid field study found that SUR/SIS and SUR/TOR outperformed non-grafted Suraj under both full and deficit irrigation, and at 60% ETc the yield reduction was only 14% for SUR/SIS versus 25% for the non-grafted control (Khapte et al., 2025). These responses are consistent with the broader mechanism proposed for grafted vegetables: drought-tolerant rootstocks improve soil exploration, osmotic adjustment, and stress buffering through deeper and more vigorous roots (Nadoda et al., 2024).

Salinity tolerance shows a similarly clear pattern. In eggplant, SH and ST rootstocks protected the scion under 80 mM NaCl by maintaining higher photosynthetic pigment concentration and chlorophyll fluorescence and by lowering Na^+ accumulation in the shoot relative to non-grafted plants (Mozafarian et al., 2023). Mechanistically, salinity tolerance depends strongly on ion partitioning, because tolerant rootstocks can retain more Na+ in roots while helping preserve higher K^+/Na^+ balance and water status in active tissues under saline. The abiotic benefits of grafting extend beyond drought and salinity. Reviews indicate that tolerant rootstocks can also reduce damage from high and low temperatures, flooding, heavy metals, and other adverse soil conditions, although the strength

of evidence in eggplant is better for drought and salinity than for all other stresses (Nadoda et al., 2024). Postharvest work also suggests that rootstock-mediated stress protection can persist after harvest, as grafting onto the cold-tolerant 'Java' rootstock reduced chilling injury, softening, and pulp browning during cold storage.

5.3 Improvement of resource use efficiency

A third major contribution of grafting is improved resource use efficiency, especially for water and nutrients. This effect is closely tied to the functional role of the rootstock, since vigorous root systems can enhance nutrient uptake, water transport, and osmoregulation under stress while sustaining productive growth (Musa et al., 2020). Root traits are central here: deep and robust roots improve access to soil water and nutrients, and anatomical studies in grafted eggplant show that *Solanum rootstocks* often produce greater root volume and wider xylem, traits that correlate positively with yield (Kappel et al., 2024). Water use efficiency is one of the best documented outcomes. In a Mediterranean greenhouse, the highest WUE for fruit production occurred in the IR50 treatment with To/Bb, showing that grafting onto *S. torvum* can convert moderate water limitation into more efficient yield formation rather than simply buffering damage (Argento et al., 2023). Under semi-arid deficit irrigation, SUR/SIS achieved 9.47 kg/m^3 water productivity and SUR/TOR 8.22 kg/m^3, both above the non-grafted control at 6.72 kg/m^3, confirming that rootstock choice can materially improve output per unit water (Khapte et al., 2025). These gains appear to arise from better soil-plant water balance, sustained PSII efficiency, and stronger antioxidative defense under water deficit (Nadoda et al., 2024).

Nutrient use efficiency also improves when grafting enhances root function and rhizosphere activity. Comparative studies report that grafting can increase tissue concentrations or uptake efficiency of major nutrients such as N, P, and K, while greater root activity and xylem development support more effective mineral translocation to the shoot (Yang et al., 2026). In grafted eggplant specifically, enhanced microbial biomass and enzyme activity in the rhizosphere indicate faster nutrient cycling and a more nutrient-rich root environment, which likely contributes to stronger growth and stress resilience (Argento et al., 2023). Resource-use benefits can be strengthened further when grafting is combined with complementary soil management. Integrating grafted plants with vermicompost under moderate drought increased shoot fresh weight by 48.81%, reduced yield loss by 96%, and improved irrigation water productivity by 62.79% in greenhouse conditions. Field and greenhouse evidence from the same research line also shows that the grafting-vermicompost combination improves soil moisture retention, mineral content, and total productivity under drought, making it a promising low-input strategy for climate-resilient eggplant production (Kıran et al., 2026).

6 Case Study: Evaluation of Grafting Effects on Eggplant Growth and Yield under Protected Cultivation

6.1 Experimental design and grafting treatments

Protected-cultivation studies on eggplant grafting generally use comparative designs that include non-grafted and self-grafted controls alongside one or more commercial or wild rootstocks, allowing the specific contribution of the rootstock to be separated from the grafting procedure itself (Sabatino et al., 2022). In unheated greenhouse and tunnel systems, common experimental materials include scions such as 'Madonna', 'Birgah', or locally adapted cultivars grafted onto *Solanum torvum*, allied *Solanum* species, or tomato rootstocks such as Optifort and Emperador (Argento et al., 2023). The cultivation environment is also a defined treatment component in these case studies. Greenhouse experiments have been conducted in soilless pot culture, polyethylene-covered tunnels, unheated plastic houses, and low-cost polyhouses, often with drip irrigation, mulching, and standardized fertigation so that treatment differences mainly reflect graft combination rather than uneven crop management (Sabatino et al., 2022). Some protected-cultivation studies explicitly add environmental or stress factors to the design, such as saline versus non-saline nutrient conditions or heated versus cold greenhouse and substrate regimes, in order to test whether grafting effects remain stable under suboptimal production environments (Mozafarian et al., 2023).

The grafting methods used are usually simple nursery techniques chosen for reproducibility and high survival. Tube grafting, splice grafting, cleft grafting, and clip fixation during healing are all represented in the eggplant

literature, followed by a healing and acclimatization period before transplanting into the protected structure (Sabatino et al., 2022). High graft success is repeatedly reported when compatible rootstocks are used, including complete success in some interspecific and wild-rootstock combinations, which supports the feasibility of protected-cultivation trials at commercial scale. Treatment structures also increasingly include integrated inputs intended to test sustainability rather than grafting alone. Recent greenhouse studies combined grafting with arbuscular mycorrhiza, microbial or seaweed-derived biostimulants, or vermicompost so that performance could be evaluated under a broader low-input management framework. This broader design logic is useful for protected cultivation, where growers are interested not only in absolute yield but also in nutrient use efficiency, reduced disease risk, and improved return on intensive infrastructure and input costs (Figure 2) (Sabatino et al., 2022).

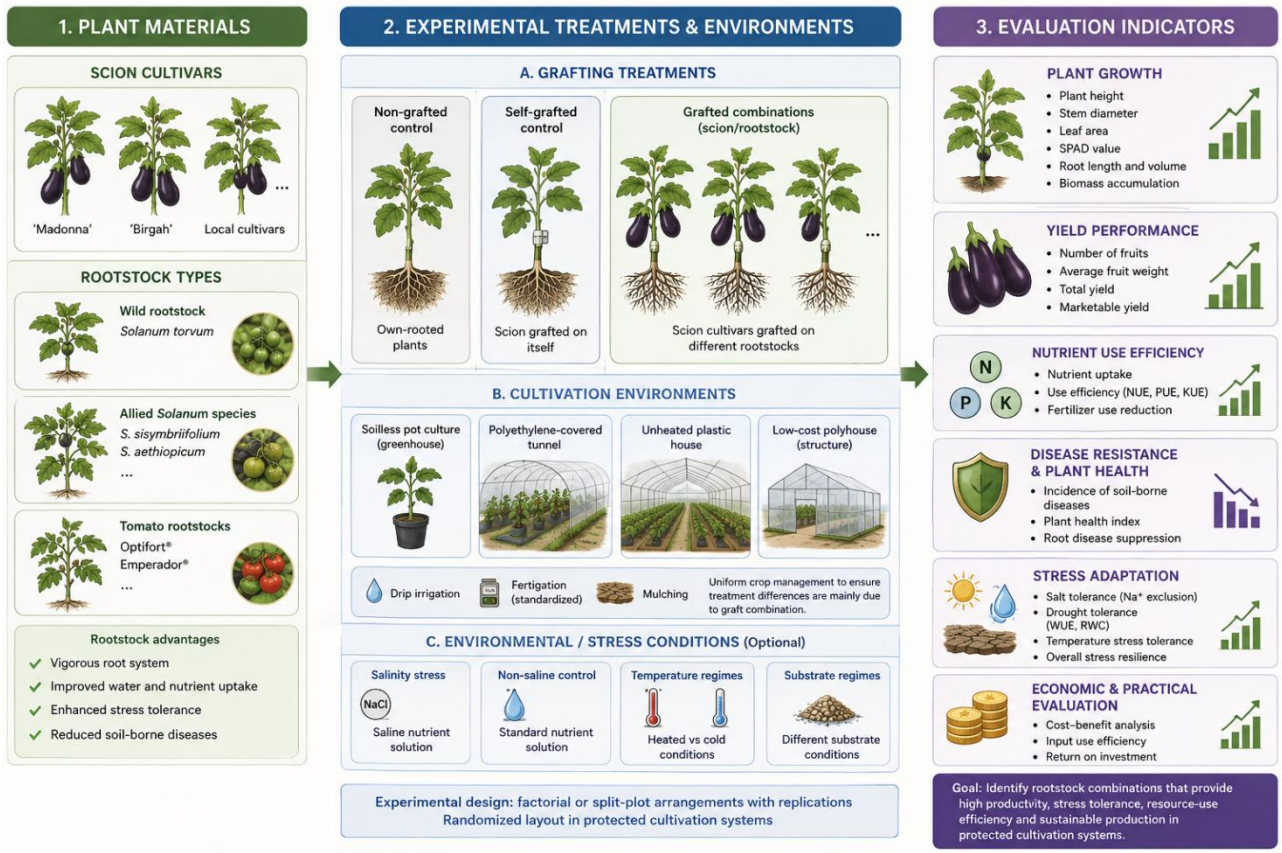

Figure 2 Experimental framework commonly used in protected-cultivation studies evaluating eggplant grafting performance

6.2 Effects of different grafting treatments on growth and physiological traits

Across protected systems, different grafting treatments produce clear differences in vegetative growth and physiological status. In greenhouse eggplant, grafted plants commonly show larger stem diameter, higher SPAD values, and greater general vigor than self-rooted controls, and these responses are strongest with vigorous *Solanum rootstocks* such as *S. torvum* or selected wild eggplant materials (Sabatino et al., 2022). In seedling and early growth analyses, rootstock effects also extend belowground, with *Solanum rootstocks* tending to produce larger root volume and wider xylem development, traits that are associated with stronger subsequent growth and better crop performance. Physiological improvement under protected cultivation is closely tied to rootstock-mediated uptake and transport processes. Stronger roots improve water and nutrient acquisition and help maintain chlorophyll status and photosynthetic efficiency, which explains why grafted plants often outperform non-grafted plants even when greenhouse management is otherwise optimized (Kappel et al., 2024). Under salinity in protected soilless culture, grafted plants maintained higher photosynthetic pigment concentrations and chlorophyll fluorescence while retaining more Na^+ in roots than in shoots or fruits, indicating that ion partitioning is a major mechanism of physiological protection (Mozafarian et al., 2023).

Not all rootstocks generate equally balanced growth responses. Some combinations stimulate desirable vigor and leaf development, while others induce excessive vegetative growth or functional imbalance, particularly when compatibility is weaker or when the rootstock is too vigorous for the scion (Argento et al., 2023). This makes rootstock selection a physiological decision as much as a disease-management one, because the best protected-cultivation combinations are those that enhance vigor without disrupting reproductive balance or plant architecture (Yan et al., 2022). Protected-cultivation studies also show that grafting can alter the rhizosphere and thereby indirectly affect plant physiology. In greenhouse trials, grafted eggplants had higher microbial biomass carbon, nitrogen, and phosphorus and higher enzyme activity in the rhizosphere, supporting a more active nutrient-cycling environment around the roots (Du et al., 2024). When grafting is combined with beneficial biological inputs such as arbuscular mycorrhiza, these physiological gains can extend to stronger nutrient assimilation and higher nitrogen use efficiency, reinforcing the functional advantage of grafted plants under protected production (Sabatino et al., 2020).

6.3 Effects on yield performance and production sustainability

Yield performance under protected cultivation generally improves when eggplant scions are grafted onto vigorous and compatible rootstocks. In an unheated polyethylene greenhouse, grafting ‘Madonna’ onto *S. torvum*, SH, or A increased total marketable yield markedly relative to self-rooted plants, with *S. torvum* reaching 3.94 kg/plant versus 1.65 kg/plant in the control (Mozafarian et al., 2023). Similarly, greenhouse work focused on rhizosphere microecology reported yield increases of up to 36.89% for plants grafted onto ‘Huimei Zhenba’ and substantial gains also for *S. torvum*, showing that the yield advantage persists across distinct protected-cropping systems (Du et al., 2024). Yield gains are usually expressed through more than one component. Depending on the graft combination, grafted plants can produce more marketable fruits, greater mean fruit weight, or both, and several studies show that the strongest rootstocks improve reproductive output without necessarily compromising apparent fruit quality (Sabatino et al., 2020). In some greenhouse studies, however, quality-related responses are mixed, with certain combinations lowering soluble solids or firmness, which means that higher yield does not automatically translate into better quality across all scion-rootstock pairs (Sabatino et al., 2022).

From a sustainability perspective, protected-cultivation grafting is valuable because it stabilizes production under disease pressure and reduces dependence on chemical soil disinfestation. Grafting emerged partly as a response to restrictions on soil fumigants, and in intensive greenhouse systems it functions as a non-chemical tool to sustain yield where continuous cropping and soil-borne diseases would otherwise reduce plant survival and marketable output (Kappel et al., 2024). This role is reinforced by direct disease outcomes in greenhouse eggplant, where superior rootstocks greatly lowered bacterial wilt incidence while simultaneously increasing yield, making grafting both a protective and productive intervention (Du et al., 2024). Production sustainability under protected cultivation also improves when grafting is integrated with complementary low-input practices. In greenhouse eggplant, combining grafting with microbial biostimulants, mycorrhiza, or vermicompost improved nitrogen use efficiency, marketable yield, and several nutritional traits, suggesting that grafting can serve as the structural platform for broader input-efficient production systems (Sabatino et al., 2020; Consentino et al., 2022).

Even so, the economic value of grafting remains treatment-dependent, because rootstock compatibility, seedling cost, and management complexity still determine whether the biological gains achieved in protected cultivation translate into a consistently scalable production strategy (Argento et al., 2023; Kıran et al., 2026). In protected cultivation, eggplant grafting is most effective when the experimental system uses well-matched rootstock-scion combinations and evaluates both yield and resource efficiency. Across the available case studies, the most reliable outcome is higher and more stable production, especially with *Solanum torvum* and related vigorous rootstocks.

7 Advances and Future Perspectives of Eggplant Grafting Technology

7.1 Optimization of rootstock selection and grafting methods

Future progress in eggplant grafting depends first on improving rootstock selection beyond the current reliance on a few standard materials. Reviews emphasize that the available rootstock pool remains narrow relative to the diversity of stresses growers face, and that rapid pathogen evolution and complex abiotic-stress traits make it

necessary to expand screening and evaluate new rootstock-scion combinations more systematically. This need is reinforced by the strong performance of alternative genetic resources, including interspecific hybrids and wild relatives, which can match or even substitute for *Solanum torvum* when compatibility and vigor are high. Recent studies show that rootstock optimization should be based not only on disease resistance or survival, but also on anatomical and physiological traits linked to final productivity. In grafted eggplant, larger root volume, wider xylem, and higher cortex cell number were associated with higher yield, indicating that internal rootstock structure can serve as a more informative selection criterion than gross compatibility alone (Kappel et al., 2024). At the same time, practical screening continues to identify robust candidates such as *S. torvum*, *S. sisymbriifolium*, and *S. incanum* for multi-disease resistance, which is directly relevant for breeding next-generation rootstocks with broader adaptation (Akanksha et al., 2025).

Optimization also extends to the grafting process itself, where high success rates depend on both biological compatibility and technical precision. In eggplant relatives, some interspecific hybrids achieved at least 90% germination and 100% graft success, showing that appropriate rootstock genetics can simplify nursery operations as well as improve field performance. Comparable evidence from alternative rootstock programs found high grafting success for selected materials such as SPA and the hybrids Msa 2/2 E7 and 460 CAL, supporting continued diversification of nursery-ready rootstocks (Du et al., 2024). Method optimization is increasingly tied to automation because manual grafting limits scale, uniformity, and labor efficiency. A fully automatic synchronized grafting machine developed for eggplant tray seedlings achieved 700 grafts per hour, about 95% average success, and zero stem damage, indicating that machine-assisted grafting can improve both throughput and seedling quality (Liu et al., 2025). A separate comparison of manual and robotic brinjal grafting likewise found faster healing, about 96.3% success, and about 689.5 grafts per hour in the best robotic treatment with *S. torvum*, suggesting that future optimization will combine superior rootstocks with precision nursery engineering.

7.2 Integration of grafting with modern agricultural technologies

A major future direction is the integration of grafting with other sustainable crop-management tools rather than using grafting as a stand-alone intervention. Combined strategies are already showing clear benefits in eggplant, particularly when grafted plants are paired with microbial or biological inputs that improve nutrient uptake, stress buffering, and fruit functional quality. This shift matters because plant responses to grafting remain genotype- and environment-dependent, so integrated protocols are needed to stabilize outcomes across production systems (Kıran et al., 2026). *Arbuscular mycorrhiza* represents one of the clearest examples of productive integration. Under greenhouse conditions, AM fungi increased marketable fruit, fruit number, and nitrogen use efficiency regardless of graft combination, while the B/T and B/P grafts combined with AMF delivered the best overall results for yield traits, mineral profile, and nutritional quality (Sabatino et al., 2020). These findings suggest that future protected-cultivation systems can use grafting as a structural platform onto which beneficial symbionts are added to enhance nutrient acquisition and functional quality.

Biostimulants and organic amendments provide a second integration pathway. In eggplant, *Azospirillum brasilense* combined with *S. torvum* or *S. aethiopicum* rootstocks enhanced growth, yield, nutritional traits, and nitrogen use efficiency, and the authors identified these combinations as useful for plug-plant production systems seeking more sustainable performance (Consentino et al., 2022). More recent drought-management work similarly showed that combining grafted plants with vermicompost improved yield, soil moisture, mineral status, and antioxidant-related quality traits under greenhouse and field stress, supporting integrated low-input strategies for climate-resilient production (Kıran et al., 2026). Digitalization and mechanization are likely to shape the next phase of adoption. Reviews of grafting technology argue that specialized nurseries equipped with advanced seeders, growth chambers, and acclimatization facilities can improve seedling uniformity, while databases, crop models, and mobile tools could help growers choose the most suitable rootstock-scion combinations (Awazade and Verma, 2024). In parallel, grafting-robot reviews conclude that further progress depends on better machine vision, artificial intelligence, and tighter integration between robotics and seedling biotechnology to produce more universal and intelligent systems (Yan et al., 2022).

7.3 Molecular and omics-based understanding of grafting responses

The molecular basis of eggplant grafting is now moving from descriptive physiology toward integrated omics. Reviews highlight that next-generation sequencing has made it possible to examine genomic interactions across the graft junction, including gene exchange, rootstock-scion communication, and large shifts in scion DNA methylation that may underlie graft-induced changes in vigor, stress responses, and quality. This is important because future rootstock selection is unlikely to become truly predictive until molecular markers are connected to agronomic performance (Tsaballa et al., 2021). Eggplant-specific evidence already shows that grafting can trigger strong epigenetic reprogramming. Heterografting-induced vigor was associated with genome-wide CHH hypomethylation and altered scion gene expression, linking improved plant performance to epigenetic remodeling rather than to anatomy alone. More broadly, epigenetic diversity is now being considered a usable source of phenotypic variability, with potential value for breeding and for designing graft combinations that are more resilient under changing environments (Tsaballa et al., 2021).

Transcriptomics and metabolomics are also clarifying how grafting alters fruit composition and stress biology. In eggplant, integrated metabolomic and transcriptomic analysis showed that the Sm64R rootstock improved fruit size and several nutritional traits, while differential metabolites and genes clustered in phenylpropanoid, phospholipid, and nucleotide metabolism pathways (Yan et al., 2022). Cold-stress transcriptomics further showed thousands of differentially expressed genes between self- and heterografted combinations, with enrichment in hormone signaling and arginine-proline metabolism, indicating that graft-mediated stress tolerance is regulated through broad transcriptional networks rather than single genes. A further frontier is the study of mobile regulatory molecules as direct mediators of rootstock effects. Under cadmium stress, grafting onto *S. torvum* reduced fruit Cd by 76% and identified five key differentially expressed miRNAs whose targets were involved in hormone signaling and ion transport, suggesting a mechanistic link between long-distance RNA signaling and safer fruit production (Chenshu et al., 2026). Together with broader evidence that grafting can involve genetic exchange and methylation changes across the union, these findings indicate that future eggplant grafting technology will increasingly rely on omics-guided rootstock design, not just empirical nursery testing (Tsaballa et al., 2021).

8 Conclusions

Across studies, grafting consistently improved eggplant vegetative vigor, root development, and reproductive performance relative to non-grafted controls. Field and greenhouse experiments reported significant increases in plant height, leaf area, chlorophyll status, fruit number, and total yield, showing that rootstocks alter both source strength and sink development rather than only protecting plants from stress. This effect appears strongest when vigorous and compatible rootstocks such as *Solanum torvum*, wild relatives, or selected interspecific hybrids are used, because these combinations enhance biomass production and support earlier or more sustained fruiting. The yield advantage of grafting is mechanistically linked to stronger root systems, improved water and nutrient uptake, and more stable physiology under stress. Soilless and deficit-irrigation studies showed that vigorous rootstocks increased plant dry weight and yield through greater root development, while also improving water productivity and reducing yield losses under drought. Fruit-quality responses were more variable than growth and yield responses, but several studies still found gains in soluble solids, chlorogenic acid, vitamin C, amino acids, antioxidants, and mineral composition when favorable rootstock-scion combinations were selected.

From a production standpoint, grafting is now a practical tool for stabilizing eggplant performance in intensive and low-input systems. It is especially valuable where continuous cropping and soil-borne diseases limit conventional cultivation, because resistant rootstocks can sharply reduce disease incidence while maintaining or increasing yield under commercial greenhouse conditions. This makes grafting relevant not only for yield improvement, but also for lowering dependence on chemical soil disinfestation and supporting more resilient production systems under protected and open-field cultivation. The technology also has clear value for resource-efficient horticulture. Vigorous rootstocks maintained higher biomass and yield even under reduced nutrient supply, suggesting that fertilizer inputs can be lowered without proportional productivity loss, while other studies documented marked gains in water use efficiency under deficit irrigation. In practice, these benefits can be

strengthened further when grafting is combined with complementary approaches such as arbuscular mycorrhiza, microbial biostimulants, or vermicompost, which improved nitrogen use efficiency, fruit nutritional quality, and drought resilience in integrated systems.

Future progress in eggplant grafting depends on moving from empirical rootstock choice to more predictive selection based on compatibility, anatomy, stress tolerance, and target production environment. Current evidence shows that rootstock performance is not universal, and some highly vigorous combinations can cause low compatibility or vegetative imbalance, making it essential to match rootstock and scion more precisely. Root anatomical traits such as xylem width, cortex cell number, and root volume also correlate with later yield, so these characteristics can serve as useful criteria in future breeding and screening programs. A second priority is deeper mechanistic and technological development. Omics studies show that grafting responses in eggplant involve transcriptomic, metabolomic, and epigenetic reprogramming linked to fruit quality and vigor, while nursery engineering studies indicate that automation can raise grafting efficiency and standardize seedling quality at commercial scale. Overall, the most promising application prospect is an integrated grafting system that combines improved rootstock breeding, precision nursery methods, and stress-adapted crop management to support sustainable eggplant production under increasingly constrained environmental conditions.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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