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Plant Gene and Trait, 2026, Vol. 17, No. 1, 1-11

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Plant Gene and Trait, 2026, Vol. 17, No. 1, 12-19

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Plant Gene and Trait, 2026, Vol. 17, No. 1, 20-35

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Plant Gene and Trait, 2026, Vol. 17, No. 1, 36-55

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Plant Gene and Trait, 2026, Vol. 17, No. 1, 56-73

Research Report

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Genetic Diversity and Genetic Relationship Analysis of *Platycladus orientalis* Germplasm Based on SSR Markers

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Abstract To investigate the genetic diversity and phylogenetic relationships among *Platycladus orientalis* germplasm resources in Zaozhuang City, simple sequence repeat (SSR) molecular markers were used to analyze genetic diversity and relatedness in 100 *P. orientalis* accessions collected from four regions. The results showed that a total of 32 allelic loci were detected using seven pairs of SSR primers, with an average of 6.571 alleles per primer pair. The mean number of alleles (N_a) was 3.714, the mean effective number of alleles (N_e) was 1.900, the mean Shannon's information index (I) was 0.818, and the mean expected heterozygosity (H_e) was 0.440, indicating relatively high genetic diversity among the 100 sampled *P. orientalis* accessions. The F_{st} value was 0.0371, suggesting a high degree of similarity among populations, small genetic distances, and low genetic differentiation. Cluster analysis based on the estimation of the optimal K value showed that the maximum ΔK occurred at $K=3$, indicating that the 100 *P. orientalis* accessions could be divided into three groups rather than clustering strictly according to geographic origin, which implies the existence of gene flow among the sampled populations. Through preliminary screening and repeated validation, seven pairs of SSR primers with clear gel electrophoresis profiles were obtained, which showed stable amplification across all populations and yielded reliable, easily interpretable results. These microsatellite markers provide a useful reference for future studies on the origin and evolution of *P. orientalis* varieties, molecular identification and classification, hybrid breeding, and parental selection for genetic mapping.

Keywords *Platycladus orientalis*; SSR; Fingerprinting; Cluster analysis

1 Introduction

Platycladus orientalis, belonging to the family Cupressaceae, subfamily Cupressoideae, genus *Platycladus* (Fu, 1982), is the coniferous tree species with the widest natural distribution in China. It has a broad ecological amplitude, strong tolerance to drought and poor soils, vigorous vitality, and a long lifespan. It is one of the most commonly used pioneer tree species for afforestation of barren mountains in northern China, possessing extremely high economic and medicinal value, and it is also an important component of historical and cultural landscapes (Wu, 1986; Yang et al., 2014a). With global climate change, under the combined effects of natural disasters, deterioration of site conditions, pests and diseases, as well as subjective factors such as human disturbance and poor management, *P. orientalis* populations have shown varying degrees of decline, including weakened growth and even near death. The conservation and utilization of *P. orientalis* germplasm resources are facing great challenges, and its ecological value has not been effectively utilized (Su, 2003; Wang et al., 2004; Yang et al., 2014b).

Since the 1970s, extensive provenance trials have been carried out for *P. orientalis*, making it one of the earliest tree species in China to undergo such trials. In recent years, many scholars have conducted substantial research on provenance testing and patterns of genetic variation in *P. orientalis* (Wang, 2011). Numerous studies have also investigated and discussed the pharmacologically active components in different parts of *P. orientalis* (Miao Hui, 2018). During cultivation, a number of regional cultivars have been formed. Due to its wide distribution, wild populations of *P. orientalis* possess rich genetic resources. By studying the genetic variation and distribution patterns of *P. orientalis*, analyzing its genetic diversity and phylogenetic relationships, further revealing kinship

and evolutionary relationships, and evaluating its genetic potential and value, a theoretical foundation can be established for the construction of core germplasm collections and the screening of superior genes. This is of great significance for the selection and utilization of elite germplasm.

With continuous advances in science and technology, methods for studying genetic diversity have also been constantly updated. Molecular marker techniques, owing to their advantages of being unaffected by environmental factors, developmental stage, or gene expression, have become important tools in genetic research (Liu et al., 2012). Geographic population variation in *P. orientalis* has shown significant effects on population selection and improvement (Jin, 2020). There is an urgent need to improve the genetic quality and adaptability of superior *P. orientalis* varieties through molecular marker technologies and to carry out research on the genetic basis of breeding populations. Among these methods, microsatellite markers (simple sequence repeats, SSRs), because of their high level of genetic information, good reproducibility and stability, and codominant inheritance (Maroof et al., 1994; Guichoux, 2011), have been widely used in studies of genetic diversity and phylogenetic relationships in forest trees (Reisch et al., 2007; Kalia, 2011; Lin et al., 2013; Huang et al., 2018). At present, sequenced genomes are mainly concentrated in cultivated plants and species with important economic value. Meanwhile, the development of new microsatellite primers is difficult and costly. However, species derived from a common ancestor often exhibit high sequence homology. Therefore, screening SSR primers required for the target species from closely related species with well-developed microsatellite primers has been widely adopted (Barbara, 2007).

In this study, SSR molecular markers were used to analyze the genetic diversity and kinship relationships of 100 *P. orientalis* samples collected from four regions. Through preliminary screening and repeated validation, seven pairs of microsatellite primers were selected from all synthesized SSR primers. These primers produced clear gel electrophoresis patterns, could be stably amplified in each population, showed relatively ideal performance, and were easy to score and statistically analyze, and were thus used for subsequent analyses.

1 Results and Analysis

1.1 PCR amplification and primer polymorphism

Through primer screening and repeated validation, seven polymorphic microsatellite primers were successfully selected from 45 pairs of SSR primers. These primers produced clear gel electrophoresis profiles, could be stably amplified in all populations, showed relatively ideal performance, and were easy to score and statistically analyze. A total of 26 allelic loci were detected by the seven SSR primers, mainly distributed in the range of 125–309 bp (Table 1). On average, each SSR primer detected 3.714 alleles. The number of effective alleles (N_e) ranged from a minimum of 1.317 for primer 18 to a maximum of 2.819 for primer SF13, with a mean of 1.9. The observed heterozygosity (H_o) of *Platycladus orientalis* populations ranged from 0.140 to 0.610, with an average of 0.406. The expected heterozygosity (H_e) varied from 0.241 to 0.645, with a mean value of 0.440. The polymorphic information content (PIC) ranged from 0.212 to 0.579. Highly polymorphic primers ($PIC > 0.5$) accounted for 28.6% of the total, primers with moderate polymorphism ($0.25 < PIC < 0.5$) accounted for 57.1%, and primers with low polymorphism ($PIC < 0.25$) accounted for 14.3%, with an average PIC value of 0.398. The Shannon information index (I) ranged from 0.405–1.194, with an average of 0.818, indicating that the genetic diversity of the population of 100 *P. orientalis* accessions was relatively low.

Table 1 Genetic diversity characteristics of different SSR loci

Locus	Genotype No.	N_a	N_e	I	H_o	H_e	uHe	PIC
4	3	2	1.688	0.598	0.350	0.408	0.410	0.325
18	3	2	1.317	0.405	0.140	0.241	0.242	0.212
SF3	5	3	1.595	0.685	0.380	0.373	0.375	0.341
SF14	12	5	2.441	1.194	0.610	0.590	0.593	0.557
SF4	7	5	1.894	0.955	0.490	0.472	0.475	0.441
SF12	6	4	1.551	0.703	0.370	0.355	0.357	0.330
SF13	10	5	2.819	1.188	0.505	0.645	0.649	0.579
Mean	6.571	3.714	1.900	0.818	0.406	0.440	0.442	0.398

1.2 Genetic diversity and kinship analysis of *Platycladus orientalis*

Genetic variation among *Platycladus orientalis* populations was analyzed using seven SSR markers. Based on GenAlix analysis, the results (Table 2) showed that at the population level, the observed number of alleles per population ranged from 2.571 to 3.571, with an average of 3.071. The effective number of alleles ranged from 1.865 to 1.987, with an average of 1.909. The Shannon information index ranged from 0.725 to 0.807, with a mean of 0.766. Observed heterozygosity ranged from 0.393 to 0.492, with an average of 0.423, while expected heterozygosity ranged from 0.431 to 0.452, with a mean of 0.439. The fixation index (F_{st}), which reflects the level of allelic heterozygosity among populations and is used to measure the degree of population differentiation, was 0.0371. This value falls within the range of 0–0.05, indicating a high degree of similarity among populations, small genetic distances, and very low genetic differentiation. Population A exhibited the highest values of polymorphism rate, N_a , and I , indicating that this population had the highest genetic diversity. It is therefore inferred that population A represents the center of genetic diversity of *P. orientalis* among the four sampling regions.

Table 2 Genetic diversity of *Platycladus orientalis*

Population	N	N_a	N_e	I	H_o	H_e	uH_e
a	68	3.571	1.902	0.807	0.394	0.431	0.434
b	9	2.857	1.883	0.761	0.492	0.452	0.479
c	4	2.571	1.987	0.725	0.393	0.433	0.495
d	19	3.286	1.865	0.771	0.414	0.438	0.450
Mean	25	3.071	1.909	0.766	0.423	0.439	0.464

1.3 Genetic Differentiation Analysis of *Platycladus orientalis*

Analysis of variance (ANOVA) was used to assess genetic variation in *Platycladus orientalis*. The results (Table 3) showed that genetic variation in *P. orientalis* was mainly derived from within populations, accounting for 91% of the total variation, while genetic variation among populations accounted for 9%. This indicates that the genetic variation of *P. orientalis* is predominantly distributed within populations.

Table 3 Molecular variance analysis of *P. orientalis* germplasm

Sources of variation	df	SS	MS	Est. Var.	Percentage of variation
Among Pops	3	4.36	1.45	0	0%
Among Indiv	96	165.74	1.73	0.15	9%
Within Indiv	100	143	1.43	1.43	91%
Total	199	313.10	1.58	1.58	100%

1.4 Genetic structure analysis of *Platycladus orientalis*

Bayesian clustering analysis of 100 individuals from four populations was performed using STRUCTURE software. The number of subpopulations (K) was preset from 2 to 10, with 10 independent runs for each K value. The value of $\ln P(D)$ continuously decreased with increasing K . When $K=3$, ΔK reached its maximum peak, indicating that division of the experimental materials into three clusters was the most appropriate (Figure 1; Figure 2).

The distribution of individuals among the three clusters (Table 4) showed a relatively even composition, with mean Q values of 0.619, 0.476, and 0.461, respectively. When $Q \geq 0.6$, the genetic background of a sample is considered relatively pure, whereas when $Q < 0.6$, the genetic background is considered complex (Falush et al., 2003). In this study, the Q value of Subpopulation 1 was ≥ 0.6 , indicating a relatively homogeneous genetic background. In contrast, Subpopulations 2 and 3 had Q values < 0.6 , suggesting that these two subpopulations integrated genetic components from multiple clusters and exhibited evident gene flow.

The first cluster contained 39 individuals, including 27 from Shanting District, 2 from Yicheng District, 2 from Shizhong District, and 7 from Tengzhou City. The second cluster comprised 28 individuals, including 18 from Shanting District, 2 from Yicheng District, and 5 from Tengzhou City. The third cluster contained 23 individuals,

including 68 from Shanting District, 9 from Yicheng District, 4 from Shizhong District, and 19 from Tengzhou City.

Using the method for estimating the optimal K value, Structure clustering analysis based on microsatellite data showed that ΔK reached its maximum at $K=3$; therefore, the optimal K value was 3. The studied populations were divided into three genetic clusters rather than being grouped according to sampling regions. This indicates the presence of gene flow among different regions, which is consistent with the results of the UPGMA clustering analysis.

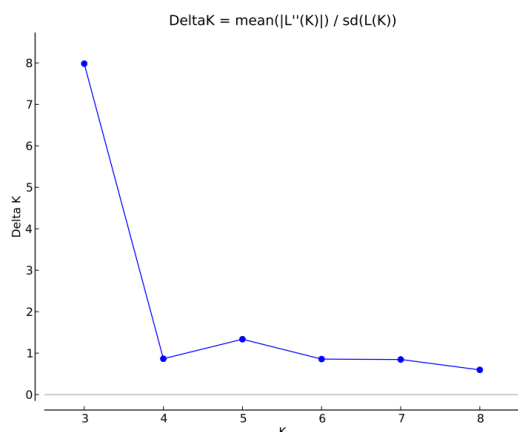


Figure 1 The deltaK (ΔK) values of structure output

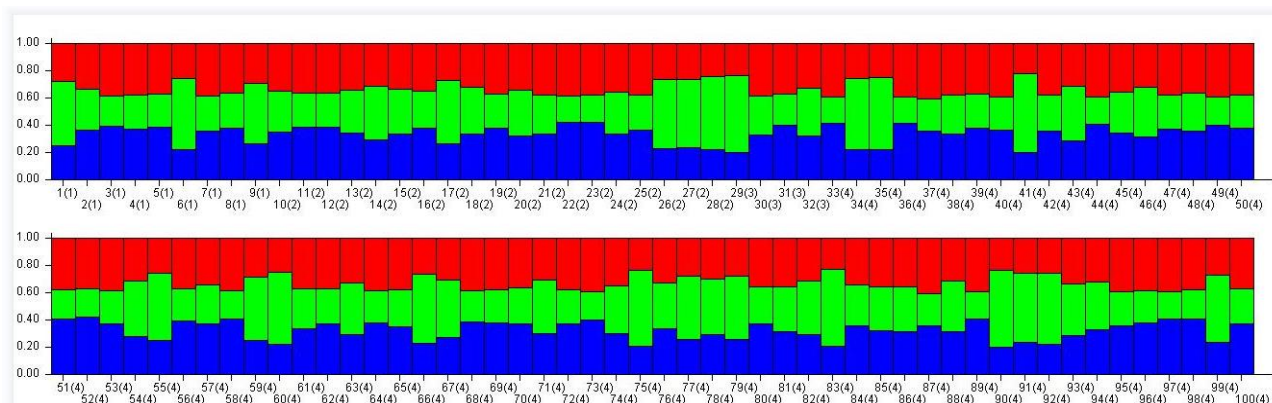


Figure 2 The structure output at $K=3$

Table 4 Distribution of *P. orientalis* germplasm subpopulations when $K=3$

Sub-population	Shanting	Yicheng	Shizhong	Tengzhou	Total	Q-Value
Sub-population 1	27	3	2	7	39	0.619
Sub-population 2	18	5	0	5	28	0.476
Sub-population 3	23	1	2	7	33	0.461
Total	68	9	4	19	100	0.5187

Using PowerMarker software, UPGMA clustering based on Nei's genetic distance was performed on 100 samples from four populations. The clustering results showed both similarities and differences compared with the three clusters identified by STRUCTURE. The main difference was that the proportions of samples from each provenance differed among the clusters. The similarity was that germplasm from the Shanting provenance was distributed across all three clusters. Based on the clustering outcomes from both methods, further analyses of kinship relationships among germplasm accessions can be conducted. To some extent, the clustering results indicate that genetic relatedness among populations is associated with geographic distribution; however, most samples did not cluster strictly according to their sampling regions, suggesting that there is a certain level of gene flow among different regions.

The clustering analysis of *Platycladus orientalis* (Figure 3) showed that red represents Cluster I, blue represents Cluster II, and yellow represents Cluster III. Cluster I contained 13 individuals, including 7 from Shanting District, 4 from Tengzhou City, and 1 from Yicheng District. Cluster II contained 23 individuals, including 21 from Shanting District and 2 from Shizhong District. Cluster III contained 64 individuals, including 40 from Shanting District, 15 from Tengzhou City, 8 from Yicheng District, and 1 from Shizhong District.

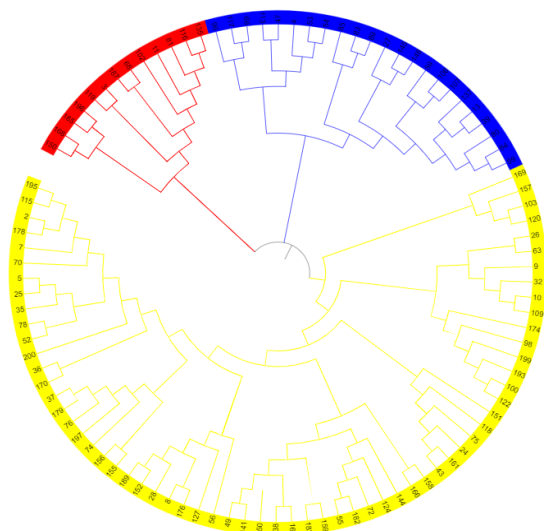


Figure 3 Phylogenetic tree of *Platycladus orientalis* based on SSR data

Using NTSYS software, UPGMA clustering analysis based on Nei's genetic distance was performed on 100 samples from four populations (Figure 4).

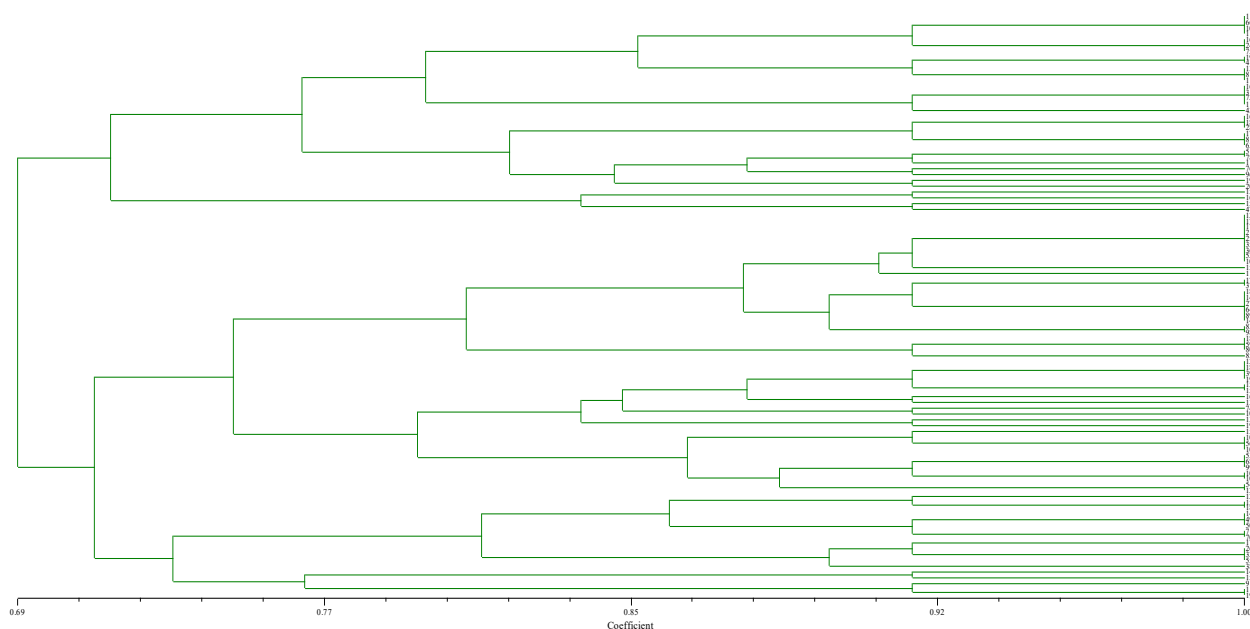


Figure 4 Dendrogram of *Platycladus orientalis* based on SSR data

At a similarity coefficient of approximately 0.69, the samples were divided into two clusters. Samples 135, 137, 141, and 145 collected from Shizhong District, which showed relatively close kinship, were grouped together with samples from other regions, while samples from the remaining regions were not strictly clustered according to their geographic origins. At a similarity coefficient of approximately 0.70, four clusters were identified, and the collected samples still did not cluster according to the four sampling regions. When the genetic similarity coefficient reached 0.77, the 100 *Platycladus orientalis* accessions were divided into eight clusters.

2 Discussion

In this study, seven polymorphic microsatellite primers with clear gel electrophoresis patterns and stable amplification across all populations were successfully selected, and corresponding DNA fingerprint profiles were established. These markers provide a valuable reference for future studies on the origin and evolution of *Platycladus orientalis* varieties, molecular-level identification and classification, hybrid breeding, and parental selection for genetic mapping. As an ideal type of molecular marker, microsatellites exhibit advantages such as good reproducibility, simplicity, efficiency, and high primer transferability in the identification of *P. orientalis* varieties. However, because the detection and application of SSR polymorphism largely depend on PCR amplification efficiency, different primers may require different reaction conditions. Therefore, it is essential to conduct preliminary optimization experiments for each primer and adopt appropriate strategies to maintain PCR reaction conditions at an optimal level.

In plant variety identification and classification studies, a more scientific approach is to integrate multiple methods and use them to complement and validate one another (Yuan et al., 2014). Identification results based on morphological traits and molecular markers are not always completely consistent. For example, some morphologically similar *P. orientalis* individuals were identified as hybrids in STRUCTURE clustering analyses. This phenomenon may be attributed to genetic variation caused by backcrossing and introgression, or to morphological variation resulting from convergent evolution and environmental selection (Rieseberg et al., 1999; Schwarzbach et al., 2001; Lexer et al., 2003). Similar patterns have also been observed in other populations exhibiting natural hybridization (Rieseberg, 1995). Any single method has inherent limitations, and relying on a single approach for species identification and classification makes it difficult to ensure the scientific rigor and reliability of the results.

Numerous studies have shown that factors such as genetic drift and gene flow have a substantial impact on population genetic structure. In recent years, parameters such as genetic differentiation coefficients have been widely used as important indicators for evaluating population genetic structure and kinship relationships among varieties (Song et al., 2011; Xu et al., 2014). Genetic variation analysis of *P. orientalis* populations using seven SSR markers showed that the average number of alleles (N_a) among the 100 samples from four sampling regions was 3.714, the average effective number of alleles (N_e) was 1.900, the average Shannon index (I) was 0.818, and the average expected heterozygosity (H_e) was 0.440. These results indicate that the 100 sampled accessions possessed relatively rich genetic diversity. The fixation index (F_{st}), which reflects the level of allelic heterozygosity among populations and is used to measure the degree of population differentiation, was 0.0371. This value falls within the range of 0–0.05, indicating high similarity among populations, small genetic distances, and very low genetic differentiation.

Results from principal coordinate analysis (PCoA), UPGMA clustering, and STRUCTURE clustering based on microsatellite data consistently showed that the 100 *P. orientalis* accessions were not strictly clustered according to their geographic origins. This suggests that the genetic backgrounds of the germplasm resources are relatively similar and that varying degrees of natural hybridization may occur among *P. orientalis* germplasm from different sampling regions. Hybridization promotes gene flow among populations and contributes to genetic evolution, thereby influencing population genetic structure and altering its overall pattern.

The formation of this spatial genetic variation pattern may be the result of the combined effects of long-distance gene flow, natural climatic conditions, and geographic isolation. In theory, geographically proximate regions tend to have similar soil conditions and environmental climates, resulting in less pronounced differences in natural selection pressures and increased opportunities for interpopulation gene exchange. Consequently, populations located closer to each other tend to have smaller genetic distances and higher genetic similarity.

At present, only a portion of *P. orientalis* resources has been collected, and the limited sample size may introduce bias into the analyses. Therefore, a more comprehensive evaluation and utilization of *P. orientalis* germplasm resources will require further investigation and research. To adapt to diverse ecological conditions and geographic environments, wild plant resources have undergone prolonged evolutionary processes involving intense survival

competition and extensive natural selection, resulting in extremely rich genetic diversity. These resources constitute complex natural gene pools that harbor many superior genes for disease resistance, pest resistance, and drought tolerance that are often absent in cultivated varieties, and thus can be used to improve the genetic basis of cultivated varieties (Tao et al., 2010; Jing et al., 2020). Influenced by artificial cultivation practices and breeding strategies, cultivated varieties have undergone substantial evolutionary changes; however, the patterns and directions of their evolution show considerable similarity to those of wild populations under natural conditions (Xu, 2005). The construction of genetic populations of *P. orientalis* and the exploration of their genetic diversity are therefore of great significance for the conservation and utilization of *P. orientalis* germplasm resources (Lei, 2018).

3 Materials and Methods

3.1 Overview of experimental material collection

In March 2021, a total of 100 *Platycladus orientalis* samples were collected from four regions in Zaozhuang City (Table 5): Shanting District (a68), Yicheng District (b9), Shizhong District (c4), and Tengzhou City (d19). A sampling strategy of collecting one mature individual at intervals of 10 m was adopted to avoid repeated sampling. Healthy upper leaves free of insect damage were collected as samples whenever possible. After collection, the samples were thoroughly dried using color-indicating silica gel, and DNA was subsequently extracted.

Table 5 Geographical location of *Platycladus orientalis* sampling sites

No.	Group	Region	Locality	Longitude	Latitude	Elevation (m)	Aspect
2	a	Shanting District	Huameizhuang	117°32'24"	35°01'05"	360	South
3	a	Shanting District	Shifosi	117°36'26"	35°01'38"	360	South
4	a	Shanting District	Shifosi	117°36'28"	35°01'39"	370	South
5	a	Shanting District	Shifosi	117°36'28"	35°01'40"	370	Southwest
7	a	Shanting District	Shifosi	117°36'28"	35°01'40"	384	Southeast
8	a	Shanting District	Shifosi	117°36'31"	35°01'39"	360	Southeast
9	a	Shanting District	Shifosi	117°36'22"	35°01'42"	410	East
10	a	Shanting District	Shifosi	117°36'37"	35°01'36"	330	South
11	a	Shanting District	Shifosi	117°36'22"	35°01'42"	390	South
13	a	Shanting District	Shifosi	117°36'29"	35°01'34"	380	South
24	a	Shanting District	Shifosi	117°36'32"	35°01'49"	326	Southwest
25	a	Shanting District	Glass Walkway South Mountain	117°35'27"	35°02'04"	340	Northeast
26	a	Shanting District	Glass Walkway South Mountain	117°35'25"	35°02'12"	330	North
27	a	Shanting District	Glass Walkway South Mountain	117°35'24"	35°02'01"	360	Northwest
28	a	Shanting District	Glass Walkway South Mountain	117°35'31"	35°02'01"	360	Northeast
32	a	Shanting District	Glass Walkway South Mountain	117°35'35"	35°01'54"	390	East
35	a	Shanting District	Mujia Cave West Mountain	117°35'54"	35°02'36"	270	Southwest
36	a	Shanting District	Mujia Cave West Mountain	117°35'55"	35°01'52"	280	West
37	a	Shanting District	Mujia Cave West Mountain	117°35'03"	35°02'19"	270	Southwest
38	a	Shanting District	Mujia Cave West Mountain	117°33'57"	35°02'02"	270	Northwest
39	a	Shanting District	Mujia Cave West Mountain	117°34'54"	35°02'36"	270	Southwest
43	a	Shanting District	Mujia Cave West Mountain	117°34'54"	35°02'36"	280	Southwest
47	a	Shanting District	East Dami Mountain	117°34'26"	35°03'00"	370	Southeast
49	a	Shanting District	East Dami Mountain	117°38'27"	35°02'59"	360	Southeast
50	a	Shanting District	East Dami Mountain	117°38'26"	35°03'04"	390	Northwest
52	a	Shanting District	Dajiao Mountain	117°38'27"	35°03'38"	390	West
53	a	Shanting District	East Dajiao Mountain	117°38'26"	35°02'51"	400	Southeast
54	a	Shanting District	Dajiao Mountain	117°38'27"	35°03'10"	246	Southwest
55	a	Shanting District	East Dajiao Mountain	117°38'25"	35°02'50"	400	East
56	a	Shanting District	Dajiao Mountain	117°38'15"	35°03'29"	400	West
59	a	Shanting District	East Dajiao Mountain	117°38'25"	35°02'48"	400	East
63	a	Shanting District	East Dajiao Mountain	117°38'25"	35°02'47"	410	Southeast
64	a	Shanting District	Northwest Mountain	117°36'31"	35°03'45"	280	East

No.	Group	Region	Locality	Longitude	Latitude	Elevation (m)	Aspect
66	a	Shanting District	Northwest Mountain	117°36'29"	35°03'40"	280	Southeast
68	a	Shanting District	Northwest Mountain	117°36'31"	35°03'43"	280	East
70	a	Shanting District	Northwest Mountain	117°36'38"	35°03'22"	280	South
72	a	Shanting District	Northwest Mountain	117°36'29"	35°03'40"	290	Southeast
74	a	Shanting District	Northwest Mountain	117°36'28"	35°03'40"	240	South
76	a	Shanting District	Northwest Mountain	117°36'27"	35°03'39"	280	Southwest
78	a	Shanting District	Northwest Mountain	117°36'40"	35°03'09"	280	South
80	a	Shanting District	Northwest Mountain	117°36'25"	35°03'38"	270	South
81	a	Shanting District	Yanggang Mountain	117°36'30"	35°02'55"	230	East
83	a	Shanting District	Yanggang Mountain	117°36'28"	35°02'57"	280	Southeast
85	a	Shanting District	Yanggang Mountain	117°36'10"	35°03'21"	300	Southwest
89	a	Shanting District	Yanggang Mountain	117°36'29"	35°02'56"	290	Southeast
96	a	Shanting District	Shengshan'an Pass	117°34'08"	35°00'19"	180	North
98	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'38"	290	Northwest
99	a	Shanting District	Yanggang Mountain	117°36'00"	35°03'22"	280	South
100	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'38"	216	Southwest
102	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'39"	213	Southwest
103	a	Shanting District	Jiguan Gu	117°36'57"	34°58'15"	270	Southwest
107	a	Shanting District	Jiguan Gu	117°37'09"	34°58'09"	260	Southwest
109	a	Shanting District	Jiguan Gu	117°36'46"	34°58'37"	300	Southwest
113	a	Shanting District	Baodu Gu	117°42'54"	34°59'11"	330	Northwest
115	a	Shanting District	Baodu Gu	117°42'55"	34°59'08"	340	Southwest
118	b	Yicheng District	Qingtian Temple	117°34'23"	34°57'39"	410	East
135	c	Shizhong District	Guishan Forest Farm	117°40'51"	34°46'43"	200	Northeast
151	d	Tengzhou City	East Mountain	117°23'13"	34°55'51"	120	East
155	d	Tengzhou City	Hutou Mountain	117°16'44"	34°53'28"	120	Northwest
166	d	Tengzhou City	Mushi Forest Farm	117°16'59"	34°58'08"	100	South
183	d	Tengzhou City	Hulutao	117°16'34"	34°52'58"	80	Northeast
200	a	Shanting District	Beiyu	117°25'53"	35°08'46"	360	Northeast

3.2 Experimental methods

3.2.1 Extraction and quality assessment of total plant DNA

Genomic DNA was extracted from the leaves of 100 *Platycladus orientalis* samples using a modified cetyltrimethylammonium bromide (CTAB) method. After the extracted DNA was completely dissolved, its quality was assessed by electrophoresis on 2% agarose gels.

3.2.2 Primer screening

In this study, SSR primers of *Platycladus orientalis* were developed based on the identification of SSR loci and primer screening from *P. orientalis* transcriptome sequences, and a total of 45 pairs of SSR primers were initially selected. Prior to large-scale PCR amplification and sequencing of all individuals, eight individuals were randomly selected for preliminary screening of the 45 microsatellite primers (Figure 5). Using the annealing temperature of 58 °C reported in the literature as a reference, PCR products were examined by 3% agarose gel electrophoresis and capillary sequencing. Primers with poor amplification efficiency or unclear banding patterns were discarded. Ultimately, seven pairs of SSR primers that produced clear gel electrophoresis profiles, could be stably amplified in all populations, showed satisfactory performance, and were easy to score and statistically analyze were selected for subsequent analyses.

3.2.3 SSR analysis

In this study, seven pairs of polymorphic primers with clear and well-resolved DNA bands were selected (Table 6). Detailed information on the PCR reaction system (Table 7) and amplification program is provided below.

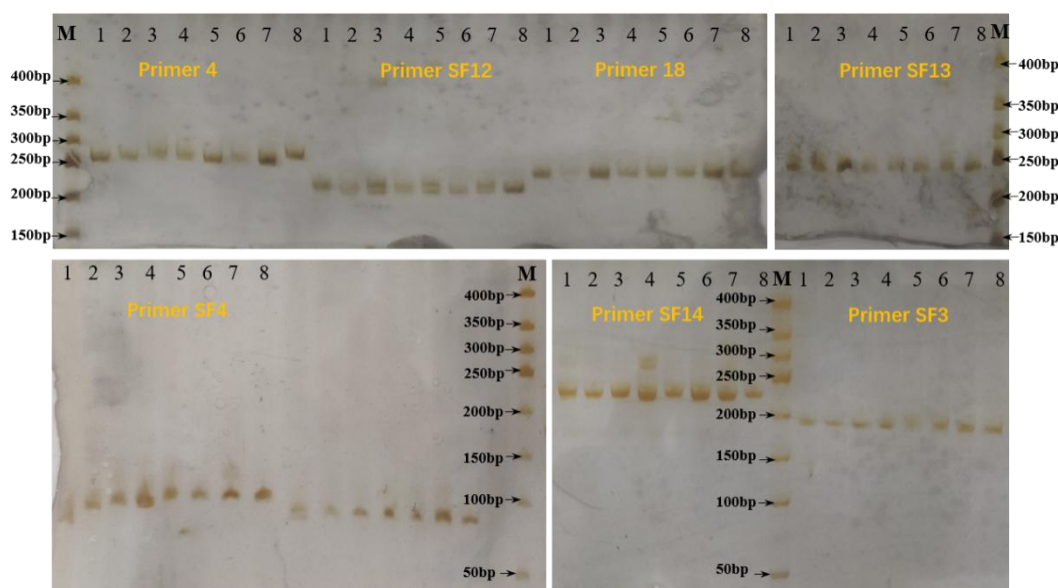


Figure 5 Amplification and detection of primers 4, 18, SF3, SF14, SF4, SF12 and SF13

Note: 1~8 The number of strains; M: 50bp DNA ladder

Table 6 Information of microsatellite primers used in this study

Locus name	Dye	Primer sequence (5'-3')	T _m (°C)	Allele size (bp)	Repeated motif
SF12	5'-FAM	F:AAACGAATGAGGCTGAATGG R:GGATGCACGCAATTTTCTTT	58	150-200	(AT) ₆
SF3	5'-FAM	F:GAGAGCTCTGCTGCCATCTT R:ATAACGTTCCCTGGCATCTG	58	150	(TC) ₆
SF4	5'-FAM	F:ATAAAAAAGTCCCCGGAGCAT R:GCCAGTGAAATTGAGGTTGC	58	100-150	(AG) ₉
18	5'-FAM	F:ACATTGATTTGCATTGGGGT R:AGAGCACATTCCGGTACCAC	58	200-250	(CA) ₆
SF13	5'-HEX	F:ACGGCCTTTGTTTTCTCTCA R:AAACCGCCAACACAGGTAAT	58	250-300	(GT) ₇
SF14	5'-HEX	F:CTTCGTCCCCGATACAAGAG R:CATCATGCCCCGATATCATCA	58	200-300	(CAG) ₆
4	5'-HEX	F:AGTGAGAGCACCTGCTGGAT R:AGCAGTGGGCTTTACCCTTT	58	300	(TTC) ₅ /(GGGTAAA) ₃

Table 7 The PCR reaction system of the microsatellite markers

Component	Volume (μL)
(Vazyme)2×Taq Master mix	12.5
Forward primer	1.5
Reverse primer	1.5
ddH ₂ O	6
DNA template	1.5
Total	20

The PCR reaction program was as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 5 min, and then held at 4 °C. After completion of the PCR reactions, the products were examined by electrophoresis on 3% agarose gels. Qualified PCR amplification products were sent to an automated sequencer (Applied Biosystems) for allele genotyping. GeneMarker software was used to read allele sizes, and genotyping results were obtained for 100 *Platygladus orientalis* individuals.

PowerMarker V3.25 software was used to analyze the genotype data, including the number of alleles, number of genotypes, heterozygosity, and polymorphic information content (PIC) for different sample combinations. Genetic distances among varieties were also calculated, and clustering was performed using the unweighted pair-group method with arithmetic means (UPGMA) (Liu, 2005).

Author Contributions

Zhou Jilei and Li Jingtao were responsible for the experimental design and the execution of the experimental research. Zhou Jilei and Zhang Liudong carried out data analysis and prepared the first draft of the manuscript. Fu Yinyin completed the experimental design and analysis of the experimental results. Chen Yong participated in sample collection for the study. Li Jingtao conceived and led the project and provided guidance on experimental design, data analysis, and manuscript writing and revision. All authors read and approved the final manuscript.

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Research Report

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Regulatory Effects of Nursery Mode and Canopy Closure on the Establishment Survival Rate of *Tetrastigma hemsleyanum* and Delineation of the Optimal Closure Range

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Abstract To clarify the regulatory factors affecting the establishment survival rate of *Tetrastigma hemsleyanum*, cutting propagation experiments and field establishment trials were conducted to compare survival performance under different nursery modes and canopy closure conditions. The results demonstrated significant differences in survival rates among the three nursery modes, with container substrate cultivation showing significantly higher survival than direct field cuttings. Establishment survival also differed significantly across canopy closure levels, exhibiting a unimodal response pattern along the closure gradient. Quadratic regression analysis indicated that the predicted maximum survival rate occurred at a canopy closure of approximately 0.67. Based on comprehensive statistical analyses and trend fitting, the optimal canopy closure range for the establishment of *T. hemsleyanum* was determined to be 0.6–0.7. These findings provide quantitative support for understory cultivation management of *T. hemsleyanum*.

Keywords *Tetrastigma hemsleyanum*; Establishment; Nursery mode; Canopy closure; Survival rate

1 Introduction

Tetrastigma hemsleyanum Diels et Gilg is a perennial climbing vine belonging to the Vitaceae family and is one of the important medicinal resource plants in southern China. Its tuberous roots are rich in polysaccharides, flavonoids, and various bioactive compounds, exhibiting considerable pharmacological potential in anti-inflammatory, antitumor, and immunomodulatory applications. With increasing market demand and continuous depletion of wild populations, artificial cultivation has become an essential approach for ensuring sustainable resource utilization. Within the cultivation system, establishment represents the critical transition from nursery production to subsequent field management. The survival rate during this stage directly affects planting costs, population structural stability, and future yield potential. Forest ecological studies have demonstrated that the seedling establishment phase often constitutes a demographic bottleneck in regeneration processes, where high mortality restricts the transition of individuals to stable populations (Chang-Yang et al., 2021; Stone et al., 2025). Under closed canopy conditions, seedlings may persist in the understory for extended periods, yet only a small proportion successfully overcome early survival constraints, thereby influencing community structure and regeneration trajectories (Lin et al., 2014). Therefore, improving establishment survival is a prerequisite for large-scale cultivation and stable production.

T. hemsleyanum is commonly distributed along forest edges or in open woodland environments and is considered a typical understory-adapted species. Canopy closure, as a structural indicator of canopy coverage, directly regulates understory light intensity, light quality, and microclimatic conditions. Under closed canopies, light availability may decline to 10%~15% of full sunlight, substantially affecting seedling photosynthetic performance and carbon balance (Zhou et al., 2023). Shade-tolerant or semi-shade-tolerant species generally exhibit higher light-use efficiency under moderate diffuse light, whereas excessive irradiance may induce photoinhibition and severe shading may reduce net photosynthetic rates due to insufficient light availability (George and Bazzaz, 1999; De Lombaerde et al., 2020). Variations in canopy gap size and openness can markedly alter early seedling survival conditions; however, increased light availability may simultaneously promote rapid expansion of

understory vegetation, generating an ecological filtering effect (Lu et al., 2021; Liu et al., 2022). Thus, canopy closure not only determines the light environment but also indirectly influences water balance and neighborhood competition intensity, thereby jointly affecting establishment survival. Nevertheless, quantitative studies examining the gradient response of *T. hemsleyanum* establishment survival to canopy closure and delineating an optimal closure range remain limited.

In addition to light conditions, nursery mode constitutes a key technical factor influencing establishment success. Global-scale restoration studies have shown that seedling size and root development quality significantly affect post-transplant survival (Andivia et al., 2021). Larger seedlings or those with well-developed root systems typically exhibit enhanced water uptake capacity and carbon reserves, thereby improving tolerance to drought or low-light stress (Wu et al., 2024). Container cultivation can improve substrate structure and rhizosphere aeration, contributing to root integrity and facilitating recovery after transplanting, whereas direct field cuttings are more susceptible to soil compaction and pathogen pressure. The degree of matching between seedling quality and stand conditions is therefore critical for successful establishment. However, in the cultivation practice of *T. hemsleyanum*, the combined regulatory effects of nursery mode and canopy closure have not yet been systematically compared or ecologically interpreted.

The present study focuses on the establishment survival rate of *T. hemsleyanum*, systematically comparing responses under different nursery modes and canopy closure conditions. By analyzing their regulatory effects and delineating the optimal canopy closure range, this study aims to provide a scientific basis for large-scale understory cultivation of *T. hemsleyanum* and offer reference insights for ecological suitability studies of other understory medicinal plants.

2 Materials and Methods

2.1 Study area

The experimental site was located in the Lühetang forest region of Shouchang Forest Farm, Zhejiang Province, China. The area is characterized by a subtropical humid monsoon climate, with a mean annual temperature of 16 °C~18 °C and abundant annual precipitation. The terrain consists primarily of low mountains and hills, with elevations ranging from 200 to 400 m above sea level. The soil type is red soil with slightly acidic properties and a soil depth generally exceeding 30 cm. The dominant forest types include *Cunninghamia lanceolata* plantations, *Phyllostachys edulis* (Moso bamboo) forests, natural broadleaf forests, and *Metasequoia glyptostroboides* stands, providing suitable ecological conditions for understory cultivation experiments.

2.2 Cutting propagation experiment design

2.2.1 Nursery mode settings

Cutting propagation experiments were conducted in spring 2021. Cuttings were collected from healthy 1-3-year-old mother plants. Each cutting contained 2-3 nodes, with a length of 10-15 cm and at least two retained leaves.

Three nursery modes were established:

- (1) Mode I: Container cultivation in a greenhouse using a self-formulated substrate composed of peat (20%), organic fertilizer (20%), rice husk powder (10%), yellow subsoil (48%), and calcium–magnesium phosphate fertilizer (2%). Container size was 6.5 cm × 6.5 cm.
- (2) Mode II: Non-woven fabric container cultivation. The substrate consisted of peat (40%), rice husk powder (10%), organic fertilizer (10%), vermiculite (10%), and perlite (25%). Container size was 5 cm × 8 cm.
- (3) Mode III: Direct cutting insertion in prepared field beds in the forest, with trenches 30 cm wide and 30 cm deep.

Each nursery mode included 50 randomly assigned cuttings per replicate, with three replicates per treatment, totaling 450 cuttings.

2.2.2 Operational procedures and variable control

Cutting size, timing (mid-March to before the plum rain season in June), and routine management practices were standardized across treatments. Container seedlings were maintained under shaded conditions with substrates kept moist but well-drained. Field cuttings received equivalent irrigation management. No additional growth regulators were applied, ensuring that treatment differences primarily reflected nursery mode effects.

2.3 Field establishment experiment design

2.3.1 Plot arrangement

From April to June 2022, container-grown seedlings produced in 2021 were transplanted into four forest stand types. The site conditions were as follows:

Plot 1: Moso bamboo forest, canopy closure 0.5, quasi-wild establishment;

Plot 2: Chinese fir forest (*Cunninghamia lanceolata*), canopy closure 0.6, quasi-wild establishment;

Plot 3: *Metasequoia glyptostroboides* stand, canopy closure 0.7, cultivation in biodegradable planting bags;

Plot 4: Natural broadleaf forest, canopy closure 0.8, quasi-wild establishment.

For quasi-wild establishment, plant spacing was 0.3 m × 0.5 m. Each plot included 100 seedlings per replicate, with three replicates, totaling 1 200 seedlings.

2.3.2 Measurement of canopy closure

Canopy closure was determined by averaging measurements from multiple sampling points within each plot. At least five evenly distributed points were established per plot. Canopy cover proportion was visually estimated at each point, and the mean value was calculated as the canopy closure index for that plot.

2.4 Survival assessment and indicator definition

Survival in the cutting experiment was defined as successful root formation accompanied by resumption of new shoot growth. Establishment survival in the field was defined as seedlings maintaining normal aboveground growth without mortality until October of the same year.

Survival rate was calculated as: Survival rate (%) = (Number of surviving individuals / Total number of individuals surveyed) × 100%.

2.5 Data analysis and statistical models

2.5.1 Comparison of survival rates

Differences in survival rates among nursery modes and forest stand treatments were analyzed using contingency table chi-square (χ^2) tests. When overall differences were significant, pairwise comparisons were performed using Fisher's exact test, with Bonferroni correction applied for multiple comparisons.

2.5.2 Effect size estimation

Effect size of survival differences between treatments was quantified using Relative Risk (RR), and 95% confidence intervals were calculated to evaluate the magnitude of treatment effects.

2.5.3 Analysis of canopy closure response trends

To analyze the response pattern of survival rate to canopy closure, a quadratic polynomial model was constructed for descriptive fitting between canopy closure and survival rate. Curve visualization was used to evaluate the presence of a unimodal response pattern and to delineate the canopy closure range associated with higher survival. All statistical analyses were conducted using proportional data, with a significance level set at $\alpha = 0.05$.

3 Results and Analysis

3.1 Differences in cutting survival among nursery modes

The survival performance of *Tetrastigma hemsleyanum* cuttings under the three nursery modes is presented in Figure 1. Each mode included 50 cuttings. Mode I had an average of 47 surviving individuals, corresponding to a

survival rate of 94%; Mode II had an average of 45 survivors, with a survival rate of 90%; and Mode III had an average of 35 survivors, with a survival rate of 70%.

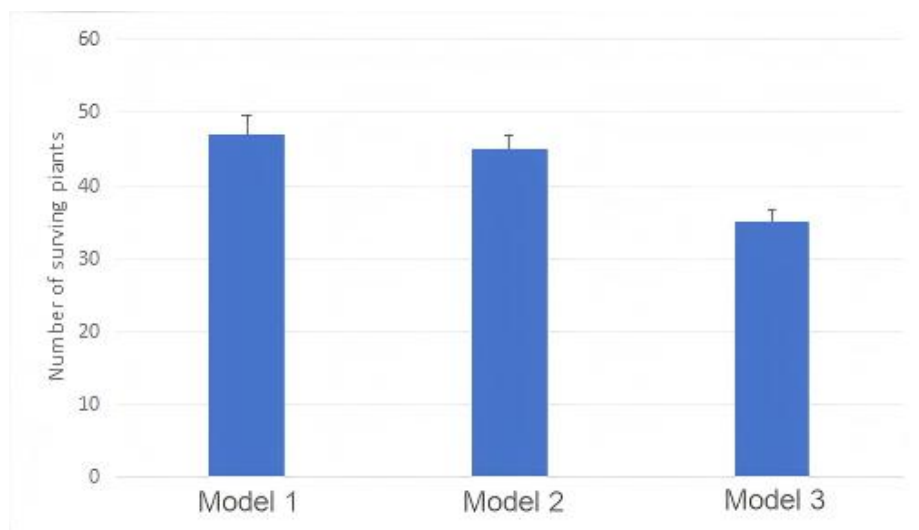


Figure 1 Survival rate under different nursery modes

The contingency table chi-square test indicated significant differences in survival rates among nursery modes ($\chi^2=12.74$, $df=2$, $p=0.0017$). Pairwise comparisons showed that the difference between Mode I and Mode III was significant (Fisher's exact test, $p<0.01$). The difference between Mode II and Mode III reached significance before correction ($p\approx 0.02$) but was not significant after Bonferroni adjustment. No significant difference was detected between Mode I and Mode II ($p>0.05$).

Relative risk (RR) analysis showed that, compared with Mode III, both Mode I and Mode II had RR values greater than 1, with 95% confidence intervals not crossing 1. Overall, container-based nursery modes exhibited approximately a 20-percentage-point higher survival rate than direct field cutting.

3.2 Effects of Canopy Closure Gradient on Establishment Survival

The establishment survival of *T. hemsleyanum* under different forest stand conditions is shown in Figure 2. Each plot included 100 transplanted individuals. In the canopy closure 0.6 stand (*Cunninghamia lanceolata* plantation), the average number of surviving plants was 85 (85% survival); in the 0.5 closure stand (Moso bamboo forest), 60 plants survived (60%); in the 0.8 closure stand (natural broadleaf forest), 75 plants survived (75%); and in the 0.7 closure stand (*Metasequoia glyptostroboides*, biodegradable planting bags), 95 plants survived (95%).

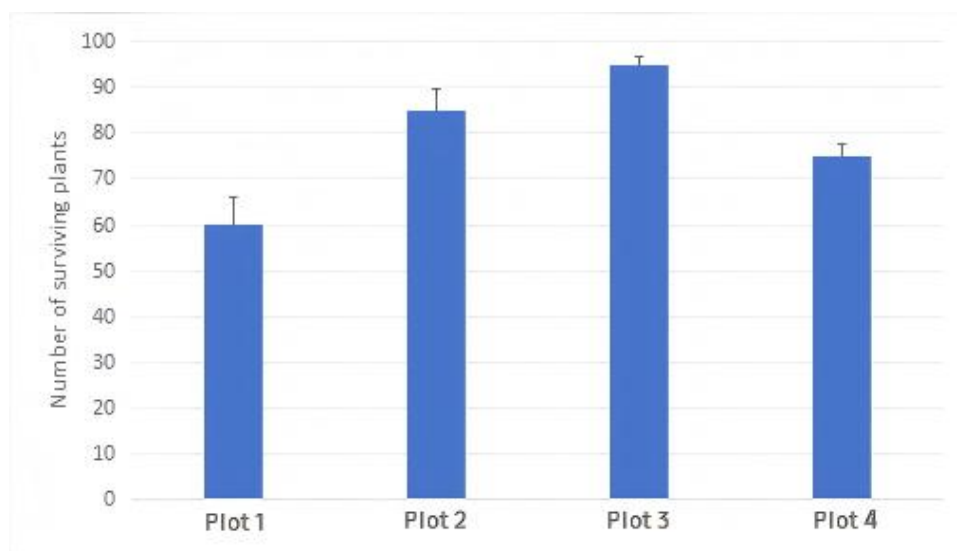


Figure 2 Establishment survival rate under different canopy closure levels

The contingency table chi-square test revealed significant differences in survival rates among the four stand conditions ($\chi^2=39.96$, $df=3$, $p<0.001$). Survival rates ranked as follows: $0.7>0.6>0.8>0.5$. Pairwise comparisons indicated that survival under canopy closure 0.7 was significantly higher than under 0.5 and 0.8 ($p<0.001$), and survival under 0.6 was significantly higher than under 0.5 ($p<0.001$). No statistically significant difference was detected between 0.6 and 0.8.

Relative risk analysis showed that the establishment survival rate under canopy closure 0.7 was approximately 1.58 times that under closure 0.5, with the 95% confidence interval not crossing 1. Overall, survival increased as canopy closure rose from 0.5 to 0.7 and declined at 0.8.

3.3 Integrated response characteristics of nursery mode and stand conditions

The stage-wise results showed that container substrate nursery modes exhibited higher survival during the cutting phase. In the field establishment experiment, survival under canopy closure levels of 0.6~0.7 was generally higher than under 0.5 or 0.8, with the highest survival rate (95%) observed at canopy closure 0.7. In contrast, survival declined under both lower and higher closure levels. These results reflect differences in establishment survival across nursery modes and stand conditions. As the experimental design did not constitute a fully factorial combination, the integrated response characteristics are based on stage-wise comparisons.

3.4 Summary of the response pattern between canopy closure and survival

To examine the relationship between canopy closure and establishment survival rate, scatter fitting was performed between canopy closure values and corresponding survival rates (Figure 3). The results showed a near-unimodal response pattern along the canopy closure gradient. Survival increased progressively as canopy closure rose from 0.5 to 0.7 and declined at 0.8.

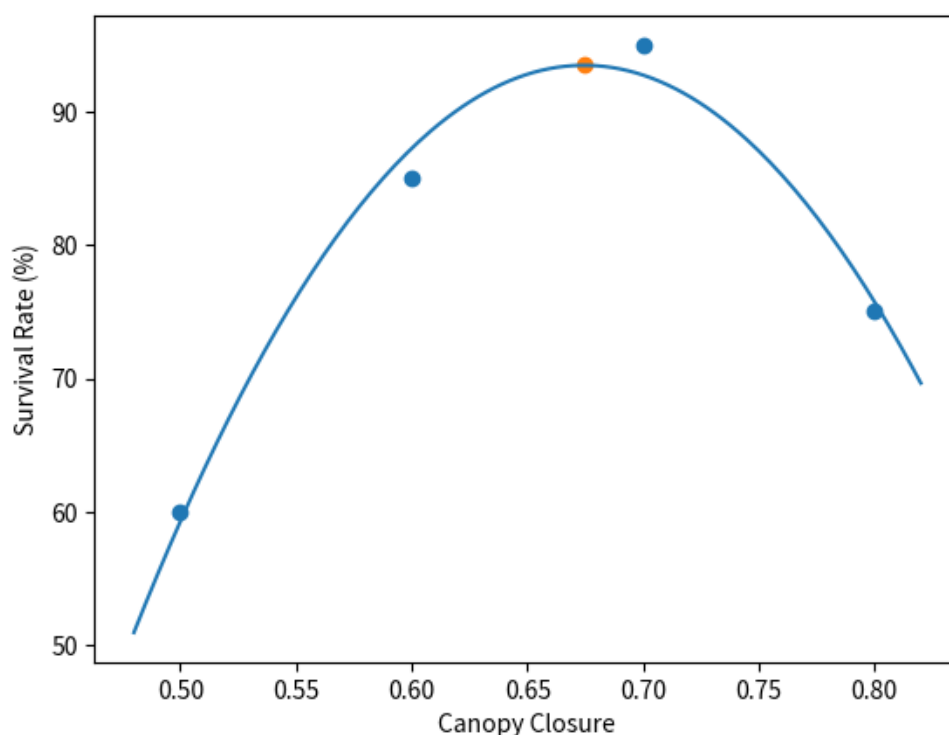


Figure 3 Response of survival rate to canopy closure

A quadratic polynomial model was applied for descriptive fitting, and the vertex of the fitted curve corresponded to a canopy closure of approximately 0.67, with a predicted survival rate of approximately 93%~94%. The fitted trend was consistent with the observed data pattern. Given that the canopy closure gradient consisted of only four discrete levels and that stand type and cultivation method differed among plots, the fitted model is primarily descriptive and used to delineate the range associated with higher survival. Based on both fitted results and observed data, the optimal canopy closure range for *T. hemsleyanum* establishment was defined as 0.6~0.7.

4 Discussion

The principal findings of this study regarding the establishment survival rate of *Tetrastigma hemsleyanum* can be interpreted within an ecological regulation framework integrating individual attributes, resource availability, and neighborhood pressure. Studies on forest regeneration have demonstrated that the transition from establishment to stable growth often constitutes a demographic bottleneck, where minor differences in individual traits and microhabitat conditions may be amplified into substantial variation in survival outcomes (Chang-Yang et al., 2021). In the present study, survival rate exhibited a pronounced nonlinear response to canopy closure, indicating that *T. hemsleyanum* is sensitive during the establishment stage to both excessive environmental stress and excessive resource limitation. This pattern is consistent with conclusions that niche differentiation and negative density dependence jointly shape seedling survival dynamics (Johnson et al., 2017). Therefore, the optimal canopy closure should be understood as a manageable operational range that reduces desiccation risk and temperature fluctuations while maintaining adequate photosynthetic returns and keeping understory competition at a moderate level. Long-term plot studies have further shown that habitat heterogeneity and density dependence exert persistent influences on seedling survival (Magee et al., 2020), providing theoretical support for the canopy closure range identified in this study.

From a mechanistic perspective, the unimodal response pattern observed here is consistent with classical interpretations of understory plant responses to light environments. Excessive shading may reduce net photosynthetic capacity and disrupt carbon balance, whereas excessive irradiance may increase transpiration and leaf temperature, leading to water deficit and photoinhibition risk. Moreover, changes in canopy structure influence ventilation and humidity conditions, thereby altering pathogen incidence. Research on understory vegetation as an ecological filter has indicated that shading and understory structural characteristics jointly regulate seedling growth and survival (George and Bazzaz, 1999). Multifactorial experiments have further demonstrated significant interactions among light, temperature, and understory cover, with combined effects often exhibiting nonlinear characteristics (De Lombaerde et al., 2020). Consequently, differences in survival under varying canopy closure levels observed in this study likely reflect the combined effects of light availability, water balance, and microclimatic stability rather than changes in light intensity alone.

Differences among nursery modes further highlight the importance of individual attributes during the establishment stage. A global meta-analysis has shown that initial seedling size and stress-resistance traits significantly affect post-transplant survival, with larger individuals generally exhibiting higher survival probabilities across most site conditions (Andivia et al., 2021). In restoration experiments conducted under extreme heat stress, initial seedling height explained survival variation more effectively than functional trait indicators (Gardiner et al., 2019). Additionally, life-history stage modulates the relative importance of neighborhood effects, with individual attributes and microhabitat conditions often playing a dominant role during early stages (Pu et al., 2020). These findings are consistent with the present results, indicating that root system quality and individual robustness developed during the nursery stage determine the capacity of seedlings to overcome the establishment bottleneck. Container-based nursery cultivation enhances rhizosphere conditions and preserves root integrity, thereby increasing tolerance thresholds to water fluctuations and shading stress and improving establishment stability.

In a broader comparative context, the conclusion that moderate canopy closure favors survival is largely consistent with observations from forest regeneration and restoration studies across multiple regions, although its applicability remains context-dependent. Research in northern coniferous forests has shown that overstory density and ground vegetation cover jointly determine seedling emergence and survival, and management must balance seedbed improvement with competition control (Kyrö et al., 2021). For *T. hemsleyanum*, an understory medicinal vine, the objective of establishment emphasizes stable survival and subsequent tuber development. Its sensitivity to strong irradiance and excessive moisture conditions may differ from that of typical tree regeneration. Therefore, under similar canopy closure levels, variation in soil moisture, ventilation, and understory vegetation structure among forest types may lead to different survival outcomes, representing an important source of inter-plot variability.

It should be noted that the canopy closure gradient in this study was relatively limited and did not constitute a fully factorial design. Accordingly, inferences regarding potential interactions between nursery mode and canopy closure should be made with caution. Future studies should refine canopy closure gradients under controlled forest-type conditions and incorporate high-resolution monitoring of microclimate and soil moisture to further disentangle the relative contributions of light, water availability, and competition. Long-term follow-up experiments are also necessary to verify whether the identified optimal establishment range can be consistently translated into subsequent growth performance and population structural advantages, thereby enhancing both theoretical interpretation and practical management guidance.

5 Conclusion

This study systematically analyzed differences in establishment survival of *Tetrastigma hemsleyanum* under different nursery modes and canopy closure conditions. The results demonstrated that nursery mode significantly affected establishment survival. Container-based substrate cultivation resulted in higher survival rates, indicating that initial seedling quality and root system development are fundamental factors determining establishment stability. Optimization of nursery practices enhances seedling adaptability to understory environments.

Canopy closure exhibited a significant gradient effect on establishment survival, with survival rates displaying a unimodal response pattern along the closure gradient. Based on statistical testing and response curve analysis, the optimal canopy closure range for *T. hemsleyanum* establishment was determined to be 0.6–0.7. Within this range, understory light and microclimatic conditions were relatively balanced, and establishment survival remained comparatively stable.

Overall, establishment success of *T. hemsleyanum* is jointly regulated by seedling quality and stand structure. Developing establishment strategies that align nursery practices with appropriate canopy closure conditions represents an effective technical pathway for improving the stability of large-scale understory cultivation. By delineating the optimal canopy closure range from an ecological regulation perspective, this study provides quantitative support for understory cultivation management of *T. hemsleyanum*.

Author Contributions

Li Jianhui and Xu Yonghong designed and conducted the experiments. Li Jianhui, Zhang Yehua, and Xu Yonghong performed the data analysis and drafted the initial manuscript. Fang Yumin and Fan Jianzhong contributed to the experimental design and analysis of the experimental results. Xu Yonghong conceived and led the project and supervised the experimental design, data analysis, manuscript writing, and revision. All authors have read and approved the final version of the manuscript.

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Case Study

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The Genetic Basis of Maple Leaf Color and Its Application in Landscape Design

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Abstract This study mainly analyzes the genetic causes of the color formation of maple leaves and its practical application in landscape design. Maple trees are an important ornamental tree species in temperate regions, and the leaf color changes significantly with the seasons. This change is mainly related to three types of pigments, namely chlorophyll, carotenoids, and anthocyanins. The content and proportion of these pigments directly affect the leaf color. Research has found that genes related to anthocyanin synthesis, such as *CHS*, *UFGT*, and *DFR*, as well as related transcription factors, such as MYB and bHLH, play an important role in the formation of leaf color. Different maple tree varieties have differences in the expression of these genes, thus showing various leaf colors such as red, yellow, and green. In addition to genetic factors, environmental conditions such as light and temperature also affect the accumulation of pigments and gene expression, thereby causing changes in leaf color. In landscape construction, maple trees are often used in parks, roads, and scenic areas. By reasonably combining different leaf-color maple tree varieties and combining with evergreen trees, water features, and other landscape elements, a landscape effect with clear layers and prominent seasonal characteristics can be formed. The relevant case in Kyoto, Japan, shows that when large areas of maple forests are combined with the natural environment, they can demonstrate high ecological value, cultural value, and economic value. In the future, using genetic information for targeted breeding is expected to provide more new maple tree varieties with both ornamental value and adaptability for urban greening.

Keywords Maple; Leaf color; Genetic basis; Anthocyanins; Landscape design

1 Introduction

Maple trees are very common ornamental tree species in temperate regions. People like maple trees mainly because of their attractive tree shape, wide range of heights, and rich leaf colors (Zhu et al., 2022). There are approximately 100 to 200 native species of maple trees, and many more are artificially selected varieties. They are commonly found along roadsides, in parks, residential areas, and botanical gardens, and are frequently used as tree species in landscaping. Maple trees grow quickly and have good shading effects. In autumn, the leaf colors become very vivid, so they play an important role in urban greening and seedling cultivation. Maple trees not only have strong ornamental value but also can improve the surrounding environment and provide habitats for animals. Therefore, they have both landscape value and ecological value (Luo et al., 2023a; Sun et al., 2024). In recent years, with the continuous emergence of new varieties such as dwarf Japanese maple and large shading maple, the application of maple trees in urban greening construction and garden cultivation has become increasingly widespread (Chen et al., 2019; Lin et al., 2022).

One of the most obvious characteristics of maple trees is that their leaf color changes significantly with the seasons. Different varieties of leaves can present various colors such as green, yellow, orange, and red. Sometimes, different colored patches can also appear on the leaves of the same tree (Chen et al., 2019; Gong et al., 2025). Many maple trees change from green to red or yellow in autumn, and this change directly affects their ornamental and economic value (Zhang et al., 2023). Studies have found that this leaf color change is mainly related to the decrease in chlorophyll content and the increase in anthocyanin content. This process also occurs in tree species such as ginkgo and red maple (Luo et al., 2023a; Fan et al., 2024; Sun et al., 2024). The same maple tree can have green, yellow, or red leaves in different seasons or branches, indicating that pigment metabolism is not only influenced by environmental conditions but also regulated by its own regulatory mechanisms (Yang et al., 2022;

Zhu et al., 2022). This leaf color change has a significant impact on the landscape effect. By rationally combining plants of different colors, the visual effect of the landscape can be enhanced (Wang, 2021; Mu et al., 2022).

With the continuous advancement of research techniques, people's understanding of the genetic mechanism of the color change of maple leaves has become more profound. Through genomic research, scientists have discovered some important genes related to anthocyanin synthesis, such as chalcone synthase and UFGT. At the same time, some transcription factors that can affect pigment accumulation and seasonal changes have also been found, such as MYB and bHLH (Sun et al., 2024). In the study of *Acer truncatum*, it was found that the phenomenon of gene duplication and the different expression levels of UFGT are closely related to the formation of red and yellow leaf varieties (Zhang et al., 2023). In the study of *Acer palmatum*, some gene combinations, such as ApWRKY26/ApERF4-ApMYB2, also participate in the synthesis process of anthocyanins in different seasons and play a regulatory role in the color change of leaves (Zhu et al., 2022; Chen et al., 2025). These studies indicate that the formation of the color of maple leaves is not only controlled by genetic factors but also influenced by environmental conditions. In the future, if the related physiological activities and gene expression can be regulated, there is hope to cultivate new ornamental varieties with more stable leaf colors or more obvious seasonal color changes (Zhao et al., 2020; Fan et al., 2024).

This study focused on analyzing the genetic basis of the color change of maple leaves and explored its application value in variety breeding and landscape design. By studying the relevant key genes and molecular markers, it is hoped to cultivate new varieties with brighter leaf colors, longer ornamental periods, and stronger adaptability. At the same time, the research on seasonal plant color combinations can also provide certain references for urban green space landscape design. Currently, the research results in leaf color genetics and the color configuration and spatial layout in landscape design still lack effective integration. This study attempts to link the leaf color formation mechanism with variety selection and seasonal configuration, providing ideas for ornamental plant breeding and further enhancing the visual, ecological and cultural value of maple tree landscapes.

2 Leaf Color Formation Mechanism

2.1 Functions of chlorophyll, carotenoids and anthocyanins

The color of maple leaves is mainly determined by three types of pigments, including chlorophyll a and b, carotenoids, and anthocyanins. Chlorophyll a and b are very important pigments for photosynthesis in plants. They mainly absorb blue light and red light, so in the season when plants are growing normally, the leaves are generally green (Lu et al., 2020). Carotenoids mainly include lutein and carotene. These pigments mainly absorb blue-violet light and their colors are usually yellow to orange. At the same time, they can also help the leaves reduce damage caused by strong light (Xie et al., 2023; Tian et al., 2024). Anthocyanins are water-soluble pigments present in the cell sap. Depending on their structure and the internal environment of the cells, they can manifest as red, purple or blue, and are particularly prominent in many colorful maple leaves (Chen et al., 2019; Jie et al., 2019; Gong et al., 2025).

These three types of pigments jointly determine the color of leaves and also participate in many normal life activities within the plant. Taking red maple as an example, chlorophyll is mainly related to photosynthesis, while carotenoids and anthocyanins are important substances that affect the color of leaves, and they can also help the plant adapt to external conditions such as strong light and temperature changes (Jie et al., 2019; Yang et al., 2022; Zhang et al., 2022; Gong et al., 2025). Studies have found that the formation process of these pigments is controlled by many genes, and the changes in the content of the three pigments, namely their increase or decrease, are the main reasons for the leaves to gradually change from green to yellow or red (Chen et al., 2019; Lu et al., 2020; Fan et al., 2024).

2.2 Diversity of maple leaf colors and pigment proportions

The colors of maple leaves vary mainly depending on the content and proportion of chlorophyll, carotenoids and anthocyanins. Taking a mutant red maple as an example, its leaves are divided into three types: green (GL), red (RL), and yellow (YL). Detection shows that the chlorophyll content is the highest in GL, medium in RL, and the lowest in YL; carotenoids are the most abundant in GL, and less and similar in RL and YL. The anthocyanin

content in red leaves is much higher than that in green and yellow leaves, presenting a red-green mixture color; yellow leaves are due to a significant reduction in chlorophyll and the appearance of carotenoids, with very little anthocyanin accumulation, thus appearing yellow (Chen et al., 2019; Lu et al., 2020). In different red maple varieties, the degradation of chlorophyll and the large synthesis of anthocyanins together are the key to forming stable red leaves (Jie et al., 2019).

This phenomenon is also common in other maple trees and many other deciduous plants with distinct leaf colors. Generally speaking, the chlorophyll content in red-leaved varieties is usually lower than that in green-leaved or yellow-leaved varieties, while the contents of carotenoids and anthocyanins are higher. Among them, anthocyanins play an important role in the formation of leaf color (Chen et al., 2019; Zhang et al., 2022; Xie et al., 2023; Gong et al., 2025). Multi-gene studies on tree species such as red maple (*Acer rubrum*) have found that during the leaf color change process, the contents of chlorophyll and carotenoids gradually decrease, while some anthocyanins, such as anthocyanin-3, 5-diglucoside, increase continuously, eventually causing the leaf color to gradually change from green to red (Fan et al., 2024). Therefore, the different colors of maple leaves are actually related to the changes in the proportion of various pigment contents: when the chlorophyll content is high, the leaves usually appear green; when there is more carotenoids and less anthocyanins, the leaves are mostly yellow; when anthocyanins accumulate in large quantities, the leaves will turn red.

2.3 Seasonal changes and the formation of red leaves

The reddening of leaves is mainly due to changes in the quantities of several pigments within the leaves. As the seasons change and the leaves gradually age, chlorophyll gradually decreases, and the original green color becomes increasingly pale, eventually disappearing completely. As a result, the less obvious color of carotenoids becomes apparent. At the same time, many maple trees will produce more anthocyanins, or increase the existing anthocyanin content (Chen et al., 2019; Zhang et al., 2022). Studies on red maples and sugar maples have shown that in autumn, the chlorophyll in the leaves significantly decreases, and the content of carotenoids also changes, while anthocyanins accumulate continuously, especially in those leaves that change color earlier and have darker colors, this situation is more obvious (Mattila and Tyystjärvi, 2023). Transcriptome and metabolome studies have also found that during the process of leaf reddening, the expression of genes related to anthocyanin synthesis increases, thereby promoting the continuous accumulation of anthocyanins in the leaves (Fan et al., 2024; Gong et al., 2025).

The external environment can also affect the color changes of the leaves. Lower temperatures, larger diurnal temperature differences, and sufficient light all facilitate the breakdown of chlorophyll and promote the formation of anthocyanins. Therefore, the leaves on the outer part of the tree crown, or those that receive more sunlight, are usually more likely to turn red (Zhang et al., 2022; Mattila and Tyystjärvi, 2023; Xie et al., 2023). Additionally, if the branches of red maple trees are cut, the nutrient transportation within the branches will change, and the distribution of pigments in the leaves above the treated area will also change, so the leaves will turn red earlier (Yang et al., 2022). In summary, the red color of autumn maple leaves is the result of the combined effects of reduced chlorophyll, changes in carotenoids, and increased anthocyanins. These changes also form the common red-orange forest landscape seen in temperate regions during autumn.

3 Case Study: Maple Landscape in Arashiyama, Kyoto, Japan

3.1 Famous autumn foliage formed by extensive maple planting

Arashiyama, on the western edge of Kyoto, is renowned for its large expanses of Japanese maple (*Acer palmatum*) and other deciduous broadleaf trees, which form a celebrated autumn foliage landscape. Historical and planning studies describe Arashiyama and its suburban mountain area as a key scenic zone where maples, cherry trees, and pines are deliberately used as “constitution trees” of the landscape, shaping the visual character of the hillsides and river valley. The mixed forest mosaic on the slopes—dominated by deciduous broadleaf species—creates large, continuous patches of red, orange, and yellow that are visible from afar and attract millions of visitors annually (Figure 1).



Figure 1 Maple leaf scenery in autumn in Lansì

Image source: Japan food guide

This autumn scenery has become a major tourism resource and a symbol of Kyoto's seasonal identity. Arashiyama is cited nationally and internationally as a representative destination for viewing autumn leaves, with forest color change playing a central role in visitor appeal and local tourism planning (Mu et al., 2022). Phenological research on Japanese maple foliage shows that the timing and duration of coloration directly influence visitor numbers, and delayed but compressed color periods can increase maple-viewing tourism volume (Liu et al., 2019). Such findings help explain why the extensive planting and conservation of maples in Arashiyama have been prioritized: dense maple stands ensure a reliable, visually striking autumn display that underpins seasonal festivals and economic activity.

3.2 Color contrast with evergreen trees and water landscapes

In Arashiyama, red and yellow maples are composed with evergreen conifers and the Katsura River to create strong color contrast and spatial layering. Landscape analyses of the Kyoto-Arashiyama area identify cherry trees, pine trees (evergreen), and maples as key structural elements whose differing forms and phenologies are intentionally combined to shape views from designated vantage points. On the mountain slopes, darker green pine and other evergreens provide a deep background against which bright maple crowns stand out in autumn, enhancing perceived saturation and depth of color patches (Mu et al., 2022). This arrangement produces a tiered vertical structure: dark evergreen canopy, mid-story deciduous maples, and lighter understory vegetation.

The presence of the river and surrounding water landscapes further strengthens visual impact. Studies of slope-forest color aesthetics show that scenes with strong red-green-yellow contrast, clear primary and secondary color patches, and diversified but balanced color distribution achieve superior visual aesthetic quality and higher public preference. In Arashiyama, reflections of red maples and green pines on the river surface multiply color patches and soften transitions, while the linear water body organizes views and guides sightlines along the valley. This composition of deciduous maples, evergreen trees, and water aligns closely with empirically supported rules for high-quality autumn color landscapes on urban and peri-urban slopes (Mu et al., 2022).

3.3 Demonstrating the value of maples in landscape color design

The Arashiyama case powerfully illustrates the design value of maple trees as strategic color elements in landscape planning. As "constitution trees," maples have been used not only for ecological functions but also as key carriers of seasonal symbolism and aesthetic identity, deliberately embedded into postwar planting and scenic-area planning in Kyoto. Long-term management of Arashiyama National Forest emphasizes sustaining a

historically and aesthetically valuable forest landscape, recognizing that carefully maintained deciduous broadleaf stands are essential to preserving the scenic quality that visitors seek. This underscores how maples can anchor regional landscape character when their seasonal color traits are systematically considered in planning and management.

More broadly, research on autumn-color slope forests shows that configurations rich in red and yellow foliage, with clear yet harmonious color contrasts, are particularly effective for improving visual aesthetic quality and supporting tourism and local economies (Liu et al., 2019; Mu et al., 2022). In Japan, autumn leaf-coloring events are timed to phenological stages of maple foliage to maximize tourism benefits, confirming that maple color dynamics are integrated into destination management and marketing strategies. Arashiyama exemplifies how the genetic and phenological properties of maples-stable, vivid autumn coloration and predictable timing-can be translated into landscape-scale color design, producing spaces that are both ecologically meaningful and culturally and economically valuable.

4 The Genetic Basis of Maple Leaf Color

4.1 The influence of genes related to anthocyanin synthesis on leaf color

The formation of maple leaf color is mainly controlled by some key genes, which are involved in the production of related enzymes during anthocyanin synthesis. Studies have shown that changes in the expression of genes such as CHS, CHI, F3H, DFR, ANS, and UFGT are closely related to the increase or decrease of anthocyanin content during the color change of leaves (Li et al., 2025). For example, 20 genes related to anthocyanins were discovered in the study of false-colored maple leaves. Most of these genes have the highest expression levels during the most red stage of the leaves, indicating that they play an important role in the formation of red leaves (Figure 2) (Gong et al., 2025). In red maples, the expression of these genes is usually higher in red leaves than in green and yellow leaves, thus affecting the accumulation of pigments and the manifestation of leaf color (Chen et al., 2019).

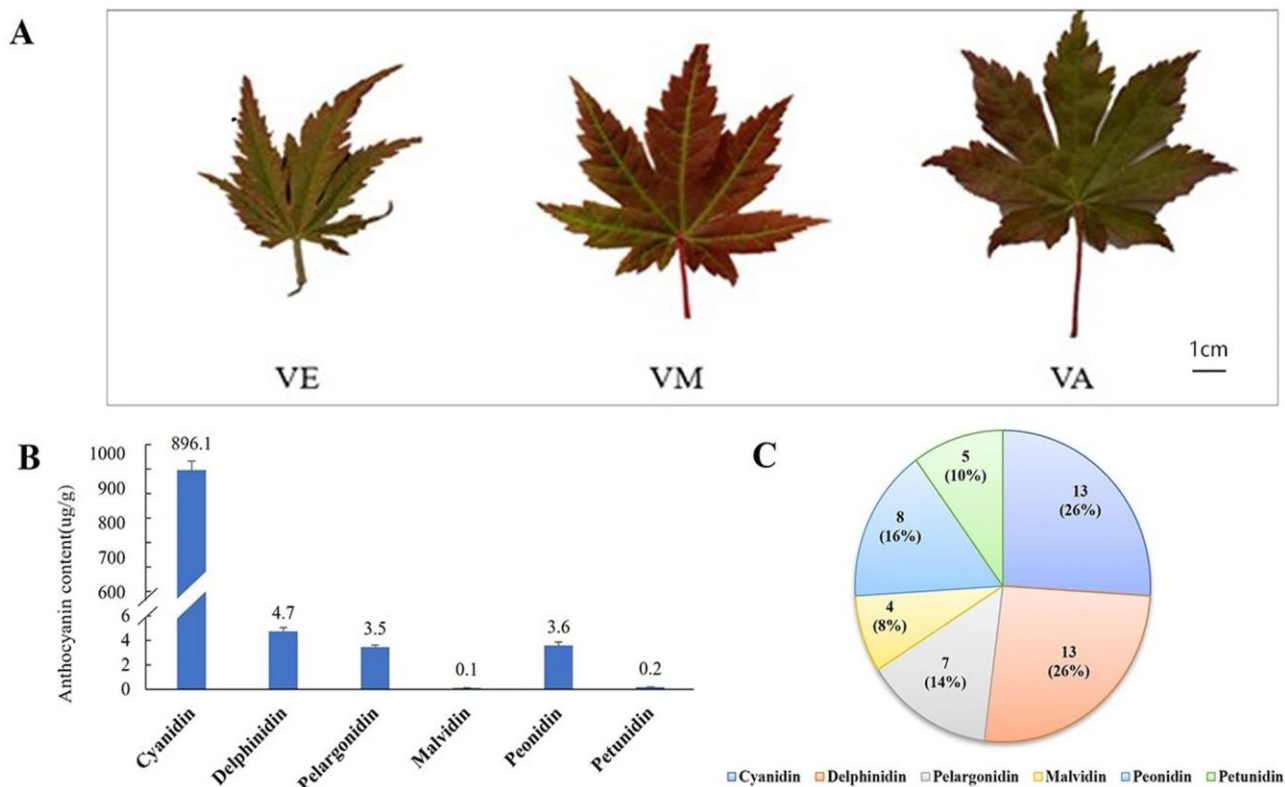


Figure 2 The leaf morphology during development (A), anthocyanin content (B) and classification (C) of *A. pseudosieboldianum* mutant (Adopted from Gong et al., 2025)

Image caption: VE (Early stages), VM (Middle stages), and VA (Late stages) represent three stages during leaf discoloration (Adopted from Gong et al., 2025)

Some genes have a more significant impact on the shade of leaf color. Comparative studies on different varieties of red maples revealed that in some red maples, there is a situation of UFGT gene duplication. When both UFGT genes maintain high expression simultaneously, the anthocyanin content in the leaves will significantly increase, indicating that this gene plays an important role in the formation of red leaves (Zhang et al., 2023). DFR is also one of the important genes affecting leaf color. When this gene undergoes mutation or its expression level decreases, the anthocyanin content will also decrease (Recinos and Puck, 2023). In maples, the expression changes of DFR and ANS usually correspond with the changes in anthocyanin content in red leaves (Chen et al., 2019; Chen et al., 2022; Gong et al., 2025; Li et al., 2025). Therefore, the quantity and expression level of these genes affect the accumulation of anthocyanins, and thereby influence the final color of the leaves.

4.2 Regulatory role of transcription factors

Apart from structural genes, transcription factors can also regulate the formation of pigments at a higher level. The production of anthocyanins is usually controlled by the MBW complex. This complex consists of three types of proteins: MYB, bHLH, and WD40, and it is activated during plant growth stages or when influenced by external environmental signals (Figure 3) (Gao et al., 2020; Yan et al., 2021; Chen et al., 2022; Fan et al., 2024; Liu et al., 2024). Studies have found that in many plants, the influence of transcription factors on pigment differences is often more significant than that of structural genes, and MYB-type transcription factors play a particularly crucial role (Recinos and Pucker, 2023). Generally speaking, MYB mainly regulates the process, while bHLH and WD40 play more auxiliary and stabilizing roles in maintaining the complex.

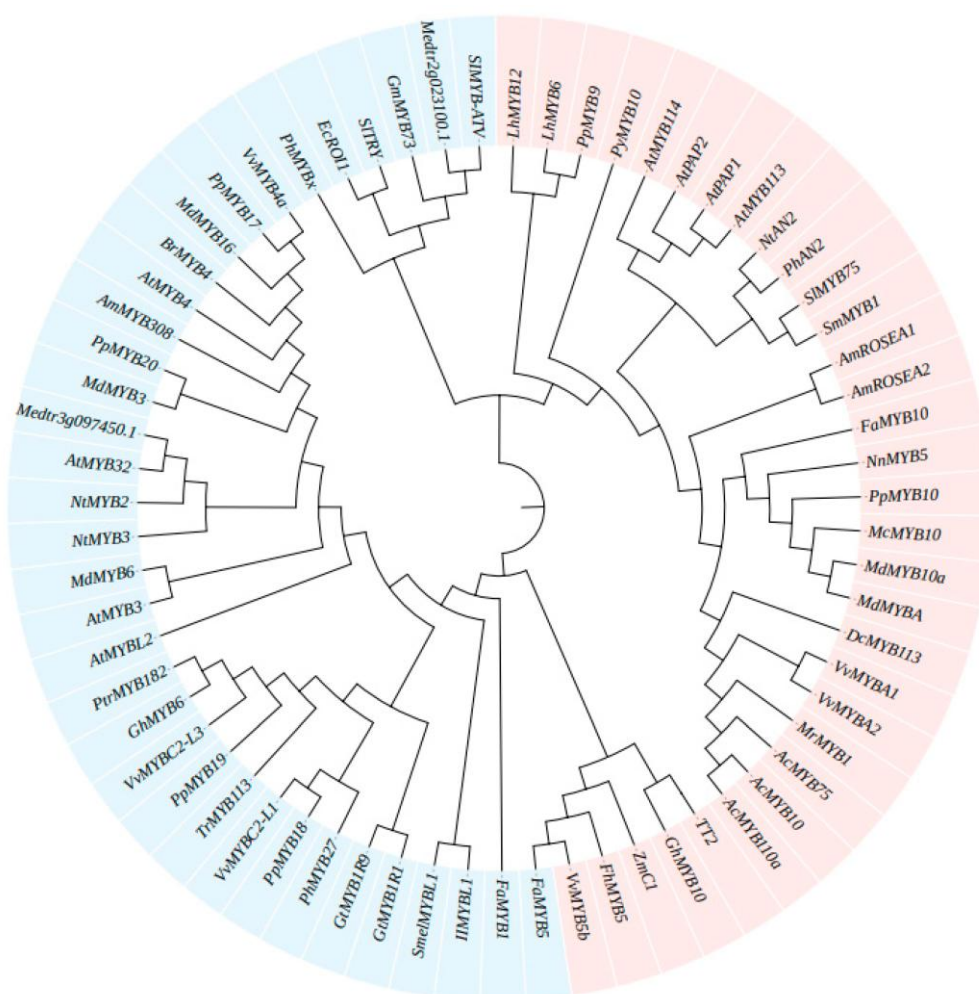


Figure 3 Phylogenetic tree of the regulation of anthocyanin biosynthesis by MYB genes (Adopted from Yan et al., 2021)

Image caption: The tree was constructed based on the entire protein sequences using MEGA 6 software. Blue represents the antagonistic effects of MYB factors in the anthocyanin biosynthetic pathway, and red represents the MYBs as activators to promote the biosynthesis of anthocyanin (Adopted from Yan et al., 2021)

Similar regulatory mechanisms were also discovered in the maple trees. When the color of the maple leaves changed, the expression levels of some *MYB* and *bHLH* genes would significantly alter. Genomic studies have shown that this species has a total of 95 R2R3-type *MYB* genes, among which some genes showed a significant increase in expression during the red leaf period, indicating that they may be involved in the regulation of anthocyanins (Figure 4) (Gong et al., 2025; Zhang et al., 2025). The transcriptome study conducted on the Jiwanfan maple tree found that genes related to anthocyanin regulation include 46 *MYB*, 33 *bHLH*, and 29 *WD40*, indicating that the formation of leaf color is influenced by multiple transcription factors (Zhu et al., 2022). In the study of red maples, after the leaves were subjected to ring excision treatment, they gradually turned red, and some transcription factors related to anthocyanins were also activated (Yang et al., 2022). These research results indicate that *MYB*, *bHLH*, and other transcription factors can integrate different signals and regulate the expression of anthocyanin-related genes.

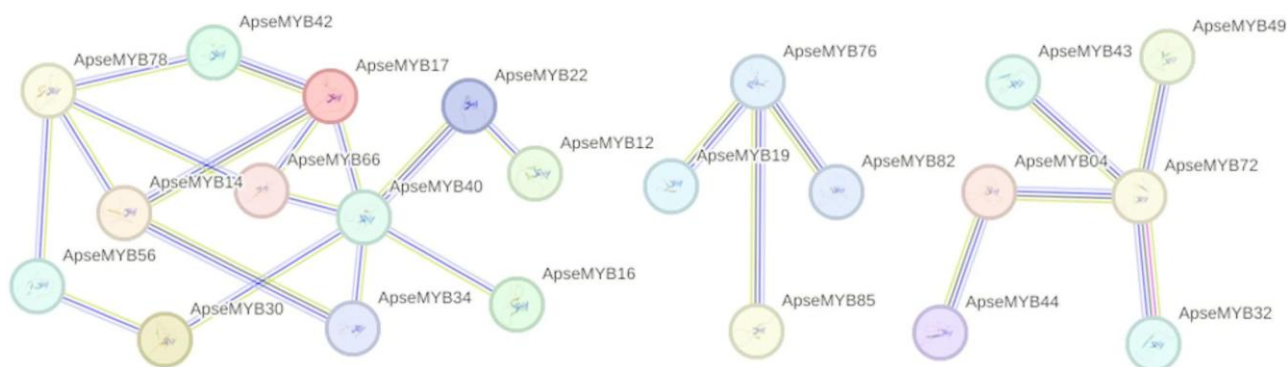


Figure 4 Analysis of the ApseMYB protein-interaction network (Adopted from Zhang et al., 2025)

4.3 Genetic expression differences lead to different leaf colors in different maple tree varieties

The differences in leaf color among different maple tree varieties are mainly related to the varying levels of gene expression, rather than a fundamental change in the metabolic pathways. After conducting a comparative study on red-leaved varieties and yellow-leaved varieties, it was found that the difference in anthocyanin content is associated with changes in the expression levels of 18 structural genes and various *MYB*, *bHLH*, and *WD40* regulatory factors. Additionally, these two types of varieties also show significant differences in their gene co-expression patterns (Zhu et al., 2022).

Similar phenomena were also observed in red maples. When comparing the mutant branches of red, yellow and green colors, it was found that most of the genes related to anthocyanins had the strongest expression in red leaves, the lowest expression in green leaves, and an intermediate level in yellow leaves. This trend of change is consistent with the changes in anthocyanin content and the depth of leaf color (Chen et al., 2019). This indicates that under the same genetic background, different expression intensities of genes can affect pigment content, thereby forming different leaf colors (Figure 5).

5 Application of Maple Trees in Landscape Design

5.1 Utilizing different leaf colors to enrich landscape colorfulness

There are many types of maple trees, and their leaf colors are also very diverse, such as yellow-green, orange, red and purple, etc. By appropriately combining different colored maple leaves, a distinctive and colorful plant landscape can be created. Research indicates that maple trees with red, orange and yellow-green leaves can enhance the overall visual appeal of the landscape to a certain extent. In landscape design, if the number of red-leaved maple trees is appropriately increased, it can make the originally green-dominated environment more eye-catching and form a clear visual center (Yang et al., 2022).

Research on the color combination of plants in autumn has also found that designs featuring warm colors or creating an appropriate contrast between warm and cool colors are more likely to be favored by the public. Relevant indicators such as the autumn color index and the ratio of warm colors to cool colors are positively correlated with the attractiveness of the landscape (Luo et al., 2023b). Therefore, in actual design, attention should

be paid to the combination of the main color and the auxiliary color, and the selection of maple trees with dark red, orange red and yellow leaves should be made instead of randomly mixing multiple colors. Proper control of the hue and saturation of the leaf color can enhance the overall color effect of parks and streets.



Figure 5 Leaves of maple trees in different colors

5.2 The extensive application of maple trees in parks and streets

Maple trees are valued for their attractive form and strong adaptability, and their vivid autumn foliage has led to their widespread use in urban greening around the world (Figure 6) (Melitopol et al., 2025). The Humble Administrator's Garden in China provides a representative example: in autumn, the maples create a richly layered red-leaf landscape, which, together with pavilions, water features, and surrounding plants, forms a classical garden scene imbued with traditional aesthetic Yijing (Figure 7). In the eastern United States, maples are among the most common deciduous street trees and are widely planted in residential areas, commercial districts, and parks. Species such as Norway maple, red maple, and silver maple grow relatively quickly, adapt well to urban environments, and possess broad canopies with pronounced seasonal visual changes, making them commonly used as street and shade trees (Lisica et al., 2023).

The actual situations in some cities of Europe and the United States demonstrate that maple trees play a significant role in urban greening. In the urban area of Belgrade, maple trees are one of the common street trees, with a total of 735 trees, occupying an important position in the local urban greening system. In Allen Park in Toronto, Canada, the tall Norwegian maple, due to its bright autumn foliage, contributes approximately one fifth of the ecological value and landscape value to the park. However, when planting maple trees on a large scale, it is necessary to carefully select suitable varieties, attach importance to the protection of genetic diversity, and strengthen the prevention and management of pests and diseases, so as to ensure that the urban greening effect remains stable for a long time (Ferus, 2023).

5.3 Create a unique seasonal landscape through plant combinations

By planting maple trees in combination with deciduous trees, flowering trees, shrubs and ground cover plants, a landscape with distinct seasonal changes can be formed. Research shows that by appropriately combining maple trees with spring-blooming tree species and evergreen coniferous trees, the landscape can maintain a continuous viewing effect throughout the year: flowers can be appreciated in spring, the trees are lush in summer, and the leaves show a rich variety of colors in autumn. Among them, the ornamental value of the autumn landscape is the most prominent. Reasonable plant arrangement can ensure that the landscape maintains a good visual effect from May to September. In May, it is mainly for flower appreciation, while from August to September, the main landscape elements are autumn leaves and fruits (Wang, 2021).



Figure 6 Application of maple in landscape design

Research on the autumn color landscape of urban parks has shown that three plant combinations are particularly effective: those dominated by warm colors, those that contrast warm and cool colors, and those with coordinated distribution of various colors. In these designs, the autumn leaves of maple trees are often used as the main background. As long as the main color and auxiliary color are reasonably controlled, and the proportion and diversity of colorful plants are managed well, a good viewing effect can be achieved (Luo et al., 2023b). Moreover, evergreen trees can connect the autumn and winter landscapes, making the overall visual effect more continuous (Wang and Zhao, 2020). By reasonably combining maple trees with other plant species, a landscape space with obvious seasonal changes and in line with public aesthetics can be formed.

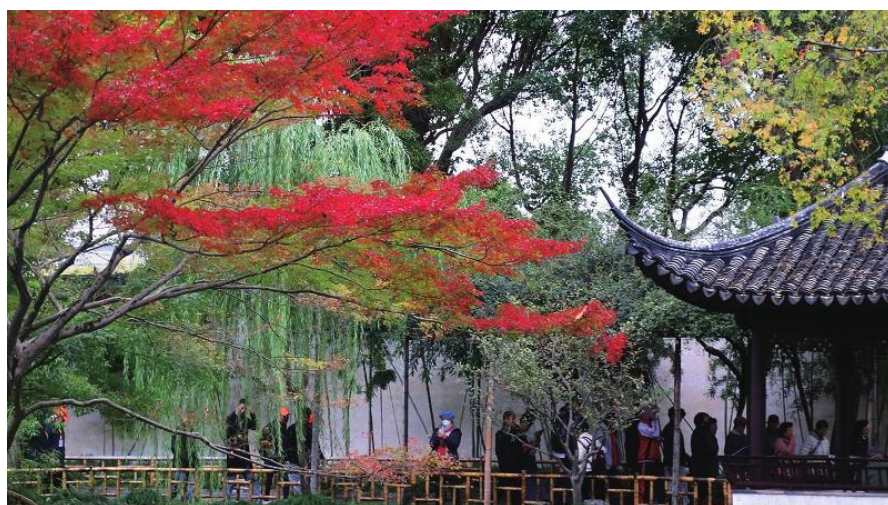


Figure 7 Maple leaves at the humble administrator's garden
 Image source: Suzhou municipal people's government website

6 Maple Variety Breeding and Genetic Improvement

6.1 Traditional breeding and molecular marker-assisted breeding

The selection and breeding of maple varieties have long relied primarily on traditional cross-breeding and seedling selection, screening based on phenotypic traits such as leaf color, tree form, and growth rate. However, leaf color traits are regulated by multiple genes and are easily influenced by environmental conditions, making traditional breeding cycles long and efficiency relatively low. In actual breeding processes, it often takes several years or even more than a decade from cross-pollination to the flowering and fruiting of seedlings, and leaf color expression requires many years of observation to confirm its stability. Furthermore, traditional breeding struggles to precisely select genes related to leaf color, and the inheritance of excellent traits involves considerable uncertainty, increasing the difficulty and cost of breeding efforts.

In recent years, with the development of molecular marker technologies, DNA molecular marker-based (such as SSR, SNP) assisted breeding has greatly improved selection efficiency. By constructing genetic linkage maps of maple, researchers have mapped QTL loci associated with leaf color, providing a basis for early screening of target traits (Zhang et al., 2023). Molecular marker-assisted breeding allows for genotype identification at the seedling stage using small amounts of leaf tissue, eliminating the need to wait many years for phenotypic observation and significantly shortening the breeding cycle. For example, molecular markers closely linked to the UFGT gene have been developed in red maple, enabling rapid identification of red-leaf genotypes and accelerating the breeding process of superior varieties (Figure 8) (Chen et al., 2019). Currently, molecular markers for several important ornamental traits in maple have been successively developed, laying a solid foundation for the genetic improvement of maple.

6.2 Application prospects of gene editing technology in leaf color improvement

With the popularization of genome sequencing technology, the genomes of various maple species have been resolved, laying the foundation for gene editing. Technologies such as CRISPR/Cas9 offer the possibility of precisely modifying genes related to leaf color. By knocking down or activating specific transcription factors (such as MYB, bHLH) or structural genes (such as DFR, ANS), the synthesis and accumulation of anthocyanins can be directionally regulated, thereby altering leaf color expression (Recinos and Pucker, 2023). Unlike random mutagenesis, gene editing technology enables site-specific modification of target genes, avoiding the linkage introduction of undesirable traits and greatly improving breeding efficiency and precision. Currently, this technology has been successfully applied in model plants such as Arabidopsis and rice for regulating anthocyanin metabolism, providing important references for the genetic improvement of maple.

Furthermore, gene editing can also be used to enhance the adaptability of maple to environmental stresses, such as improving drought tolerance or disease resistance, allowing excellent leaf color traits to be stably expressed in complex urban environments. Stress factors in urban environments, such as drought, soil salinization, and air pollution, often lead to premature leaf senescence and disrupted pigment metabolism, affecting the red leaf landscape effect. By editing transcription factors or functional genes related to stress response, the stress resistance of maple can be enhanced, ensuring normal color change under adverse conditions. Currently, the genetic transformation system for maple is not yet fully developed, but related research is progressing. Methods such as Agrobacterium-mediated transformation and particle bombardment have been successfully applied in some Aceraceae species. With the improvement of genetic transformation efficiency and the optimization of gene editing technologies, precise improvement and directional breeding of maple leaf color traits are expected in the future.

6.3 Promotion and adaptability evaluation of superior varieties

The promotion and application of new varieties require comprehensive consideration of their ecological adaptability, ornamental stability, and cultivation management requirements. Different maple varieties respond differently to environmental factors such as light, temperature, and soil pH, which directly affects leaf color expression and growth vigor. For example, some red-leaf varieties show reduced anthocyanin accumulation and greener leaves under insufficient light; while yellow-leaf varieties may shed leaves prematurely under high

temperatures, significantly shortening the ornamental period (Xie et al., 2023; Zhou, 2025). Soil conditions are equally important; alkaline soils may hinder iron absorption, causing leaf yellowing and affecting the landscape effect. Therefore, multi-site, multi-year regional trials are needed before promotion to evaluate leaf color stability, pest and disease resistance, and growth adaptability, clarifying the optimal planting areas and cultivation conditions for each variety.

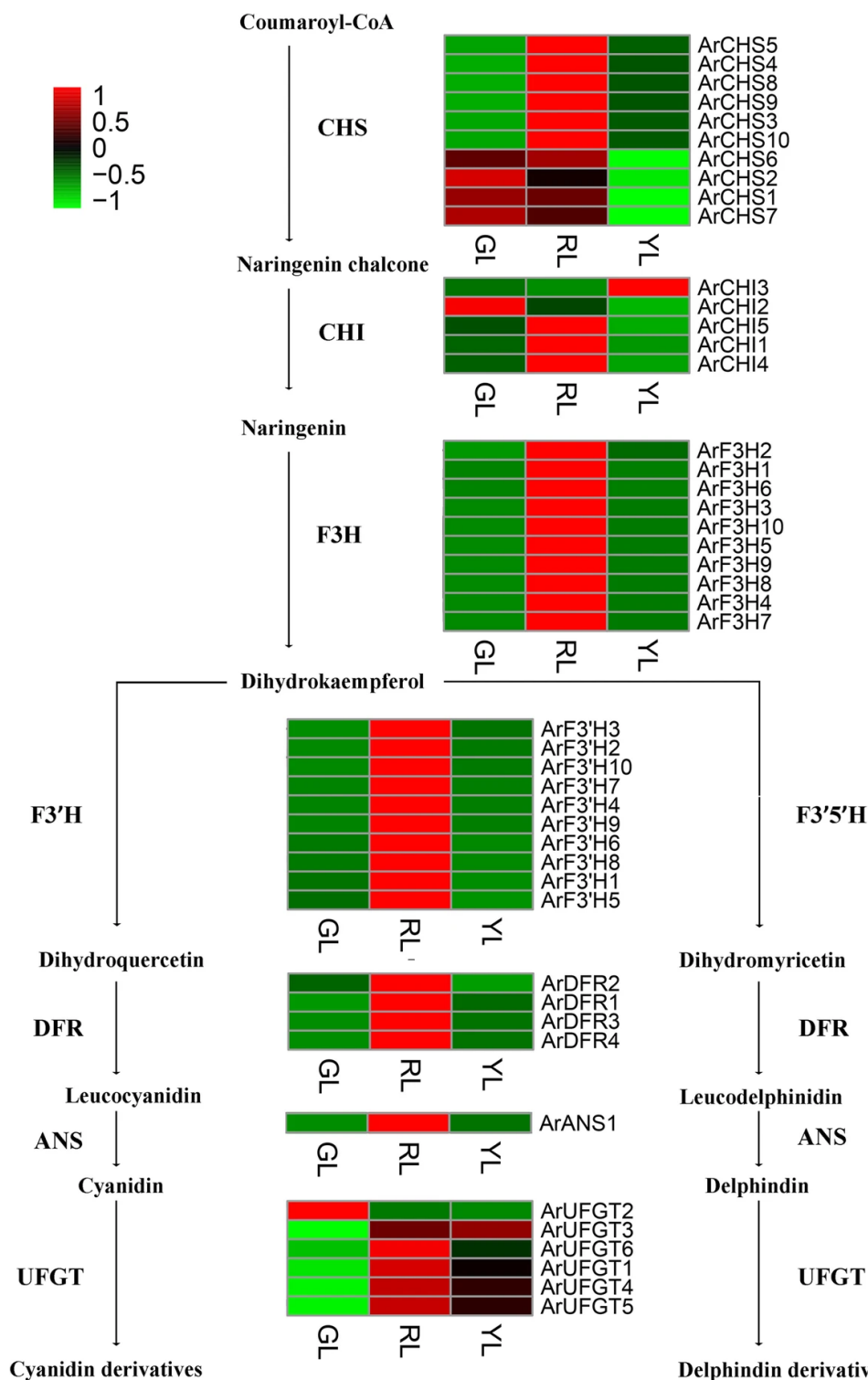


Figure 8 Heatmap of differentially expressed genes (DEGs) related to anthocyanin biosynthesis (Adopted from Chen et al., 2019)
Image caption: GL, green leaves; RL, red leaves; YL, yellow leaves; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavanone 3'-hydroxylase; F3'5'H, flavanone 3'5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanin synthase; UFGT, anthocyanidin 3-O-glucosyltransferase (Adopted from Chen et al., 2019)

Simultaneously, establishing asexual propagation systems (such as cuttings, tissue culture) to ensure the stable inheritance of excellent traits is a crucial step in variety industrialization (Lin et al., 2022). Cutting propagation is simple and low-cost, but some maple varieties have difficulty rooting, resulting in unstable survival rates; although tissue culture is technically demanding, it allows rapid propagation of superior seedlings while maintaining the excellent traits of the mother plant. Currently, tissue culture techniques for important ornamental maples like Japanese maple and red maple have matured, providing a foundation for the large-scale production of superior varieties. Furthermore, during the promotion process, cultivation technical guidance needs to be strengthened, including proper water and fertilizer management, shaping and pruning, and pest and disease control, to ensure that new varieties display their optimal ornamental effects in different regions. By establishing a comprehensive superior variety propagation and promotion system, the application of excellent maple varieties in urban greening can be accelerated, enhancing the overall quality of autumn landscapes.

7 Challenges and Future Trends in Maple Landscape Design

7.1 Impacts of urban environment on maple growth and leaf color expression

The urban environment presents specific challenges such as high temperatures, drought, soil compaction, and air pollution, which impact maple growth and leaf color expression. Research indicates that the urban heat island effect can delay autumn phenology, shorten the red leaf period, and even affect anthocyanin synthesis (Liu et al., 2019). Nighttime temperatures in city centers are significantly higher than in suburban areas, disrupting the temperature signals plants use to perceive seasonal changes, leading to delayed or insufficient leaf coloration. Additionally, air pollutants like sulfur dioxide and ozone can directly damage leaf tissue, accelerate leaf senescence, and cause leaves to fall before displaying their full red color, severely impacting the landscape effect.

Soil compaction and poor aeration limit root system development, subsequently affecting nutrient absorption and leaf pigment metabolism. Soil in urban roadsides and squares often becomes compacted due to construction traffic, reducing porosity and restricting root growth, leading to weakened tree vigor and dull leaf color. Simultaneously, the large area of impermeable pavement hinders rainwater infiltration, causing unstable soil moisture supply and frequent drought stress (Lu and Wang, 2025). Therefore, in landscape design, it is necessary to select adaptable varieties, such as Norway maple and silver maple, which tolerate urban conditions, and adopt cultivation measures like soil improvement, supplemental irrigation, and the use of mulches to maintain good leaf color effects. Furthermore, rationally configuring tree species and avoiding planting maples in severely polluted or poorly lit areas can maximize their ornamental value. Technical measures such as soil improvement in planting pits, permeable pavement around tree pits, and regular deep aeration can significantly improve the growing conditions for maples.

7.2 Challenges of climate change and maple phenological shifts for landscape design

Global climate change leads to increased temperature fluctuations and frequent extreme weather events, directly affecting the timing and duration of maple leaf coloration. For example, autumn warming can delay color transition, while early frosts may cause leaves to fall before changing color, diminishing the landscape effect (Mattila and Tyystjärvi, 2023). Over the past few decades, the peak autumn foliage period in famous maple-viewing areas like Kyoto, Japan, has shown a trend of delay and increased interannual variability, posing challenges for tourism organization and landscape management. Furthermore, changes in precipitation patterns can affect anthocyanin accumulation and leaf senescence processes; in drought years, red leaves may fall prematurely, while in rainy years, insufficient light may lead to duller leaf colors.

This requires landscape designers to consider climate change factors during planning, select varieties with stable phenological responses, and employ diverse tree species combinations to buffer the impact of climate anomalies on autumn color landscapes. For instance, combining maple varieties with different coloration periods can extend the red-leaf viewing period from 2-3 weeks for a single species to over a month; introducing other autumn color tree species like ginkgo and tulip tree creates composite landscapes with interwoven yellow, orange, and red hues, maintaining overall landscape quality even if one species performs poorly. Simultaneously, establishing long-term phenological monitoring networks, combined with meteorological data to predict leaf color change trends,

provides a scientific basis for landscape management and visitor services. Japan has established a nationwide autumn foliage forecasting system, predicting optimal viewing periods across regions using phenological models to guide visitor travel and scenic area operations, an experience worthy of reference and promotion.

7.3 Application of digitalization and intelligence in maple landscape planning

With the development of Geographic Information Systems (GIS), remote sensing technology, and artificial intelligence, the planning and design of maple landscapes are progressively transitioning towards digitalization and intelligence. Through high-resolution remote sensing imagery and drone aerial photography, the species distribution, leaf color dynamics, and health status of large maple forests can be rapidly acquired, providing data support for landscape assessment and optimization (Mu et al., 2022). Multispectral and hyperspectral remote sensing technologies can invert leaf anthocyanin and chlorophyll content, quantitatively assess red leaf color saturation, and identify early signs of abnormal coloration. Combined with visitor preference surveys and scenic beauty estimation models, designers can simulate the visual effects of different configuration schemes, achieving precise design. Through virtual reality technology, decision-makers and the public can experience autumn color landscapes firsthand during the planning and design phase, participating in scheme comparison and optimization.

Furthermore, IoT-based intelligent irrigation and maintenance systems can automatically regulate water supply based on soil moisture and meteorological conditions, ensuring healthy maple growth and extending the red-leaf viewing period. Soil moisture sensors monitor root zone water content in real-time; combined with weather forecast data, intelligent control systems can initiate irrigation before a drought occurs, avoiding premature leaf fall caused by water stress. For precious ancient maple trees, monitoring equipment such as trunk sap flow sensors and leaf chlorophyll fluorescence detectors can be installed to assess tree physiological status in real-time, promptly detecting anomalies and implementing maintenance measures. In the future, digital twin technology holds the potential to construct virtual maple landscape platforms, integrating real-time monitoring data with 3D landscape models to dynamically display leaf color change processes, aiding public engagement and cultural dissemination. Visitors can access real-time autumn foliage information via mobile apps to plan optimal viewing routes, while scenic area managers can use this data to regulate visitor flow and arrange events, further enhancing the comprehensive value and cultural influence of maple landscapes.

8 Concluding Remarks

The formation of maple leaf color is not caused by a single factor but is the result of the combined effect of genetic factors and environmental conditions. From a genetic perspective, certain genes and regulatory factors influence the production and content of chlorophyll, carotenoids, and anthocyanins, causing leaves to exhibit different colors such as green, yellow, or red. Simultaneously, external environmental conditions, such as light intensity and temperature, also affect the accumulation of these pigments and influence the expression of related genes. Therefore, the leaf color of the same maple tree may vary under different environmental conditions, and the pattern of color change may not be entirely consistent. Research also indicates that some artificial measures, such as girdling, can affect the pigment accumulation process and the expression levels of related genes, thereby causing changes in leaf color. In recent years, with the rapid development of molecular biology techniques, the understanding of the mechanisms underlying maple leaf coloration has deepened, providing a solid theoretical foundation for maple variety improvement and landscape application.

Research on the genetic mechanisms of leaf color has also promoted the development of ornamental plant breeding. Through multi-omics research methods, researchers have discovered many important genes and transcription factors related to pigment synthesis, such as MYB and bHLH. Simultaneously, studies have shown that gene duplication phenomena may also affect the stability of leaf color. The discovery of leaf color mutant materials and the application of molecular marker technology have provided new methods for breeding new varieties with specific leaf colors and the ability to exhibit changing characteristics in different seasons. Due to the rich genetic diversity in maple leaf color, through directional selection, various maple varieties with different color series, such as red series and yellow series, have been cultivated, further enhancing their ornamental value. With the maturation of gene editing technology and the improvement of genetic transformation systems, precise

regulation of maple leaf color is expected in the future, cultivating more excellent varieties possessing both ornamental value and environmental adaptability.

With technological advancements and the increasing demands of urban greening, the application of maple trees in landscape design is becoming increasingly widespread, but it also faces challenges such as climate change and urban environmental stress. In the future, molecular breeding based on genetic information and gene editing technology are expected to cultivate more superior varieties with stable leaf color and strong adaptability. Simultaneously, combined with digital planning tools and intelligent maintenance systems, precise design and sustainable management of maple landscapes can be achieved, enabling them to play a greater role in beautifying cities, inheriting culture, and promoting tourism. Through interdisciplinary integration, organically combining genetics, ecology, landscape design, and smart city technologies, this traditional ornamental tree species will surely radiate new vitality and vigor in modern urban landscapes, bringing people richer and more colorful autumn visual feasts.

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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

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Trait Basis and Management Strategies for Stable High Yield in Greenhouse Tomato Production

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Abstract This study explores the key agronomic trait basis, physiological-ecological mechanisms, and regulatory pathways underlying high and stable yield formation in protected tomato production. Protected tomato cultivation plays an important role in ensuring year-round vegetable supply and increasing yield per unit area; however, yield stability is often affected by environmental fluctuations, suboptimal canopy structure, and mismatched management practices. Based on a systematic review of recent studies, this study summarizes the major trait foundations associated with high and stable yield from the perspectives of plant growth, flowering and fruit set, fruit development, and root characteristics. It focuses on the roles of plant architecture, internode length, the balance between vegetative and reproductive growth, inflorescence number and pollen viability, single fruit weight and fruit enlargement dynamics, as well as root vigor and health in determining yield and its stability. Meanwhile, the effects of environmental factors such as temperature, light, humidity, and CO₂ on tomato growth and fruit set are reviewed, and the contributions of integrated management practices-including optimal planting density, pruning, fertigation, supplemental lighting, pollination techniques, and grafting-are discussed. Finally, future trends in intelligent environmental control, breeding of high-yield and stress-resistant varieties, and green efficient production systems are highlighted, achieving high and stable yield in protected tomato production depends on the coordinated optimization of traits, environment, and management.

Keywords Protected tomato; High and stable yield; Cultivation traits; Environmental regulation; Integrated water and fertilizer management

1 Introduction

Protected agriculture, as an important component of modern agriculture, plays a crucial role in ensuring the year-round stable supply of vegetables, improving land-use efficiency, and enhancing agricultural productivity. Tomato (*Solanum lycopersicum* L.), owing to its high nutritional value, strong adaptability to processing, and stable market demand, has become one of the most widely cultivated and economically valuable vegetable crops worldwide (Banoo et al., 2024; Avasiloaiei et al., 2025). With the intensification of climate change and increasing constraints on arable land resources, traditional open-field vegetable production is facing growing uncertainties. In contrast, protected cultivation can partially control crop growth conditions by regulating environmental factors such as temperature, humidity, and light, thereby extending production cycles and increasing yield per unit area (Banoo et al., 2024). Within global protected horticulture systems, tomato is not only one of the crops with the largest planting areas but is also regarded as a representative model crop for production intensification and technological innovation in controlled-environment agriculture.

However, compared with open-field production, protected environments provide controllable cultivation conditions while simultaneously creating a more complex growth regulation context. Environmental factors such as temperature, light, humidity, and vapor pressure deficit (VPD) are highly coupled across spatial and temporal scales, which can easily lead to microclimatic variations and consequently affect plant growth and fruit development (Dewapriya et al., 2024; Šalagovič et al., 2024). In practical production, protected tomatoes often exhibit fluctuations in fruit set and uneven fruit expansion, resulting in unstable yields and marketable fruit rates. In addition, extreme climatic events, particularly heat stress, can significantly reduce fruit set and the number of fruits per plant. Even in high-tech greenhouse systems, yield losses under extreme high-temperature conditions

may still reach 6%-53% (Kürklü et al., 2025). Insufficient or fluctuating light conditions can also reduce dry matter production and affect fruit quality.

In addition to environmental factors, protected tomato production systems generally require high inputs of water, fertilizers, and energy. If management practices do not match crop requirements, resource-use efficiency may decline and environmental pressure may increase (Avasiloaiei et al., 2025). Population structure and canopy management practices, such as plant spacing, topping, and leaf pruning, can influence light interception capacity, source-sink relationships, and fruit load, thereby regulating yield and quality formation. From the perspectives of crop production science and genetic breeding, yield stability in protected tomatoes depends on key traits such as population structure, light interception efficiency, and dry matter production capacity. These traits are not only controlled by genetic background but are also regulated by the protected environment and cultivation management. Different tomato varieties show significant differences in their ability to maintain fruit set and yield under variations in temperature and microclimatic conditions (Ali et al., 2025).

This study aims to explore the key cultivation traits and their regulatory mechanisms involved in achieving high and stable yields in protected tomato production. It particularly focuses on analyzing the linkage between traditional cultivation experience and findings from modern genomics research. From the perspective of plant traits, the study systematically summarizes the main factors influencing yield stability and analyzes their underlying regulatory bases through genetics and physiological ecology, thereby providing a theoretical basis for constructing a high-yield and stable-production technological system integrating traits-genes-management practices. In addition, this study also examines the roles of integrated regulation strategies-such as climate control, supplemental lighting, integrated water and fertilizer management, and intelligent monitoring and decision-making technologies-in protected tomato production to improve production efficiency, enhance resource-use efficiency, and stabilize fruit quality. Through a systematic summary of cultivation traits related to high and stable yields and their regulatory points in protected tomato production, this study aims to provide scientific references for optimizing protected cultivation management strategies, promoting the breeding of facility-adapted varieties, and developing precision cultivation technologies.

2 Plant Growth Trait Foundations for High and Stable Yields in Protected Tomato Production

2.1 Effects of plant vigor and architecture on canopy photosynthetic efficiency

The achievement of high and stable yields in protected tomato production depends on both strong plant vigor and well-optimized plant architecture. Plant vigor is reflected in traits such as stem elongation rate, leaf expansion capacity, internode formation rhythm, and the ability to sustain branching, flowering, and fruiting. Together, these traits determine the efficiency of plant acquisition of light, spatial resources, and nutrients. The greenhouse environment partially buffers external climatic variability, allowing tomatoes to maintain strong vegetative growth potential. However, excessive vegetative vigor often leads to canopy closure, increased self-shading in upper leaves, and insufficient light penetration to middle and lower canopy layers, ultimately reducing canopy photosynthetic efficiency and dry matter accumulation. Even under similar total leaf area, canopy photosynthesis remains highly sensitive to structural traits, including internode length, leaf size, leaflet morphology, inclination angle, and spatial arrangement. Yield stability therefore depends not on leaf area alone, but on whether a structurally optimized effective leaf area can establish a balanced light distribution within the canopy. Moderate increases in internode length, improved leaf length-to-width ratio, and optimized leaflet arrangement can create a more open canopy and enhance vertical light penetration, thereby improving overall photosynthetic efficiency and dry matter production.

From a population perspective, plant architecture influences not only light interception at the individual level but also the distribution and utilization of radiation within the entire canopy. An ideal architecture typically features an upright stem, moderate internode length, evenly distributed leaves, and upper leaves that are extended but not excessively horizontal, promoting a balanced vertical light gradient. The research conducted by Zhang et al. (2022) based on the functional-structural plant model (FSPM) indicates that the combination of longer internodes and

narrower leaves reduces excessive shading in the upper canopy and redistributes light toward middle canopy layers, improving photosynthetic performance at both plant and population scales. This has led to the concept of a photosynthetic ideotype for greenhouse tomato cultivation. Further studies demonstrate strong interactions between planting configuration and plant architecture. Planting pattern, spacing, and row orientation often exert greater effects on canopy radiation interception and photosynthesis than individual traits alone. When optimized together with appropriate architecture, these factors produce synergistic effects (Zhang et al., 2024a).

In high-density, long-cycle production systems, plant architecture plays a critical role in maintaining canopy photosynthetic efficiency. Excessive density combined with luxuriant growth accelerates light attenuation within the canopy, leading to premature senescence of lower leaves and reduced photosynthetic contribution, ultimately compromising fruit set continuity and yield stability. Conversely, optimizing plant architecture and planting configuration—such as adjusting row spacing, orientation, and canopy thickness—can significantly enhance light interception, promote dry matter accumulation, and improve fruit quality. Yield increases of approximately 3.92%–9.78% have been reported under optimized configurations across different seasons (Li et al., 2024). Therefore, achieving high and stable yields in protected tomato production requires moderate and stable plant vigor combined with a well-balanced architecture that supports both efficient light interception in the upper canopy and adequate light penetration to lower canopy layers.

2.2 Regulatory effects of internode length and leaf distribution on ventilation and light conditions

Internode length is a key structural trait shaping plant architecture in protected tomatoes. It determines the three-dimensional distribution of leaves and influences canopy porosity, light penetration, and the surrounding microclimate. Moderate internode length promotes uniform vertical leaf stratification, reduces leaf overlap, and enhances the penetration of scattered light into lower canopy layers. When internode length increases from approximately 7 cm to 10–12 cm, the canopy becomes more open, vertical light penetration improves, and canopy photosynthetic efficiency can increase by about 10%, particularly under high radiation conditions. Moreover, the combination of longer internodes and narrower leaves facilitates the redistribution of light from upper to middle canopy layers and fruit-bearing zones, enhancing their photosynthetic contribution without substantially reducing total light interception. These findings indicate that internode length functions not only as a morphological trait but also as a key structural regulator of canopy light distribution.

Leaf distribution patterns further regulate canopy ventilation and light conditions. Due to their large and compound structure, densely arranged tomato leaves can create localized shading and high-humidity zones. A balanced leaf arrangement maintains sufficient photosynthetic area while creating interleaf spaces that enhance air circulation, reduce humidity, and shorten leaf wetness duration, thereby lowering the risk of diseases such as gray mold and leaf mold. Variations in leaf area distribution, leaflet inclination, and plant-to-plant structural heterogeneity influence the uniformity of light absorption within the canopy. Although their effects on total canopy photosynthesis may be smaller than those of planting density or spacing, they can significantly alter local microenvironments and inter-plant variability, ultimately affecting yield stability. Therefore, leaf distribution uniformity and canopy ventilation capacity should be considered key criteria in evaluating plant architecture in protected tomato systems.

Beyond light regulation, canopy openness also influences airflow, heat dissipation, and temperature stratification. As canopy closure increases, ventilation efficiency declines, leading to the accumulation of heat and humidity—especially under high-temperature conditions. Li et al. (2025) conducted field measurements and aerodynamic studies show that moderate removal of older leaves can modify airflow pathways, increase within-canopy wind speed, and reduce localized heat accumulation, indicating that leaf management is essentially a process of microclimate optimization rather than simple defoliation. Leaf area index (LAI) serves as an integrative parameter linking leaf distribution, light interception, and ventilation. Excessively low LAI limits photosynthetic capacity, whereas excessively high LAI intensifies shading. Maintaining LAI within an optimal range of approximately 3.0–3.5 allows a balance between light interception and internal light penetration. Thus,

internode length and leaf distribution jointly regulate both canopy structure and microclimate, making them critical determinants of stable yield formation.

2.3 Balance between vegetative and reproductive growth

Maintaining a dynamic balance between vegetative and reproductive growth is fundamental for achieving high and stable yields in protected tomato production. Vegetative growth, including stem and leaf development, leaf area expansion, and root vitality, provides the basis for assimilate production. Reproductive growth, encompassing flower differentiation, pollination, fruit set, and fruit enlargement, directly determines yield formation. Excessive vegetative growth may result in continuous allocation of assimilates to stems and leaves, suppressing flower differentiation and reducing fruit set stability. Conversely, excessive reproductive load can lead to insufficient leaf area, reduced root activity, and premature senescence, ultimately limiting sustained fruit production. Therefore, vigorous growth alone does not guarantee high yield; rather, the key determinant is the maintenance of balanced “source-sink” relationships across growth stages.

Semi-determinate tomatoes exhibit a moderately extended vegetative phase compared with determinate types, while avoiding excessive vegetative growth typical of indeterminate types. This growth habit facilitates coordination between leaf capacity and fruit demand, improving yield-related traits and water use efficiency. These findings suggest that optimal plant types are characterized by a coordinated structural-functional state combining moderately sustained growth with stable reproductive translocation. Light regulation further influences biomass partitioning. Supplemental LED lighting generally increases total biomass and fruit yield, while specific spectral combinations can enhance dry matter allocation to fruits. By regulating leaf thickness, internode elongation, and plant morphology, light quality reshapes the balance between vegetative and reproductive growth. Thus, this balance should be regarded as a dynamic trait jointly shaped by genotype, growth habit, and environmental regulation.

Under stress conditions, this balance becomes even more critical. High temperatures not only affect photosynthesis and vegetative growth but also directly impair pollen viability, stigma development, and fruit set, leading to yield loss even when vegetative growth appears vigorous. Reproductive traits such as flower number, pollen performance, and fruit set rate show closer relationships with final yield than many vegetative indicators (Graci and Barone, 2024). Similarly, under water stress, genotypic differences in vegetative performance do not necessarily translate into stable yields if reproductive development is compromised. Some genotypes maintain strong vegetative growth under stress but still exhibit significant yield reduction due to impaired fruit formation. This highlights that stable assimilate allocation to fruits during later growth stages is a key determinant of yield stability.

3 Traits Related to Flowering and Fruit Set in Protected Tomato Production

3.1 Fundamental role of inflorescence number and flower number in yield formation

The number of inflorescences and the number of flowers directly determine the potential number of fruits in protected tomato production and are among the most fundamental quantitative traits underlying yield formation. In greenhouse tomatoes, the number of marketable fruits per unit area largely depends on how many inflorescences each plant can produce and how many fully functional flowers each inflorescence can ultimately form and retain. Analyses of yield components in indeterminate tomato materials have shown that the number of flowers per inflorescence and the number of successfully fruit-set flowers are significantly positively correlated with fruit number and total yield, and these traits often determine yield potential more directly than plant size alone. The path analysis conducted by Ramana et al. (2025) also demonstrated that both flower number and fruit number have significant direct positive effects on yield. These findings indicate that achieving high and stable yields in protected tomato production depends not only on vigorous vegetative growth, but also on the effective transition from vegetative to reproductive growth, namely, the formation of a sufficient number of high-quality inflorescences and flowers.

At the production level, inflorescence number is usually closely associated with plant vigor, nutrient supply, growth habit, and pruning or branch-retention practices, whereas the number of flowers within each inflorescence determines the upper limit of its fruiting potential. Different inflorescences on the same plant do not contribute equally to yield. Continuous inflorescence studies in greenhouse cherry tomato have found that middle-position inflorescences often outperform some upper or lower inflorescences in terms of flower number, production efficiency, and related biochemical performance, indicating that inflorescence position, local light and thermal environment, and assimilate supply jointly affect the efficiency with which flower number is converted into fruit number (Jerca et al., 2024). Therefore, in protected cultivation, maintaining high differentiation quality of all inflorescences, especially effective middle and upper inflorescences-through rational dense planting, pruning, nutritional regulation, and inflorescence load management is a key step for improving yield stability (Jerca et al., 2024).

From the perspectives of developmental biology and genetic regulation, inflorescence structure itself determines how many flowers can be formed and thus sets the limit for potential fruit number. Tomato inflorescences have a compound branching structure, and the transition rhythm from inflorescence meristem to floral meristem is a major basis for differences in inflorescence branching degree and flower number (Lippman et al., 2008). SINGLE FLOWER TRUSS (SFT), SELF PRUNING (SP), COMPOUND INFLORESCENCE (S), ANANTHA (AN), and related MADS-box transcription factors jointly regulate inflorescence branching and floral organ formation, thereby influencing the number and arrangement of flowers within the inflorescence (Graci and Barone, 2024). More recent studies further indicate that the SEPALLATA-class transcription factor SIMBP21 acts as a negative regulator, and that suppression of its expression can increase the number of flowers per inflorescence and improve fruit yield. At the same time, the miR156a-SPL13 pathway can alter the trade-off among inflorescence number, flower number, and fruit size by regulating inflorescence morphogenesis and lateral inflorescence formation. This indicates that the more inflorescences-more flowers-higher yield pattern in protected tomato production is not a simple linear relationship, but is regulated by the genetic network controlling inflorescence development and exists in dynamic balance with fruit size and resource allocation efficiency (Zhang et al., 2024b).

3.2 Effects of pollen viability and pollination conditions on fruit set rate

Although inflorescence number and flower number determine the upper limit of potential yield in protected tomato production, the actual fruit set rate largely depends on pollen performance and pollination conditions. Pollen production, pollen viability, germination ability, and pollen tube growth rate are all important reproductive indicators affecting tomato fruiting. Higher pollen viability is usually associated with higher fruit set rates and a greater number of fruits per plant. Under prolonged moderate high-temperature conditions, the number of inflorescences itself may not decrease significantly, but declines in pollen viability and the number of effective flowers per inflorescence directly restrict fruit formation, indicating that under fluctuating protected-environment conditions, male reproductive capacity is often a key constraint on stable yield. At the same time, high temperature typically reduces pollen viability and induces flower drop, whereas heat-tolerant materials can maintain higher pollen viability and thus form fruits more steadily under high-temperature conditions.

Although protected tomatoes are typical self-pollinating crops, their anther-cone structure means that effective pollen release often depends on a certain degree of vibration or external assistance. Under protected cultivation conditions, insufficient airflow, excessively high humidity, or environmentally induced stress on floral structures may all reduce pollen release, stigma pollination, and fertilization efficiency. The research conducted by Stroh et al. (2025) indicates protected cultivation conditions can significantly affect pollen quantity, and the first inflorescence often produces less pollen or poorer-quality pollen because of stress during the seedling stage or early reproductive stage. Meanwhile, mechanical vibration, bumblebee pollination, or other supplementary pollination methods can increase the amount of pollen received by the stigma, thereby improving fruit set rate and yield performance (Stroh et al., 2025). This indicates that improving fruit set depends not only on pollen quality, but also on the efficiency with which pollen arrives.

Successful pollination is not equivalent to final fruiting; pollen germination and pollen tube penetration into the ovary are essential processes for initiating fruit development. Tomato fruit initiation is jointly influenced by pollination, pollen tube growth, and fertilization, and that the ability of the pollen tube to pass normally through the style and enter the ovary makes an independent contribution to subsequent fruit development. If pollen cannot germinate or cannot form a normal pollen tube, effective fruit development cannot be initiated even if the flower has already opened and apparent pollination has occurred (Kantoglu, 2024). Therefore, in protected tomato production, simply observing flower opening or pollen release is not sufficient to judge pollination quality; attention must also be paid to pollen germination ability and the continuity of fertilization from the style to the ovary stage.

3.3 Regulatory effects of environmental factors such as temperature and light on flower and fruit drop

Environmental factors such as temperature and light have decisive effects on flowering and fruit set in protected tomatoes, and their regulation of flower and fruit drop is especially sensitive during the flowering and young fruit formation stages. High temperature can interfere with pollen development, stigma and style function, and normal ovary development, thereby reducing fruit set rate and increasing the abscission of flowers and young fruits. Meanwhile, low night temperature can also inhibit pollen viability and pollen germination, induce parthenocarp, and promote pedicel abscission. Under heat stress, reproductive traits such as pollen viability, fruit set rate, pollen tube growth, and stigma exertion are all significantly impaired and are closely related to yield reduction. Therefore, flower and fruit drop in protected tomatoes is not simply a result of unfavorable climate, but rather the consequence of coordinated interference by environmental stress with floral organ development, hormonal balance, and the fertilization process (Figure 1) (Graci and Barone, 2024).

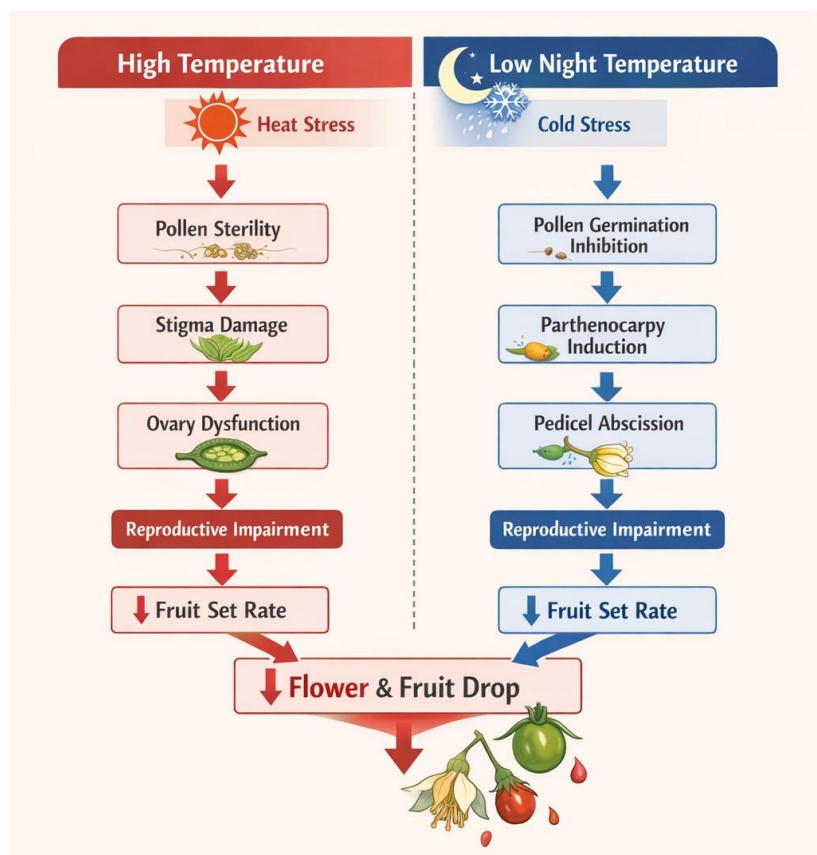


Figure 1 Effects of high temperature and low night temperature stress on reproductive processes and fruit set in tomato

Image caption: High temperature and low night temperature reduce fruit set in tomato by impairing reproductive processes, although through different pathways; High temperature mainly causes pollen sterility, stigma damage, and ovary dysfunction, whereas low night temperature primarily inhibits pollen germination and promotes pedicel abscission. Ultimately, both stresses lead to increased flower and fruit drop, thereby reducing yield formation and stability

Light affects not only the supply of photosynthetic assimilates, but also directly participates in the regulation of floral organ retention through specific signaling pathways. Under low-light conditions, the transport of assimilates to inflorescences and young fruits decreases, which can easily lead to failure in floral organ competition and subsequent abscission. Recent studies have found that the small peptide signaling molecule SIIDL6 and its downstream calcium-dependent protein kinase SICPK10 constitute an important regulatory module in low-light-induced tomato flower abscission; this pathway promotes flower drop by altering Ca^{2+} signaling status in abscission-zone cells (Fu et al., 2024). This suggests that the problem of flower drop under winter-spring protected cultivation or cloudy low-light conditions is not only the result of insufficient carbon supply, but may also involve the active activation of signaling transduction in the abscission zone. Therefore, from cultivation management to molecular breeding, regulation can be directed toward stabilizing flower retention ability under low-light conditions.

In addition to average environmental conditions, spatial microclimate heterogeneity within the greenhouse can further aggravate differences in fruit set among inflorescences. Different inflorescences on the same plant may experience different light, temperature, and humidity conditions, causing some inflorescences to show higher flower number, pollination efficiency, and fruit retention capacity, while others are more prone to flower drop or small fruit formation. This difference is more obvious in continuous-fruited systems, in large canopies, or under high-density protected cultivation. Therefore, reducing flower and fruit drop in protected tomato production should not be limited to regulating average daily temperature or a single light indicator, but should also emphasize microclimate homogenization within the canopy, improved light exposure around inflorescences, and optimized local ventilation conditions (Jerca et al., 2024).

In addition, humidity and airflow can indirectly influence flower and fruit drop by affecting pollen release, stigma pollination, and disease occurrence. Excessively high humidity may reduce pollen dispersal efficiency, while insufficient airflow weakens anther vibration and canopy heat dissipation, and can easily create a combination of localized high humidity and high temperature, ultimately damaging the fruit set process. Therefore, stable fruit set requires coordinated management of temperature, light, humidity, and airflow. In protected production practice, the risk of flower and fruit drop can be jointly reduced and the temporal stability of fruiting improved through measures such as ventilation, supplemental lighting, regulation of day-night temperature differences, improvement of canopy structure, and the use of mechanical vibration or insect pollination (Fu et al., 2024).

4 Fruit Development and Yield Component Traits in Protected Tomato Production

4.1 Effects of single fruit weight and fruit enlargement rate on yield formation

In greenhouse tomato production, total yield can usually be divided into two major components: fruit number per unit area and average single fruit weight, among which single fruit weight directly affects both per-plant yield and population yield formation. Management practices capable of increasing assimilate supply to fruits or enhancing fruit sink strength often increase single fruit weight and overall yield. For example, supplemental lighting and CO_2 enrichment can significantly enhance plant photosynthesis and dry matter production, while also increasing single fruit weight and per-plant yield, indicating that yield improvement does not rely entirely on increasing fruit number, but to a large extent on strengthening the growth capacity of individual fruits (Figure 2).

At the cultivation management level, single fruit weight is not necessarily better when larger, but should be maintained within an appropriate range coordinated with fruit number, truss load, and late-stage plant vigor. If individual fruits become excessively large while source supply, water transport, or root absorption capacity is insufficient, subsequent trusses may experience intensified competition, leading to uneven fruit enlargement, increased malformed fruits, or reduced yield in later stages. Conversely, under relatively stable fruit set conditions, moderately increasing single fruit weight is often one of the most direct ways to raise yield. Average single fruit weight and aboveground biomass are important positive indicators affecting final yield, and that moderate water deficit treatments can maintain relatively high marketable fruit yield while improving water-use efficiency (Figure 3).

In addition to final fruit weight, the rate of fruit enlargement, its day-night variation, and its developmental dynamics also profoundly influence yield formation. Inter-canopy LED supplemental lighting can increase the relative growth rate of fruits at night and raise the final weight of fruits in middle and later fruit positions, thereby improving fruit size uniformity within the same truss. In contrast, when fruit sink number is increased by increasing branch number, average single fruit weight often declines, even when carbon supply per unit leaf area does not decrease synchronously. This suggests that smaller fruits are not necessarily caused by source limitation, but may also result from reduced sink capacity of individual fruits or intensified competition among multiple fruit sinks. In addition, fruit enlargement rate is jointly influenced by plant hydraulic status and hormonal regulation, further indicating that single fruit weight formation is the result of the coordinated action of carbon assimilation, transport efficiency, and sink activity.



Figure 2 Maturation and attachment characteristics of long-elliptical tomato fruits under facility cultivation condition

4.2 Roles of fruit shape and fruit uniformity in marketability

Fruit shape and uniformity are important appearance traits affecting the marketability of protected tomatoes. For fresh-market tomatoes and cluster-harvested tomatoes, yield does not necessarily equate to high commercial value. Whether fruits are well rounded, whether the shoulders are symmetrical, whether the fruit shape index is stable, and whether fruit size is uniform all directly affect grading, packaging efficiency, transportation damage rates, and consumer acceptance. Traits such as fruit weight, shoulder height, and height-to-width ratio are closely related to commercial classification. Although environmental effects on fruit shape parameters are usually smaller than their effects on chemical traits such as sugar content and dry matter, clear genotype \times environment interactions still exist for fruit size and shape stability. Therefore, under protected cultivation conditions, selecting varieties with stable fruit-shape performance is of great significance for maintaining consistent market quality. Fruit uniformity is not only related to appearance, but also directly affects harvest rhythm and commercial management efficiency.

If fruit enlargement rates differ greatly among fruits on the same truss or the same plant, uneven ripening is likely to occur, increasing the number of harvest rounds, raising the difficulty of manual grading, and reducing packaging standardization. In greenhouse tomato quality control systems, uniform fruit shape, smooth fruit surface, and fewer mechanical or physiological defects are usually important prerequisites for fruits to enter high-grade commercial circulation systems. Surface defects such as fruit cracking, scarring, zippering, and wind scars can significantly reduce commercial value, and fruits may be downgraded even if their weight meets the standard. Therefore, the marketability of protected tomatoes is not determined solely by large fruit, but is instead the combined result of fruit shape stability + size uniformity + skin integrity.

From the perspective of genetic basis, fruit shape, fruit size, and susceptibility to commercial defects are interrelated. GLOBE locus on chromosome 12 can significantly affect the difference between oblate and spherical fruit shapes in large-fruited fresh-market tomatoes, while also influencing fruit size, pedicel morphology, and the tendency for skin defects to occur (Sierra-Orozco et al., 2021). Among these, spherical-fruit materials often have larger fruits, but are also more prone to surface cracking and epidermal defects, thereby creating a trade-off among fruit shape, fruit weight, and marketability (Sierra-Orozco et al., 2021). This indicates that in protected tomato breeding and cultivation, simply pursuing larger fruit does not necessarily lead to higher market returns; fruit shape stability and defect control must also be considered simultaneously. With the development of phenotypic analysis and digital agriculture technologies, evaluation of fruit shape and uniformity is gradually shifting from experience-based judgment to standardization and intelligence. Machine-learning classification systems established using standardized fruit-shape parameters from tools such as Tomato Analyzer have already shown greater stability and consistency than manual evaluation in tomato fruit-shape classification, and can be used for breeding material screening, fruit grading, and supply-chain quality control (Vazquez et al., 2024). In addition, some preharvest growth-regulation measures can improve fruit shape and internal quality to a certain extent. For example, benzylaminopurine (BAP) and gibberellin treatments can alter the fruit shape index of small-fruited greenhouse tomatoes, making fruits shift from elongated forms to more rounded ones, accompanied by changes in soluble solids and some nutritional indicators. This suggests that fruit shape is plastic to a certain extent and can be optimized through physiological regulation (Eissa et al., 2025).

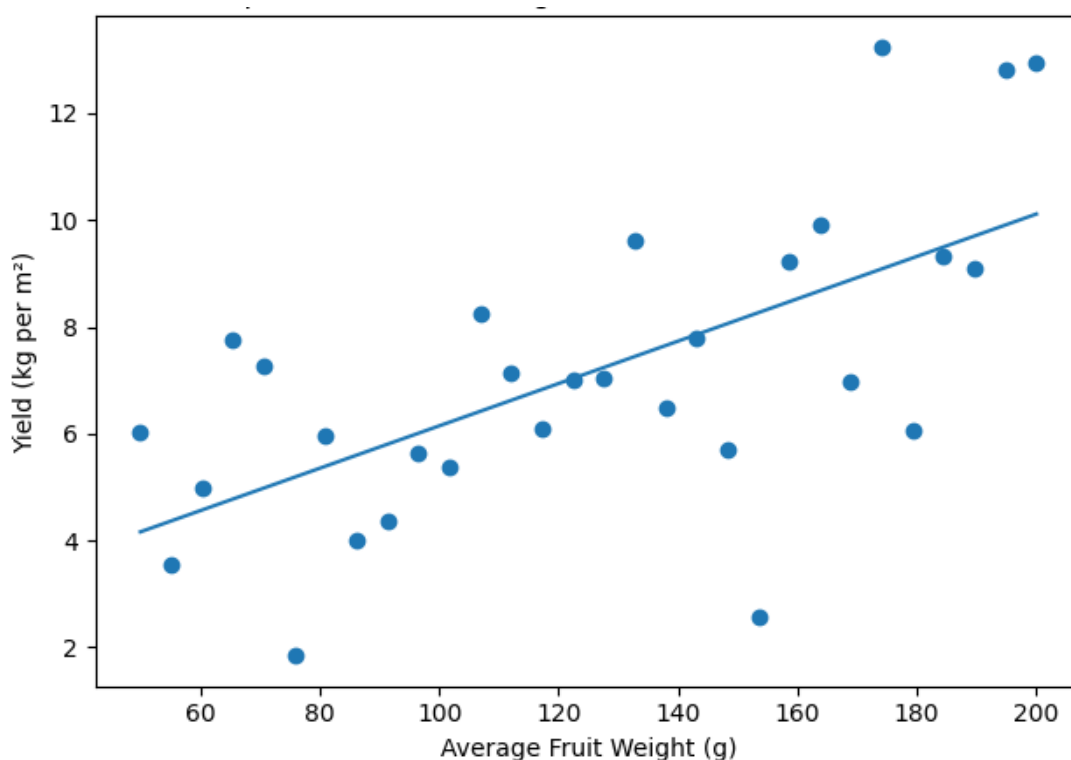


Figure 3 Relationship between average fruit weight and total yield of greenhouse-grown tomatoes under different irrigation treatments

4.3 Characteristics of nutrient allocation and assimilate accumulation during fruit development

Fruit development in greenhouse-grown tomatoes is essentially a continuous process of assimilate translocation and accumulation toward the fruit as the primary sink. During the late growth stage, fruits become the dominant site of dry matter accumulation, accounting for up to 73% of aboveground dry biomass under organic cultivation conditions, indicating a clear shift to fruit-centered resource allocation. Nitrogen and potassium are key macronutrients for maintaining sink activity, particularly under continuous fruiting. Nutrient demand varies across developmental stages: early fruit development is characterized by cell division and sink establishment, regulated by assimilate supply and hormonal signals, whereas the rapid expansion stage depends on sustained inputs of sugars and mineral nutrients to determine fruit weight and quality. Appropriate supplemental lighting and nutrient supply can enhance both yield and quality, while excessive electrical conductivity (EC) may improve quality concentration but inhibit growth, reflecting a trade-off between biomass production and quality (Xie et al., 2024).

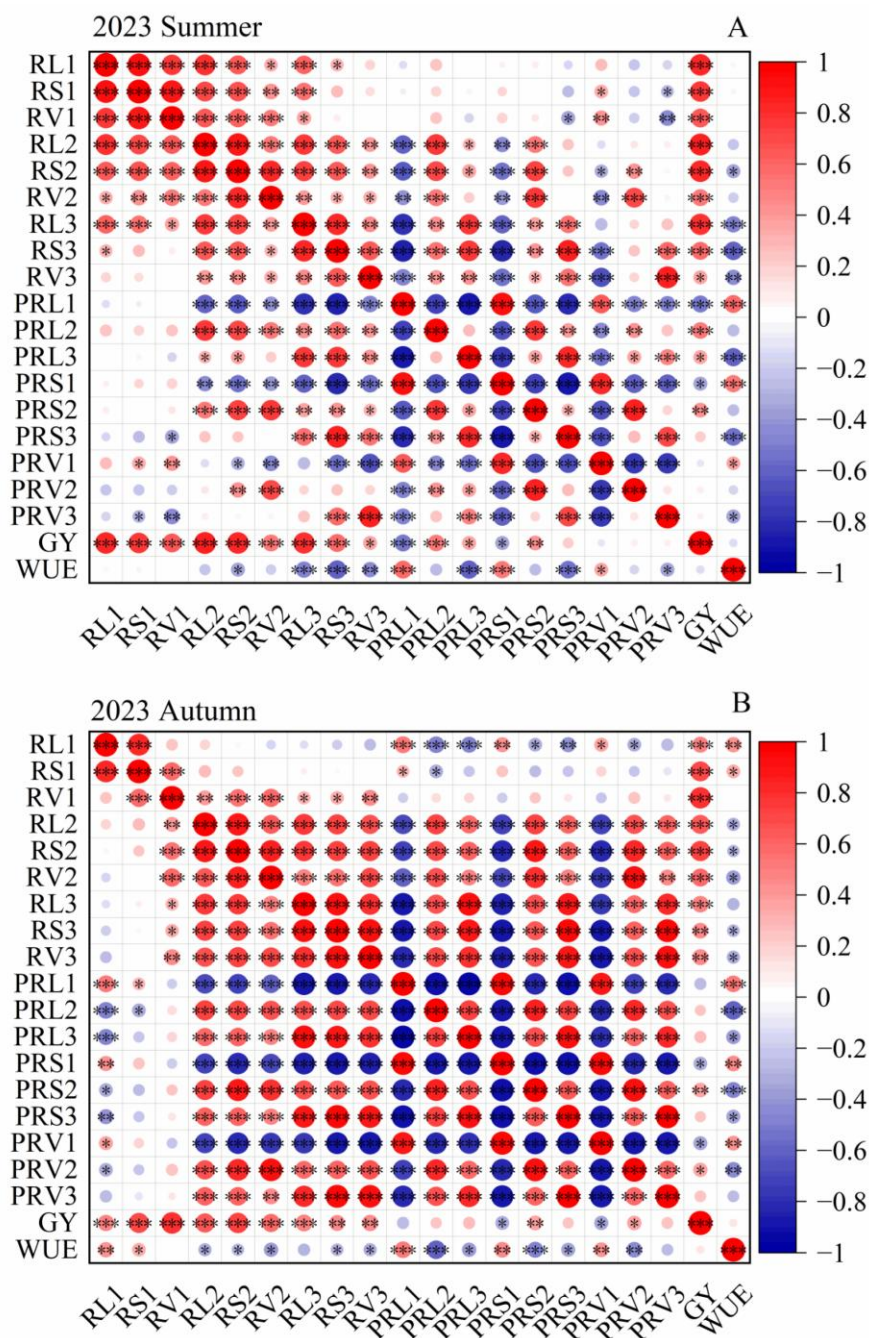
At the single-fruit level, assimilate accumulation involves not only sugars and dry matter but also dynamic changes in mineral nutrients and secondary metabolites. Increasing the proportion of blue light can enhance soluble sugars, lycopene, and β -carotene contents while maintaining fruit fresh weight, indicating that carbon allocation affects both fruit size and composition. Mineral elements continue to change during fruit development and postharvest ripening, suggesting that fruit maturation is a dynamic process involving both import and internal redistribution. Thus, fruit quality formation results from the coordinated interaction of carbon assimilation, mineral transport, and metabolic regulation. Environmental and nutrient management further shape these processes by regulating source strength and assimilate transport. Moderate deficit irrigation may reduce yield but improve quality attributes, whereas supplemental lighting and CO₂ enrichment enhance carbon supply, increasing fruit weight and overall yield. The research conducted by Su et al. (2025) demonstrated that appropriate Cl⁻ supply can improve photosynthetic performance and sucrose metabolism, promoting sugar transport to fruits and increasing soluble sugar content (Su et al., 2025). Overall, the coordinated improvement of yield and quality depends not only on total assimilate production but also on their allocation patterns within the fruit.

5 Root Traits and Nutrient Uptake Capacity in Protected Tomato Production

5.1 Effects of root activity and root distribution on water and fertilizer uptake efficiency

Root activity is one of the key factors determining water and nutrient uptake efficiency in protected tomato production. Highly active root systems usually exhibit a higher root tip growth rate, a more developed fine-root system, a larger absorptive surface area, and higher root metabolic activity. As a result, they can respond more rapidly to changes in root-zone water and nutrient availability and convert these resources into the supply needed for plant growth and fruit development. In intensive protected cultivation systems, roots are not merely passive absorbing organs, but the core interface linking irrigation, fertilization, and yield formation. Under drip fertigation, increases in root length, root surface area, and root volume in different soil layers are significantly positively correlated with tomato fruit yield and water-use efficiency. Under appropriate water-nitrogen management conditions (100% ET_c + 250 kg N·ha⁻¹), compared with the no-nitrogen treatment, root length, root surface area, and root volume increased by about 40%-150%, while yield increased by 31.6% and water-use efficiency by 34.4% (Figure 4) (Feng et al., 2024). This indicates that improved root activity is not simply reflected in having more roots, but rather in more efficient resource capture and utilization.

The fine-root system is especially important in this process. Active fine roots with smaller diameters usually account for the main absorptive function of the root system, and their length, surface area, and branching degree directly determine the intensity of contact between roots and the root-zone solution. Studies on aerated irrigation further show that increasing dissolved oxygen levels in the root zone can significantly increase the length and surface area of fine roots (≤ 2 mm), and these fine-root traits are significantly positively correlated with aboveground biomass, fruit yield, nitrogen-use efficiency, and irrigation water-use efficiency (Zhang et al., 2023). Therefore, the high and stable yields of protected tomatoes do not depend simply on a large root mass, but on an efficient root system characterized by abundant fine roots, active absorption, and strong renewal capacity.



* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

Figure 4 Correlation of root characteristics with fruit yield and water use efficiency (Adopted from Feng et al., 2024)

Image caption: RL1: root length belong to the 0-10 cm soil layer, RS1: root surface area belong to the 0-10 cm soil layer, RV1: root volume belong to the 0-10 cm soil layer, RL2: root length belong to the 10-20 cm soil layer, RS2: root surface area belong to the 10-20 cm soil layer, RV2: root volume belong to the 10-20 cm soil layer, RL3: root length belong to the 20-30 cm soil layer, RS3: root surface area belong to the 20-30 cm soil layer, RV3: root volume belong to the 20-30 cm soil layer, PRL1: Radio of root length belong to the 0-10 cm soil layer to total root length, PRS1: Radio of root surface area belong to the 0-10 cm soil layer to total root surface, PRV1: Radio of root volume belong to the 0-10 cm soil layer to total root volume, PRL2: Radio of root length belong to the 10-20 cm soil layer to total root length, PRS2: Radio of root surface area belong to the 10-20 cm soil layer to total root surface, PRV2: Radio of root volume belong to the 10-20 cm soil layer to total root volume, PRL3: Radio of root length belong to the 20-30 cm soil layer to total root length, PRS3: Radio of root surface area belong to the 20-30 cm soil layer to total root surface, PRV3: Radio of root volume belong to the 20-30 cm soil layer to total root volume, GY: fruit yield, WUE: water use efficiency. (A) 2023 Summer (B) 2023 Autumn (Adopted from Feng et al., 2024)

In addition to root activity, the vertical and horizontal distribution patterns of roots also profoundly affect water and fertilizer uptake efficiency. If roots are excessively concentrated in localized zones, water and nutrients may be depleted too rapidly in those areas, increasing spatial heterogeneity of resources in the soil or substrate and reducing overall use efficiency. In contrast, if roots can form a relatively broad and uniform distribution within the root zone, they are better able to continuously acquire water and nutrients from different spatial locations and buffer short-term fluctuations in supply. In soil-grown greenhouse tomatoes, mulching practices, emitter placement, and irrigation level all significantly alter root length density distribution. For example, under plastic mulch, more roots are concentrated in the top 0-20 cm of soil, whereas without mulch, roots are more often distributed at a depth of about 20 cm, which is related to the more suitable temperature and moisture conditions in the surface soil under mulching (Ge et al., 2025). At the same time, deficit irrigation can cause roots to redistribute either upward or deeper into the soil profile. In potted cherry tomato, increasing the number of emitters per plant can encourage roots to expand more widely, while deficit irrigation promotes deeper root extension. The combination of two emitters + deficit irrigation can create a root distribution that is both wide and deep, thereby increasing root length density, root weight density, yield, and water-use efficiency (Figure 5).

5.2 Fundamental role of root health in plant growth and stress resistance

In addition to root system size and distribution, root health itself is also fundamental to sustained growth and stress resistance in protected tomato production. Root health includes not only intact root structure, high cellular activity, and undamaged absorptive tissues, but also a stable rhizosphere environment, adequate oxygen supply, low pathogen pressure, and a relatively balanced beneficial microbial community. Healthy roots can maintain high water and nutrient uptake capacity and support continuous shoot growth and high photosynthetic assimilation by influencing hormonal signaling, osmotic regulation, and antioxidant systems. Under high-temperature stress, tomato genotypes that maintain higher root dry weight and a higher root-to-shoot ratio usually show higher yield and harvest index, indicating that a larger, healthy root biomass is an important basis for heat tolerance and sustained carbon assimilation (Mohammed et al., 2025).



Figure 5 Spatial distribution of tomato fruits at different maturity stages on plants and management status

The role of root health in stress resistance is especially evident under water stress and nitrogen stress. Drought stress significantly reduces root length, root biomass, and root activity, thereby weakening water and nutrient uptake capacity and leading to reduced shoot growth and photosynthesis. Under high nitrate stress, root structure is also inhibited, with development of both coarse and fine lateral roots being restricted and membrane stability declining, thereby affecting overall uptake and growth performance. Correspondingly, exogenous regulatory measures that improve root structure and vitality often have clear mitigating effects. For example, melatonin and sodium nitroprusside treatments can promote the growth of both coarse and fine lateral roots, increase root activity and antioxidant enzyme activity, and thereby enhance plant adaptation to high-nitrate conditions. Similarly, tannin-based biostimulants can increase root length and root weight under salt stress and upregulate the expression of genes related to root development and salt tolerance, thereby improving nutrient uptake and root salt tolerance.

Root health also determines the plant's basic resistance to biotic stress. Roots are a major entry point for soil-borne pathogens. If roots are damaged, if the rhizosphere microecology is imbalanced, or if aeration is poor, diseases such as root rot and wilt can be easily induced, thereby threatening whole-plant growth stability. Conversely, inoculation with beneficial microorganisms can enhance root health by promoting root growth, improving antioxidant capacity, enhancing rhizosphere nutrient cycling, and competitively suppressing pathogens. *Trichoderma asperellum* or plant growth-promoting rhizobacteria can significantly increase tomato root and shoot biomass, improve soil enzyme activity and nutrient availability, and effectively suppress soil-borne diseases such as *Fusarium oxysporum* (Zhang et al., 2025b). This indicates that root health is not only an inherent plant trait, but is also strongly influenced by the state of the rhizosphere ecosystem.

5.3 Improvement of root development and yield stability through grafting cultivation

In recent years, grafting cultivation has become one of the key technologies in protected tomato production for improving root performance, enhancing stress resistance, and increasing yield stability. Its core mechanism lies in using superior rootstocks with strong root systems, stress tolerance, and disease resistance to compensate for deficiencies of scion cultivars in root absorption, rhizosphere adaptation, and environmental stress tolerance. Through grafting, tomato plants can develop greater total root length, more root tips, a higher root-to-shoot ratio, and stronger root activity by relying on the rootstock, thereby enhancing water and nutrient uptake capacity and improving growth stability under adverse conditions such as low temperature and salt stress. Different rootstocks differed significantly in root traits, and grafted combinations using superior rootstocks increased yield by 14.6%-17.2% compared with nongrafted plants without reducing fruit quality; in some cases, lycopene and ascorbic acid contents were also increased.

The improvement in root development brought about by grafting is not limited to an increase in root quantity, but is more importantly reflected in optimized root function. Superior rootstocks usually have stronger root branching capacity, higher mineral nutrient uptake efficiency, and better osmotic adjustment ability, enabling them to maintain stable resource supply under unfavorable conditions. For example, in coconut coir-based cultivation systems, some tomato rootstocks can increase root and shoot biomass, chlorophyll content, and the uptake of mineral elements such as K, Ca, Mg, Fe, Mn, and Cu, thereby improving both yield and fruit quality. This indicates that grafting can fundamentally strengthen the growth basis of protected tomatoes by improving root growth and mineral nutrient acquisition.

Grafting is particularly valuable in buffering environmental stress and disease pressure. Under deficit irrigation and partial root-zone drying, grafted tomatoes can still maintain relatively high vegetative growth, mineral nutrient uptake, and fruit yield, even with a 30%-40% reduction in irrigation water, while achieving higher water-use efficiency. This indicates that the enhanced root absorption and water regulation capacity provided by the rootstock can significantly improve the resilience of resource use under protected cultivation conditions. In addition, when wild *Solanum* species are used as rootstocks, grafted plants show improvements in plant height, branch number, fruit number, average fruit weight, and yield per plant, while also exhibiting significant resistance to soil-borne diseases such as bacterial wilt (Kamble et al., 2025). Regarding adaptation to high temperatures,

recent studies also indicate that heat tolerance can to some extent be transferred through grafting, although the effect depends on appropriate matching between rootstock and scion (Biermann et al., 2025).

6 Regulatory Effects of the Protected Environment on High and Stable Tomato Yield

6.1 Effects of temperature regulation on tomato growth, development, and fruit set

Temperature is one of the key environmental factors affecting growth, development, and yield stability in protected tomato production. Tomatoes have different temperature requirements at different growth stages, but overall, their growth, flowering, pollination, and fruit development depend on suitable and stable temperature conditions. Tomatoes generally exhibit good growth and fruiting performance when daytime temperatures are about 18 °C-29 °C with relatively lower nighttime temperatures, whereas excessively high or low temperatures can disrupt plant physiological processes and reduce yield stability (Arshad et al., 2024). Under high-temperature conditions, early plant growth rates may temporarily increase, but pollen viability, pollen germination, and pollen tube growth are often inhibited, resulting in poor pollination, reduced fruit set, and increased flower and fruit drop (Jerca et al., 2024). When the average temperature increases from 14 °C-26 °C, the period from flowering to fruit maturity can be significantly shortened, but excessively high temperatures often lead to smaller fruits and reduced fruit set.

Low temperatures can also limit protected tomato production. Under low-temperature conditions, leaf photosynthesis and assimilate transport capacity decrease, plant growth slows, and flower bud differentiation may be inhibited, leading to delayed flowering (Adams et al., 2001). In early spring or winter protected cultivation, excessively low night temperatures often impair reproductive growth and reduce fruit marketability. In addition to average temperature, microclimatic differences within the greenhouse can also influence yield performance. Studies show that even when the difference in average daily temperature within the same greenhouse is only about 3 °C, noticeable differences in plant growth rate and fruit truss weight may occur (Šalagovič et al., 2024). Furthermore, maintaining temperatures around 18 °C-22 °C during flowering and early fruit set is more favorable for inflorescence productivity (Jerca et al., 2024), while maintaining relatively higher air temperature and suitable root-zone temperature during the seedling stage promotes root development and leaf area formation.

6.2 Effects of light intensity and photoperiod on photosynthesis and yield formation

Light is the primary energy source driving photosynthesis and dry matter accumulation in tomatoes, and therefore directly influences the potential yield of protected tomatoes. Light intensity, daily light integral (DLI), and light distribution within the canopy all affect leaf carbon assimilation capacity and fruit development. Although light distribution in large greenhouses is relatively uniform, spatial variation still leads to differences in plant growth and yield; plants located in areas with better light conditions generally achieve higher yields (Šalagovič et al., 2024). Adequate and sufficient light promotes photosynthesis and dry matter accumulation and provides the energy required for flowering and fruit enlargement. The optimal light intensity for inflorescence development and high yield formation in cherry tomato is about 360-384 W·m⁻², whereas insufficient light reduces inflorescence number and fruit set efficiency (Jerca et al., 2024). Under winter greenhouse conditions, increasing light intensity can also significantly promote plant growth and yield formation (Arshad et al., 2024).

When natural light is insufficient, artificial supplemental lighting has become an important technology in protected tomato production. A meta-analysis showed that LED supplemental lighting can increase greenhouse tomato yield by about 40% on average and significantly enhance photosynthetic capacity and chlorophyll content. Inter-canopy lighting not only increases total radiation input but also improves light distribution within the canopy, enhancing light-use efficiency of lower leaves and promoting uniform fruit development. In addition, photoperiod and the allocation of light-dark cycles may also influence yield formation. Under the same daily light integral, extending the light-dark cycle may increase yield in some cases; however, when the total light input remains constant, changes in photoperiod have a limited effect on yield. Therefore, in protected tomato production, ensuring sufficient daily light integral (DLI) and properly scheduling supplemental lighting periods is generally more important (Shibaeva et al., 2024).

6.3 Regulatory effects of air humidity and CO₂ concentration on tomato growth in protected systems

Air humidity and CO₂ concentration are important factors influencing tomato growth in protected environments, and they regulate plant physiological activity by affecting stomatal behavior, transpiration, and carbon assimilation processes. Appropriate air humidity helps maintain stomatal opening and transpiration-driven transport, whereas excessively high or low humidity can negatively affect plant growth. When the air is too dry and the vapor pressure deficit (VPD) is high, transpiration increases and stomata may close, reducing CO₂ assimilation rates. Conversely, excessive humidity restricts transpiration, affects mineral nutrient transport, and increases the risk of physiological disorders such as blossom-end rot. Even when VPD differences within the greenhouse are only about 0.6 kPa, significant differences in plant and fruit growth rates may occur (Šalagovič et al., 2024). In addition, humidity fluctuations can influence stomatal conductance and photosynthetic rates under fluctuating light conditions (Shi et al., 2024).

CO₂ concentration is another important regulatory factor that enhances photosynthetic potential in protected environments. Increasing environmental CO₂ concentration from approximately 400 ppm to 800-1 000 ppm can significantly increase leaf area, chlorophyll content, and net photosynthetic rate, thereby promoting dry matter accumulation and yield formation (Amarasinghe et al., 2025). This stimulatory effect is more pronounced under suitable light and temperature conditions, because photosynthesis responds more effectively to increased carbon supply under these conditions. However, excessively high CO₂ concentrations are not always beneficial. In cherry tomato production, the optimal CO₂ concentration under moderate light conditions is approximately 450-510 ppm, whereas excessively high CO₂ may reduce certain quality parameters through dilution effects (Arshad et al., 2024). Therefore, in protected tomato production, CO₂ regulation must balance the improvement of yield potential with the maintenance of fruit quality.

7 Cultivation Management Measures for Achieving High and Stable Yields in Protected Tomato Production

7.1 Optimization of canopy structure through rational plant density and pruning

Rational plant density and pruning (including side-shoot removal) are important cultivation measures for achieving high and stable yields in protected tomato production. Their core objective is to optimize canopy structure so as to coordinate the relationship among yield per unit area, individual plant light interception, and assimilate distribution. The canopy structure of protected tomatoes directly affects canopy light interception efficiency, the distribution of temperature and humidity within the canopy, and the balance between vegetative and reproductive growth, thereby exerting a significant influence on yield stability (Figure 6). Planting arrangements-such as row orientation, plant spacing, row spacing, and furrow spacing-often have a greater impact on canopy radiation interception, temperature distribution, and dry matter accumulation than individual plant structural traits. Moderately increasing plant spacing can improve light interception by individual plants and enhance fruit development. In protected production systems, moderate dense planting can increase leaf area per unit area and improve canopy light interception. However, excessively high planting density can deteriorate ventilation and light conditions, intensify competition among plants, and increase the risk of disease. In solar greenhouses, adopting an east-west row orientation combined with relatively wider row spacing can significantly enhance canopy light interception and photosynthetic capacity, increasing yields by approximately 4%-10% across different seasons (Li et al., 2024).

In recent years, dynamic planting density strategies have provided new approaches to canopy management in protected tomato production. By maintaining relatively high planting density during early growth stages to maximize canopy light interception, and then reducing density in later stages, it is possible to alleviate problems such as reduced fruit size and quality caused by excessive crowding, thereby balancing high yield and fruit quality (Karpe et al., 2024). Pruning and side-shoot removal further optimize canopy structure by controlling branch number and leaf area distribution, improving canopy ventilation and light penetration while promoting assimilate allocation to flowers and fruits. In high-wire cultivation systems, training a plant into two main stems can increase yield per plant and improve spatial utilization efficiency without increasing plant number. In addition, appropriate leaf removal is also an important component of refined canopy management. Studies show that data-driven leaf

pruning strategies based on light environment monitoring can reduce pruning frequency by approximately 35%-42%, while maintaining yield and increasing soluble solids content (Kim and Kubota, 2025). Under winter conditions, leaf pruning and LED supplemental lighting exhibit clear synergistic effects, with supplemental lighting significantly increasing yield and accelerating fruit maturation.



Figure 6 Demonstration of cultivation management and growth status of greenhouse-grown tomatoes

7.2 Effects of integrated water and fertilizer management on nutrient supply and yield stability

Integrated water-fertilizer management is an important technique for efficient protected tomato cultivation. Its core principle is the precise coupling of irrigation and fertilization to achieve dynamic matching between water and nutrient supply in the root zone, thereby maintaining stable plant growth and improving resource-use efficiency. Compared with traditional fertilization methods, integrated water-fertilizer management emphasizes adjusting water and nutrient supply according to crop growth stages and environmental conditions in order to avoid growth imbalance caused by water stress or excessive nutrient supply. Tomatoes exhibit different water and nutrient requirements at different growth stages. During the seedling and vegetative growth stages, adequate water supply is needed to promote root development and leaf area expansion, whereas during flowering and fruiting stages, stable supplies of nitrogen, phosphorus, and potassium are required to maintain fruit set and fruit enlargement. Under drip irrigation in solar greenhouses, combining soluble organic fertilizers with chemical fertilizers and applying appropriate irrigation levels can significantly increase nitrogen uptake, yield, and water-use efficiency, while also providing more stable economic returns.

Clear synergistic effects exist between irrigation level and fertilization rate. Research indicates that under micro-seepage irrigation, a combination of moderate irrigation and moderate fertilization can achieve higher photosynthetic rates, greater dry matter accumulation, and higher yields, while also improving fertilizer-use efficiency (Liu et al., 2024). In substrate cultivation systems, initiating irrigation when substrate moisture declines to about 70% of its capacity significantly improves water-use efficiency and enhances fruit soluble solids and vitamin C content compared with irrigation at higher moisture levels. In addition, irrigation frequency and real-time monitoring also influence water and nutrient use efficiency. Shorter irrigation intervals under drip irrigation help maintain stable root-zone moisture conditions and promote root growth (Zhang et al., 2025a). Intelligent irrigation control using soil moisture sensors can further reduce irrigation water consumption while improving yield and nutrient uptake efficiency (Wang et al., 2024).

7.3 Application of growth regulators and pollination techniques to improve fruit set

Fruit set rate is an important limiting factor in yield formation of protected tomatoes, especially under suboptimal temperature, light, or humidity conditions. Therefore, the use of plant growth regulators, biostimulants, and assisted pollination technologies has become an important strategy for stabilizing yield in protected tomato production. Certain biostimulants can enhance plant vigor and increase yield by regulating endogenous hormone levels, promoting cell division, and stimulating chlorophyll formation. Applying biostimulants such as Albit and

Turboroot in greenhouse tomato production can increase chlorophyll content and promote vegetative growth; when combined with soil improvement measures, yields can increase by approximately 16%-45% (Avasiloaiei et al., 2025). At the same time, appropriate levels of NPK fertilization are also important for stable fruit set, with moderate fertilization levels optimizing photosynthetic rates and fruit quality.

In addition to growth regulators, canopy structure and environmental conditions also influence fruit set stability. Excessively high planting density or excessive branching can intensify resource competition among fruits, resulting in reduced single fruit weight and increased fruit drop (Karpe et al., 2024). Therefore, rational plant density and pruning management can increase assimilate supply to inflorescences, thereby promoting fruit set and fruit retention. Improving the light environment can also increase fruit set rates. For example, LED supplemental lighting has been shown to increase fruit set rate by about 46% and accelerate fruit maturation. Under protected cultivation conditions, weaker air movement and reduced insect activity often result in lower pollination efficiency compared with open-field conditions. Therefore, pollination efficiency can be significantly improved through manual vibration, mechanical vibration, or bumblebee pollination, which enhance pollen release and fertilization success. These measures help reduce flower and fruit drop and stabilize yield.

8 Future Development Trends in High and Stable Yield Cultivation of Protected Tomatoes

With the rapid development of sensor technology, automation, artificial intelligence, and data science, protected tomato production is gradually shifting from experience-driven management to data- and model-driven systems. A key characteristic of future high-yield and stable greenhouse production will be the use of intelligent environmental control technologies to monitor and regulate key factors such as temperature, humidity, light intensity, CO₂ concentration, irrigation, and nutrient solution supply in real time through closed-loop control systems. This approach enables more precise production management. Currently, high-tech greenhouses have increasingly integrated technologies such as water and fertilizer sensors, supplemental lighting systems, and microclimate control software, forming automated production systems that combine environmental sensing, decision analysis, and operational control. Compared with traditional experience-based management, intelligent control systems can dynamically optimize environmental parameters by integrating real-time and historical data, thereby improving resource-use efficiency and stabilizing yield levels. In recent years, greenhouse control platforms and predictive models based on the Internet of Things (IoT) have further promoted the transition of protected agriculture from passive regulation to proactive prediction. Meanwhile, emerging concepts such as autonomous greenhouses and digital twin greenhouses are providing important directions for the future intelligent upgrading of protected tomato production. In addition to improving yield, intelligent environmental regulation can also reduce environmental burdens by optimizing energy and water-fertilizer utilization efficiency, thereby providing technical support for sustainable protected tomato production.

Against the backdrop of climate change and increasing intensification of protected cultivation systems, the breeding of high-yield and stress-resistant varieties, together with grafting technology, will become important foundations for stable tomato production. Modern tomato breeding is gradually shifting from a single focus on high yield to the improvement of multiple traits including high yield, superior quality, stress tolerance, and adaptability to protected cultivation environments. This transition is increasingly supported by technologies such as marker-assisted selection, molecular design breeding, and gene editing, which are used to develop new varieties resistant to high temperature, salinity, and diseases. Tolerance to stresses such as high temperature and waterlogging in tomato is associated with multiple genetic effects, and some superior hybrid combinations can still maintain relatively high fruit set and yield under adverse conditions. Therefore, future breeding efforts should not only focus on fruit quality and marketability, but also incorporate root system traits, reproductive stability, and stress-resistance physiological characteristics as key selection criteria. At the same time, grafting cultivation can combine high-quality scions with stress-resistant rootstocks, improving plant adaptability to complex environments and extending the fruiting period. Recent studies have also revealed that certain stress-resistance traits can be regulated by rootstocks and transferred to the scion, providing new directions for targeted rootstock breeding and research on rootstock-scion interactions.

Future protected tomato production will not only aim to achieve high and stable yields but must also emphasize efficient resource utilization and environmental sustainability. Consequently, green and efficient protected agriculture systems will become an important development direction. This model emphasizes the integration of technologies such as precision water-fertilizer management, nutrient recycling, renewable energy coupling, and biological pest control to maintain high productivity while reducing carbon emissions and environmental pressure. Compared with open-field production, protected systems often achieve better resource-use efficiency due to higher water-use efficiency and greater yield per unit area. In China's solar greenhouse and plastic tunnel systems, measures such as drip irrigation, integrated water-fertilizer management, and controlled-release fertilizers can significantly reduce carbon emissions, with fertilizer management considered a key factor influencing the environmental footprint of greenhouse production. Furthermore, high-tech soilless cultivation systems in greenhouses demonstrate the potential for circular and low-carbon production through nutrient solution recirculation systems and the use of clean energy. In the future, protected tomato production will also place greater emphasis on integrated pest management (IPM) and biological control technologies, while adapting to different greenhouse structures and regional conditions to develop diversified and sustainable green production systems.

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Conflict of Interest Disclosure

The authors affirm 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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Review and Progress

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Trait Basis and Breeding Strategies for the Coordinated Improvement of Yield and Sugar Content in Sugarcane

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Abstract This study explores the trait basis, genetic mechanisms, and breeding strategies for the coordinated improvement of yield and sugar content in sugarcane. Yield and sugar content are the two key traits determining sugar yield per unit area and industrial value, and their formation is jointly influenced by agronomic traits, physiological processes, and molecular regulatory networks. Cane yield is mainly determined by millable cane number, single stalk weight, plant height, and stalk diameter, while sugar content is characterized by quality traits such as Brix, Pol, CCS, juice purity, and fiber content. Photosynthetic efficiency, dry matter accumulation, source-sink relationships, and assimilate partitioning constitute the fundamental physiological basis linking biomass formation and sucrose accumulation. Genetically, the complex polyploid genome of sugarcane makes both traits typical quantitative traits controlled by multiple genes, allele dosage effects, and strong environmental interactions. This study further summarizes breeding strategies, including conventional hybrid breeding, marker-assisted selection, genomic selection, and gene editing, as well as the roles of agronomic management such as water and fertilizer regulation, population structure optimization, and proper harvesting time. Overall, achieving high yield and high sugar content relies on integrating yield- and quality-related traits and their underlying mechanisms within a genotype-environment-management framework to promote multi-trait coordinated improvement.

Keywords Sugarcane; Yield; Sugar content; Source-sink relationship; Coordinated improvement

1 Introduction

Sugarcane (*Saccharum* spp.) is one of the most important sugar crops globally, contributing approximately 80% of the world's sugar supply, and also serves as a major source of biomass energy and bio-based materials (Wu et al., 2024). In tropical and subtropical countries such as Brazil, India, and China, the sugarcane industry not only supports the sugar production system but is also closely linked to rural economic development, employment, and regional agricultural stability. With the advancement of biorefinery systems, sugarcane is no longer merely a traditional sugar crop, but has evolved into an integrated industrial feedstock capable of producing sugar, fuel ethanol, electricity, and various bio-based products. Its strategic importance in the circular economy and low-carbon bioeconomy continues to increase (Wang et al., 2025). Under the pressures of global population growth, limited arable land, and climate change, improving the efficiency with which sugarcane converts solar energy into fermentable sugars and structural biomass has become a key scientific challenge for sustainable agricultural and energy systems (Lu et al., 2024; Mehdi et al., 2024).

In sugarcane production systems, sugar yield per unit area is determined by both cane yield and sucrose content, making it a core indicator of cultivar value and cultivation efficiency. Theoretically, maximizing industrial benefits requires simultaneous improvement in biomass accumulation and sugar concentration. However, in practical breeding, increases in sugar yield have relied more on improvements in cane yield, while gains in sugar content have progressed more slowly. This is closely associated with the physiological and genetic trade-offs between the two traits: high-biomass genotypes tend to allocate more assimilates to structural carbon pools, whereas high-sugar genotypes may divert carbon toward storage tissues at the expense of sustained vegetative growth. Thus, although yield and sugar content jointly determine final sugar yield, their formation processes are not fully synchronized and often exhibit complex coordination and trade-offs. This carbon assimilation, transport,

and allocation-driven mechanism constitutes a major bottleneck in achieving coordinated improvement of high yield and high sugar content in sugarcane breeding (Lu et al., 2024; Wu et al., 2024).

From a breeding perspective, modern sugarcane cultivars are mainly derived from interspecific hybridization between *Saccharum officinarum* and *S. spontaneum*, followed by repeated backcrossing. This system has significantly improved yield, adaptability, and stress resistance, leading to the development of many widely adopted and representative cultivars (Li et al., 2024; Wu et al., 2024). However, in recent years, genetic gains for complex traits in sugarcane breeding programs have shown signs of plateauing. This is largely due to the highly polyploid and heterozygous genome of sugarcane, long breeding cycles, complex genotype \times environment \times management interactions, and limitations in high-throughput and precise phenotyping (Lu et al., 2024). These factors constrain the effective utilization of genetic variation and have led traditional breeding to remain biased toward yield improvement rather than coordinated trait optimization. With the development of whole-genome sequencing, genome-wide association studies (GWAS), multi-population QTL mapping, and multi-omics approaches, researchers have gradually elucidated the genetic architecture of key traits such as cane yield, plant height, stalk diameter, tiller number, and sucrose content. In addition, key enzymes and regulatory networks involved in sucrose metabolism have been identified as critical factors in sugar accumulation (Li et al., 2024; Mehdi et al., 2024). Meanwhile, emerging technologies such as genomic selection, RNA interference, and gene editing provide new molecular tools to improve selection efficiency and precisely regulate carbon allocation (Brant et al., 2025; Wang et al., 2025).

This study aims to explore the theoretical basis and technical pathways for the coordinated improvement of sugarcane yield and sucrose accumulation. Although previous studies have reviewed sugarcane improvement from perspectives such as breeding history, genomics, or biorefinery applications, there is still a lack of an integrated framework that systematically links the multi-trait basis of yield and sugar accumulation, their genetic and physiological interactions, and coordinated improvement strategies under different environmental conditions. Therefore, developing a coordinated optimization framework centered on multi-trait (NTrait) integration has become a key direction for advancing efficient sugarcane breeding. This study analyzes the genetic associations and regulatory networks between yield- and sugar-related traits, summarizes advances in QTL mapping, association analysis, marker-assisted selection, and genomic selection, and further discusses multi-trait-driven breeding strategies and ideotype design. The objective is to provide a clearer theoretical foundation and methodological framework for the coordinated improvement of high yield and high sugar content, and to support the efficient utilization of sugarcane in sugar production and bioenergy systems.

2 Trait Basis of Sugarcane Yield and Sugar Content Formation

2.1 Key agronomic traits related to sugarcane yield formation

Sugarcane yield is a typical complex quantitative trait, commonly expressed as tons of cane per hectare (TCH), and primarily determined by millable cane number and single stalk weight. Traits such as plant height, stalk diameter, and internode characteristics further influence yield by affecting single stalk biomass (Tolera et al., 2024). Multi-environment trials and path analysis consistently indicate that millable cane number and single stalk weight exert the strongest direct effects on yield, making them key selection targets in breeding. Thus, yield improvement depends on the coordinated optimization of multiple component traits rather than reliance on any single trait.

At the population level, millable cane number represents the fundamental determinant of yield, integrating germination rate, emergence uniformity, tillering ability, and stalk formation efficiency. Strong early germination and tillering promote rapid canopy establishment, while a high stalk formation rate ensures effective conversion of tillers into harvestable canes, thereby stabilizing yield per unit area (Tolera et al., 2024; Vennela et al., 2024). Accordingly, genotypes combining high tillering potential with stable stalk formation capacity are more likely to achieve consistently high yields.

Single stalk traits and resource-use efficiency largely determine the upper limit of biomass accumulation. Longer and thicker stalks with well-developed internodes generally exhibit greater fresh weight and dry matter

accumulation, contributing substantially to cane yield (Navya et al., 2025). In addition, physiological traits such as leaf area index, canopy structure, stay-green ability, root distribution, and nutrient balance support yield formation by enhancing photosynthetic efficiency and resource acquisition (Lu et al., 2025). Genetic studies further indicate that key yield-related traits-including stalk number, plant height, stalk diameter, and single stalk weight-are polygenically controlled and have been consistently targeted during sugarcane improvement (Li et al., 2024).

2.2 Major quality traits related to sugar content accumulation

Sugar content is a key indicator of cane quality, processing efficiency, and sugar yield, and is typically evaluated using traits such as Brix, Pol, juice purity, commercial cane sugar (CCS), and fiber content. Among these, Brix reflects total soluble solids, Pol represents sucrose concentration, purity indicates the proportion of sucrose within soluble solids, and CCS more directly reflects industrial value. These traits are generally positively correlated with each other and with sugar yield, suggesting a shared genetic and metabolic basis (Eltaher et al., 2025).

Physiologically, sucrose accumulation mainly occurs in stem internodes during the maturation stage and depends on continuous assimilate supply, phloem transport, and sink storage capacity. Leaves function as the primary carbon source, while assimilates are transported in the form of sucrose to stem sink tissues, where they accumulate in parenchyma cells. Therefore, sugar accumulation depends not only on assimilate production but also on transport efficiency, internode maturation, and sink strength. Genotypic differences in maturity timing and sugar accumulation patterns underpin the classification of early-, mid-, and late-maturing varieties.

At the biochemical and genetic levels, key enzymes such as sucrose phosphate synthase (SPS), sucrose synthase (SuSy), and invertases (INV) regulate sucrose synthesis, degradation, and storage, while cell wall-related processes influence carbon partitioning between structural and storage carbohydrates. High-sugar genotypes typically exhibit enzyme activity and expression patterns favorable for sucrose accumulation, along with structural features such as higher stem maturity, greater parenchyma proportion, and appropriate fiber content (Lu et al., 2025). Genome-wide and candidate gene studies have identified numerous loci associated with Brix, Pol, CCS, and fiber content, providing a molecular basis for improving sugar-related traits (Li et al., 2024; Eltaher et al., 2025).

2.3 Common trait basis for coordinated improvement of yield and sugar content

Although sugarcane yield and sugar content may exhibit negative correlations under certain genotypes and environments, they are not independent breeding targets. Instead, they share a common trait basis involving plant structure, source-sink relationships, carbon allocation patterns, and genetic networks. Sugar yield is the combined outcome of cane yield and sugar content; therefore, traits that enhance biomass production while maintaining or increasing sugar concentration are key targets for coordinated improvement.

Photosynthetic efficiency and assimilate production serve as the common source for both yield formation and sugar accumulation. Traits such as higher leaf area index, optimized canopy structure, prolonged functional leaf duration, and enhanced photosynthetic efficiency increase total carbon assimilation, providing substrates for both stem growth and sucrose accumulation (Lu et al., 2025). In addition, assimilate transport and partitioning efficiency represent the key physiological link between these traits. Efficient transport to stem tissues, coupled with progressive allocation toward sucrose storage, enables the simultaneous improvement of stalk weight and sugar concentration (Singh et al., 2024).

Furthermore, maturation dynamics and shared genetic bases play critical roles in coordinated improvement. An optimal maturation pattern allows extended biomass accumulation followed by efficient sucrose deposition, reducing the risk of either insufficient biomass or delayed sugar accumulation (Singh et al., 2024). Multivariate and genome-wide analyses have identified SNPs and haplotypes associated with both yield-related traits (e.g., stalk number, plant height, and diameter) and sugar-related traits, indicating a shared genetic basis (Li et al., 2024).

3 Physiological Basis of Sugarcane Yield and Sugar Content Formation

3.1 Effects of photosynthesis and dry matter accumulation on yield and sugar formation

Photosynthesis is the fundamental physiological basis for sugarcane yield and sugar accumulation. As a typical C₄ crop, sugarcane possesses high photosynthetic efficiency and carbon assimilation capacity, enabling it to maintain high net photosynthetic rates under conditions of high temperature and strong light. It efficiently converts solar radiation into dry matter, providing the primary carbon source for cane yield and sucrose accumulation. Assimilates fixed by leaves are converted into sucrose through primary metabolism and transported via the phloem to the stem, supporting stalk elongation, tissue development, and subsequent sugar deposition. Therefore, parameters such as net photosynthetic rate, stomatal conductance, chlorophyll content, leaf area index, and radiation use efficiency are closely associated with biomass production and final yield (Figure 1).

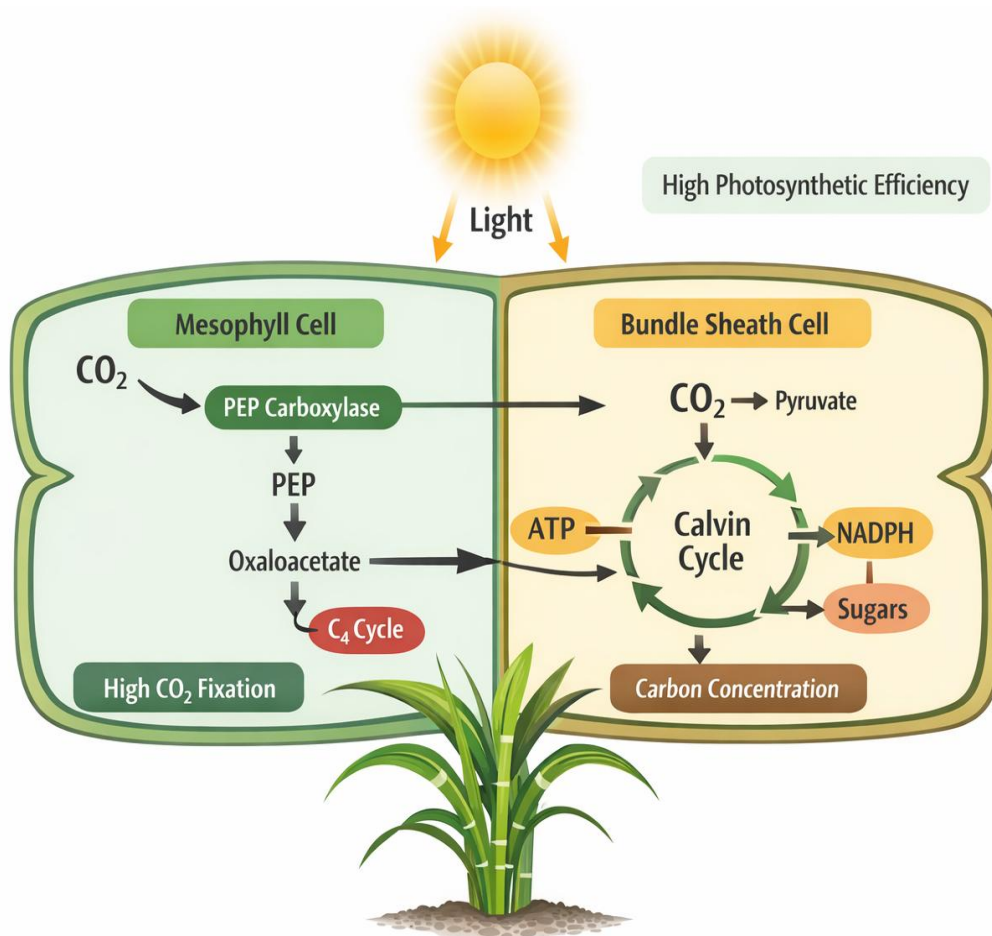


Figure 1 Schematic representation of the C₄ photosynthetic pathway in sugarcane

Image caption: CO₂ fixation in mesophyll cells and carbon concentration in bundle sheath cells to enhance photosynthetic efficiency

Dry matter accumulation serves as the key link between photosynthesis and both yield and sugar formation, reflecting the dynamic balance among carbon assimilation, respiratory consumption, and structural construction costs. During the early growth stage, sugarcane is dominated by vegetative growth, and photosynthates are mainly used for the development of leaves, stems, and roots. In the mid- to late-growth stages, as internodes mature, assimilates shift from structural carbon synthesis to soluble sugar accumulation, leading to gradual sucrose enrichment in parenchyma tissues (Martins et al., 2024; Mehdi et al., 2024). Thus, higher dry matter production efficiency, longer functional leaf duration, and sustained photosynthetic capacity are generally favorable for maintaining both high biomass and high sucrose accumulation. In essence, dry matter accumulation determines not only how many stalks are produced but also how much sugar can be stored within them.

In addition, canopy structure and ecological regulation further influence photosynthetic efficiency and dry matter production. Populations with more erect leaves, uniform canopy distribution, and good ventilation and light penetration typically exhibit higher canopy photosynthetic efficiency and biomass production (Mehdi et al., 2024). Water and nitrogen are key environmental factors affecting this process: adequate supply supports leaf area development and photosynthetic activity, whereas severe drought or nitrogen deficiency significantly suppresses photosynthetic efficiency, stalk formation, and yield (Mehdi et al., 2024). Under certain maturation stages, moderate stress may promote carbon allocation toward stem sugar storage, but excessive stress can simultaneously reduce biomass and sugar accumulation. Therefore, sustained high photosynthetic capacity, stable dry matter accumulation, and proper ecological regulation are essential prerequisites for achieving high yield and high sugar content in sugarcane.

3.2 Role of source-sink relationships and assimilate partitioning in sugar accumulation

Sugar accumulation in sugarcane is regulated by a typical source-sink relationship. Leaves act as the primary source, fixing CO₂ and synthesizing carbohydrates, while the stem serves as the main sink, particularly mature internodes that accumulate high concentrations of sucrose (Önder et al., 2025). Therefore, the capacity of source assimilation, phloem transport efficiency, unloading mechanisms, and sink storage capacity collectively determine sugar accumulation. High sugar accumulation in sugarcane is not merely the result of increased sugar production in leaves, but rather the coordinated balance between source supply and sink demand.

This source-sink relationship varies significantly across developmental stages. During early growth, leaves and young stems function mainly as growth sinks, and assimilates are primarily used for cell division, organ formation, and structural dry matter production. As internodes elongate and mature, stem sink strength increases, and mature internodes become the main carbon sinks where sucrose is extensively accumulated. Strong sink capacity allows continuous uptake and storage of sucrose from leaves, enhancing sugar content. Conversely, if transport or unloading is limited, sugars may accumulate in leaves and feedback-inhibit photosynthesis, reducing overall productivity. Thus, high photosynthetic capacity does not automatically translate into high sugar yield; the key lies in whether the sink has sufficient pull strength.

At the molecular level, source-sink coordination is finely regulated by sucrose transport, unloading, and metabolic pathways. Key enzymes such as SPS, SuSy, and various invertases determine whether sucrose entering the stem is directly stored, degraded for respiration and growth, or converted into structural carbohydrates. High-sugar genotypes typically exhibit higher SPS activity and lower acid invertase activity during maturation, favoring sucrose storage. In contrast, high-biomass genotypes often show higher SuSy and invertase activities, supporting rapid growth and cell wall synthesis but potentially reducing sugar concentration per unit fresh weight (Martins et al., 2024). Therefore, assimilate partitioning efficiency is the key link between biomass formation and sugar accumulation. If early growth prioritizes population establishment and stem elongation, followed by a gradual shift toward sugar storage in later stages, it is possible to enhance sugar accumulation without significantly compromising biomass, thereby achieving coordinated high yield and high sugar content.

3.3 Effects of hormonal regulation and stress responses on high yield and high sugar formation

Plant hormones are key integrators regulating sugarcane growth, maturation, stress adaptation, and carbon allocation. Hormones such as indole-3-acetic acid (IAA), gibberellins (GA), and cytokinins (CK) are primarily involved in stem elongation, cell division, tillering, and maintenance of leaf function, thereby influencing population structure, biomass accumulation, and sustained photosynthetic capacity (Ain et al., 2024; Lu et al., 2025). Appropriate levels of GA promote internode elongation, while IAA and CK support organ development and functional leaf maintenance, collectively forming the physiological basis for high yield. However, optimal performance depends not on the increase of a single hormone, but on the balance among different hormones and their coordination with metabolic networks (Lu et al., 2025).

Hormones closely related to maturation and carbon metabolism, such as abscisic acid (ABA), ethylene, and jasmonic acid (JA), play critical roles in sugar accumulation and stress responses. ABA is involved in maturation induction, sugar metabolism regulation, and stress signaling integration, and can promote assimilate transport to

the stem and enhance sucrose accumulation at specific stages. Ethylene is often associated with maturation promotion and increased sink strength, improving sugar storage capacity in some low-sugar genotypes (Lu et al., 2025). In contrast, sustained high levels of defense-related hormones such as JA and salicylic acid may redirect resources toward defense metabolism, inhibiting growth and sugar accumulation (Lu et al., 2025). Therefore, from the perspective of coordinated improvement, the key lies in balancing growth promotion, maturation initiation, and defense responses, rather than enhancing a single hormonal pathway.

Environmental stresses further influence yield and sugar formation through hormonal signaling and redox regulation. Drought, high temperature, nutrient deficiency, and biotic stresses can reduce photosynthesis, disrupt reactive oxygen species (ROS) balance, and alter carbon metabolism and sugar partitioning via ABA, ethylene, JA, and calcium signaling pathways (Mehdi et al., 2024). Moderate water deficit during certain maturation stages may promote sucrose accumulation, but excessive or premature stress can lead to stomatal closure, impaired electron transport, and reduced net photosynthesis, thereby decreasing both biomass and sugar accumulation (Mehdi et al., 2024). Additionally, exogenous regulators such as ethylene, seaweed extracts, and indole-3-butyric acid can improve high-yield and high-sugar performance by enhancing sink strength, promoting root development, and improving photosynthesis and antioxidant capacity (Zhang et al., 2025). Overall, the formation of high yield and high sugar content in sugarcane results from the coordinated interaction of hormonal balance, stress adaptation, and carbon allocation, and can only be fully realized under suitable environmental conditions and proper management.

4 Genetic Basis of Sugarcane Yield and Sugar Content

4.1 Characteristics of the complex sugarcane genome and its impact on trait studies

Sugarcane is a typical complex polyploid crop. Modern cultivars are mainly derived from hybridization and backcrossing between the high-sugar species *Saccharum officinarum* and the wild species *S. spontaneum*, resulting in a genome that combines high sugar content with strong adaptability but is also highly complex and mosaic in nature (Healey et al., 2024; Brant et al., 2025). Compared with most diploid crops, sugarcane exhibits higher ploidy levels, strong heterozygosity, and pronounced aneuploidy. Each chromosome often has multiple homologous copies and is accompanied by extensive structural variations, copy number variations, and large-scale rearrangements (Healey et al., 2024). This makes sugarcane one of the most genetically complex cultivated crops.

In terms of genome size, elite sugarcane hybrids typically possess genomes of approximately 8-10 Gb, rich in repetitive sequences and structurally complex regions. Although sugarcane shares partial micro-collinearity with sorghum, the relationship is not strictly one-to-one, making it difficult to directly use the sorghum genome to precisely dissect complex sugarcane traits (Healey et al., 2024). The combination of high ploidy, high repetition, and high heterozygosity has long posed major challenges for genetic map construction, allele dosage estimation, variant detection, and reference genome assembly, leading to slower progress in sugarcane genetic research compared with crops such as rice, maize, and sorghum.

This complex genomic background directly affects the study of quantitative traits such as yield and sugar content. These traits are typically controlled by numerous minor-effect loci, allele dosage effects, and non-additive genetic interactions rather than a few major genes. Ignoring ploidy variation and dosage effects in linkage analysis, GWAS, or genomic prediction can reduce the accuracy of effect estimation. In recent years, advances in high-throughput sequencing, whole-genome resequencing, and reference genome assembly—such as the release of R570 and *S. spontaneum* reference genomes—have greatly improved variant detection and candidate gene identification, providing a solid foundation for dissecting complex traits and advancing molecular breeding in sugarcane (Healey et al., 2024; Brant et al., 2025).

4.2 Advances in genes, QTLs, and molecular markers related to yield and sugar content

Sugarcane yield and sugar content are typical polygenic traits, whose genetic basis is determined by multiple QTLs, QTNs, and complex regulatory networks. In recent years, significant progress has been made in QTL mapping, association analysis, and molecular marker development for traits such as plant height, stalk number, single stalk weight, cane yield, Brix, and fiber content. Early linkage maps constructed using TRAP, DArT, SSR,

and GBS-based single-dose markers identified multiple QTLs associated with yield and quality traits, some of which showed stability across environments and crop cycles (Figure 2).

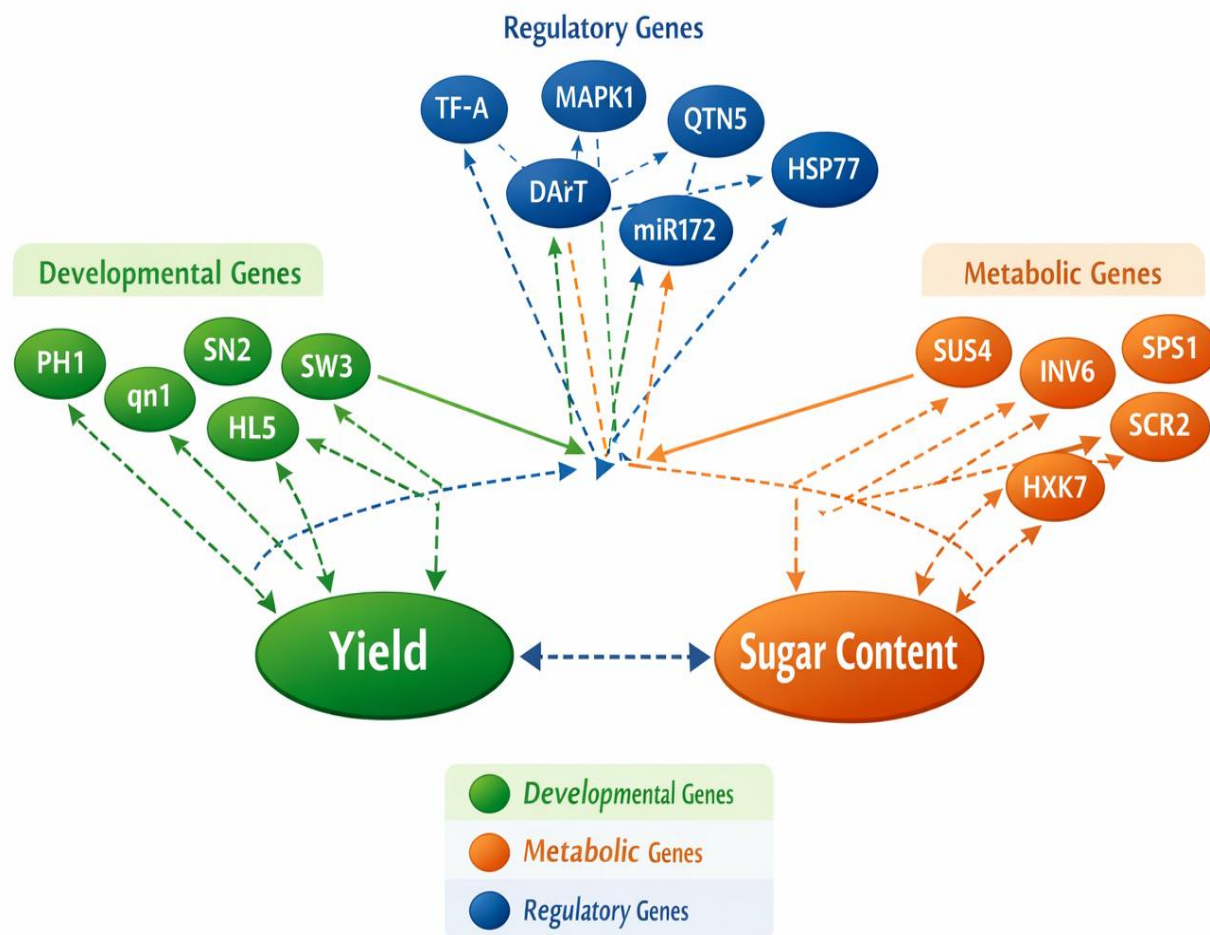


Figure 2 Conceptual model of the polygenic architecture underlying sugarcane yield and sugar content
Image caption: The interactions among multiple QTLs, QTNs, and regulatory networks

With the application of high-density SNP data, GWAS has greatly improved the resolution of genetic analysis. In Brazilian sugarcane germplasm populations, multiple marker-trait associations related to Brix, plant height, stalk number, stalk weight, and cane yield have been identified, and strong genotypic correlations among these traits suggest shared developmental and metabolic pathways (Barreto et al., 2019). In Zhang's (2023) study, more than 100 QTLs related to Brix and other yield traits were identified across the entire genome., including 35 candidate genes involved in internode development, cell wall formation, signal transduction, and carbon metabolism. These findings indicate that sugarcane yield formation is regulated by multiple developmental and metabolic processes.

For sugar content and quality traits, high-density GWAS and candidate gene analyses have identified numerous SNPs associated with Brix, Pol, CCS, sucrose content, and sugar yield (Li et al., 2024; Eltaher et al., 2025). Notably, a functional SNP in the sucrose synthase gene SoSUS1 (mSoSUS1_SNPCh10.T/C) shows significant associations with Pol, CCS, Brix, fiber content, and sugar yield across multiple environments, demonstrating clear pleiotropy and serving as an important candidate marker for selecting high-sugar genotypes with appropriate fiber content (Li et al., 2024). Meanwhile, studies on fiber-related QTLs further reveal the genetic trade-off between sugar accumulation and biomass formation (Chen et al., 2025b). With the transition to high-density SNP arrays and high-throughput sequencing platforms, QTL mapping, functional marker development, and genomic prediction are jointly driving sugarcane breeding from single-marker selection toward multi-locus integrated prediction.

4.3 Application of transcriptomics, metabolomics, and multi-omics in trait dissection

With the advancement of omics technologies, transcriptomics, metabolomics, and multi-omics integration have become essential tools for dissecting complex quantitative traits in sugarcane. Due to the highly complex genome, traditional forward genetics faces significant limitations in gene identification and functional analysis. Therefore, approaches focusing on gene expression regulation and metabolic networks have become effective strategies for studying yield and sugar formation mechanisms. In particular, RNA-seq enables systematic comparisons of gene expression across tissues, developmental stages, and genotypes, facilitating the identification of key regulatory factors associated with yield and sucrose accumulation.

Transcriptomic studies comparing high- and low-sugar materials, as well as elite and control varieties, have identified numerous differentially expressed genes involved in carbon fixation, starch and sucrose metabolism, plant hormone signaling, secondary metabolism, and cell wall formation. Pan-transcriptome analyses have further distinguished core and variable gene clusters and identified important candidate genes (Li et al., 2024). In the study by Chen et al. (2025a), ScHCT has been proposed as a key regulator of lignin biosynthesis, showing a negative correlation with sugar content and a positive correlation with lignin content, indicating a genetic coupling between secondary cell wall formation and sugar accumulation. This provides molecular insight into the balance between high sugar-low fiber and high biomass-high fiber traits.

Metabolomics reveals the dynamic processes of carbon allocation and sugar formation at the metabolite level. High-sugar varieties are enriched in sugars and sugar-phosphate intermediates related to sucrose accumulation, while secondary metabolites such as phenylpropanoids and flavonoids can indirectly regulate sugar formation by influencing carbon allocation and stress responses. In recent years, multi-omics integration has combined genomic, transcriptomic, metabolomic, and phenotypic data to dissect regulatory networks of yield and sugar content at multiple levels. Such studies have identified over 18 000 differentially expressed genes and 175 differentially accumulated metabolites, and highlighted around 100 key genes that may significantly influence high-yield and high-sugar phenotypes (Li et al., 2024). Additionally, transcription factor families such as MYB, WRKY, bHLH, NAC, TIFY, and C2C2-Dof have been identified as key regulatory nodes coordinating sugar metabolism and secondary metabolism.

5 Key Constraints on the Coordinated Improvement of Sugarcane Yield and Sugar Content

5.1 Negative correlation or trade-off between yield and sugar content

In sugarcane breeding, yield and sugar content often exhibit a certain degree of negative correlation, which is a key constraint on their coordinated improvement. Cane yield is generally positively correlated with traits such as stalk length, stalk diameter, and single stalk weight, but negatively correlated with quality traits such as Brix, sucrose percentage, juice purity, and CCS%. This indicates that although yield and sugar content jointly determine final sugar yield, they do not always increase simultaneously during trait formation, and high yield does not necessarily correspond to high sugar content.

From a physiological perspective, this trade-off reflects competition in carbon allocation between structural growth and sugar storage. Yield mainly depends on biomass accumulation in the stalk, whereas sugar content depends on sucrose concentration and storage within the stem. During vigorous vegetative growth, assimilates are preferentially allocated to cell division, elongation, and cell wall synthesis to support stalk development. In contrast, during the maturation stage, carbon flow gradually shifts toward sucrose accumulation. Under limited carbon resources, biomass increase and sugar storage tend to compete with each other.

However, this negative relationship is not absolute. Long-term breeding results indicate that increases in sugar yield have mainly been driven by biomass improvement, while gains in sugar content have been relatively slower. Nevertheless, this does not imply a permanent antagonism. Mehdi et al. (2024) mentioned in their study that there is no fixed negative trend between biomass and sugar content; instead, their relationship is strongly influenced by environmental conditions. Under favorable environments, sugarcane can simultaneously achieve high biomass and high sugar content (Mehdi et al., 2024). Therefore, this trade-off should be regarded as a conditional constraint influenced by environment, maturity process, and genetic background.

5.2 Effects of environmental factors on the stability of yield and sugar accumulation

Sugarcane yield and sugar content are not only controlled by genetic background but are also strongly influenced by environmental factors. Temperature, light, water availability, soil nutrients, and biotic stresses jointly affect photosynthesis, dry matter accumulation, carbon allocation, and maturation processes, thereby altering the stability of biomass formation and sugar accumulation (Figure 3) (Mehdi et al., 2024). Suitable temperatures and sufficient light generally enhance photosynthetic efficiency and sucrose synthesis, whereas extreme temperatures can inhibit photosynthesis, increase respiratory consumption, and disrupt sugar storage, ultimately reducing both yield and quality.

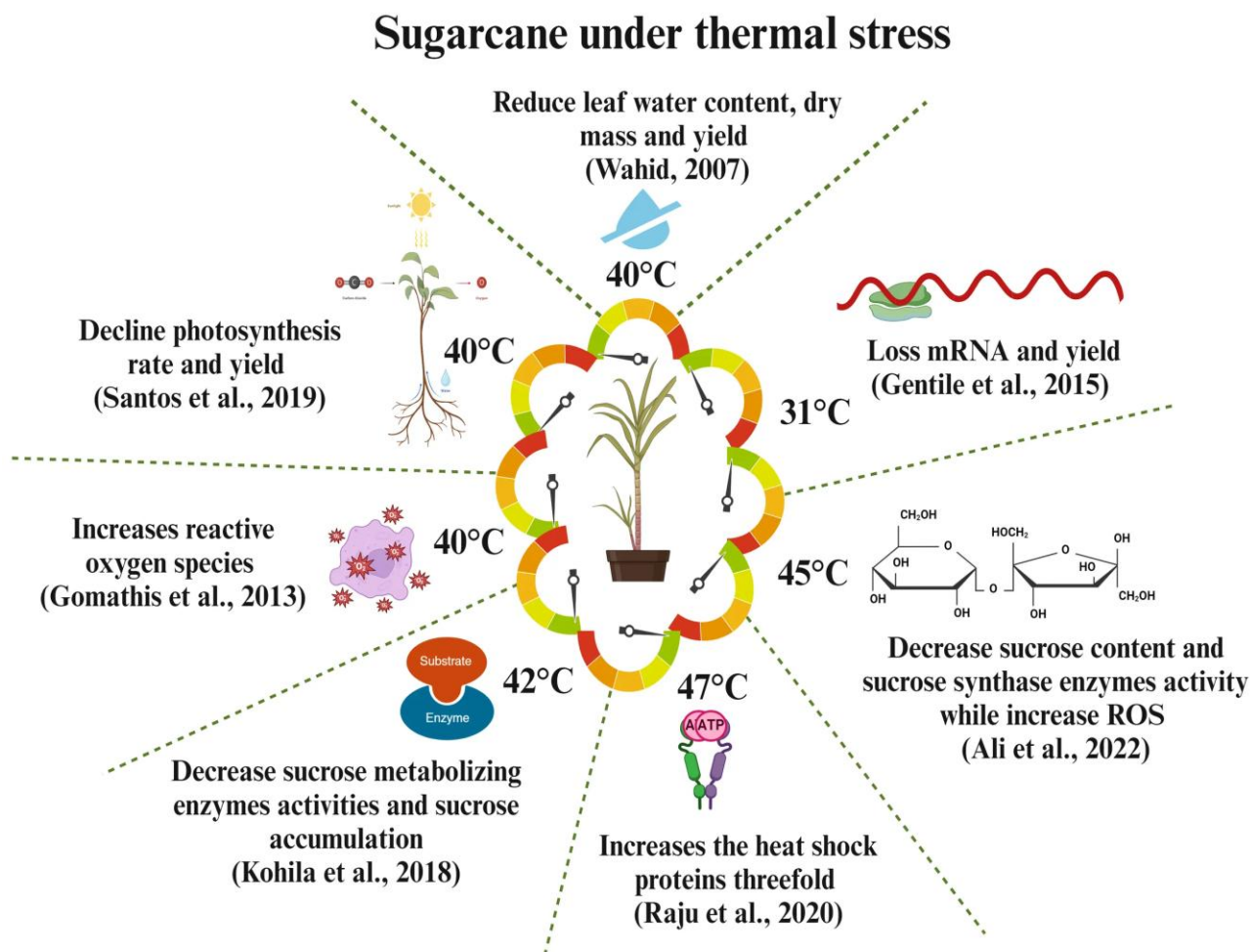


Figure 3 Response of sugarcane crop to different thermal stress conditions (Adopted from Mehdi et al., 2024)

Image caption: High temperatures can affect molecular, physiological, and biochemical processes in sugarcane, leading to reduced yield and sucrose production (Adopted from Mehdi et al., 2024)

Water conditions are particularly critical for the coordinated formation of high yield and high sugar content. Water deficit during the growth stage reduces stomatal conductance and radiation use efficiency, thereby limiting biomass production. Conversely, excessive rainfall or high soil moisture during the maturation stage may increase fresh weight but dilute sucrose concentration in the stalk, leading to the phenomenon of high yield but low sugar. It was mentioned in the article by Saavedra-Diaz et al. (2024), in humid production regions, high rainfall in the late growth stage promotes stalk growth but significantly reduces sucrose accumulation, resulting in lower sugar yield per unit area (Saavedra-Diaz et al., 2024). In addition, nutrient imbalance can disrupt coordination between yield and sugar content, as excessive nitrogen delays maturity and reduces sugar content, while nutrient deficiency suppresses overall growth.

Environmental variation also amplifies genotype×environment (G×E) interactions, leading to unstable performance of high-yield and high-sugar genotypes across regions, years, and ratoon cycles. Improvements in sugar content are generally smaller than those in biomass and are less responsive to environmental improvements, making it more difficult to maintain stable sugar levels across different ecological conditions (Amaresh et al., 2025). With increasing climate variability, factors such as drought, heat waves, extreme rainfall, and pest pressures are expected to further exacerbate instability. Therefore, multi-environment trials, environment-specific ideotype design, and refined environmental characterization are essential for improving the stability of yield and sugar content (Mehdi et al., 2024).

5.3 Breeding challenges arising from complex genetic backgrounds

The highly complex genetic background of cultivated sugarcane is another major constraint on the coordinated improvement of yield and sugar content. Modern sugarcane is a highly polyploid, aneuploid interspecific hybrid with a large genome, high heterozygosity, and complex homologous chromosome composition (Kumar et al., 2024; Wang et al., 2024; Amaresh et al., 2025). Most commercial cultivars derive the majority of their genome from *S. officinarum*, with a relatively smaller contribution from *S. spontaneum*. While this composition helps maintain high sugar traits, it also results in a relatively narrow genetic base, limited available variation, and reduced adaptive potential (Lu et al., 2024).

This complex genetic structure means that most economically important traits in sugarcane are not controlled by single major genes but by numerous small-effect QTLs, accompanied by allele dosage effects and complex interactions (Kumar et al., 2024). For traits such as high biomass and high sucrose accumulation, which may involve inherent trade-offs, combining sufficient favorable alleles within a single genotype is inherently slow and stochastic. In addition, the long breeding cycle of sugarcane, typically 10-15 years and largely reliant on clonal selection, further reduces the efficiency of improving complex traits (Amaresh et al., 2025).

Moreover, reproductive biology and quantitative genetic characteristics further complicate breeding. Asynchronous flowering, partial sterility, and limited effective crosses reduce recombination opportunities and hinder the rapid accumulation of favorable alleles. At the same time, early-stage phenotypic evaluation of yield and sugar content is highly influenced by environmental factors, reducing selection accuracy. These traits often exhibit low narrow-sense heritability and strong non-additive genetic effects, limiting the effectiveness of traditional marker-assisted selection (MAS). Although advances in reference genomes, high-density SNP platforms, and high-throughput sequencing have accelerated molecular breeding, challenges remain in accurate genotyping, allele dosage modeling, and integration of high-quality phenotypic data in polyploid contexts (Amaresh et al., 2025).

6 Breeding Strategies for the Coordinated Improvement of High Yield and High Sugar in Sugarcane

6.1 Conventional hybrid breeding and parental optimization strategies

Conventional hybrid breeding remains the core approach for sugarcane improvement, and most widely cultivated varieties are derived from this system. The basic process includes parental selection, artificial crossing, seedling population establishment, and multi-stage clonal selection and regional trials over 1014 years. In early generations, selection focuses mainly on yield-related traits such as tillering, stalk number, and vigor, while in later stages, greater emphasis is placed on sugar content, maturity, and resistance. Long-term practice shows that although this system can continuously increase yield, the gains mainly come from biomass improvement rather than significant increases in sugar content, reflecting its limitations in achieving coordinated high yield-high sugar improvement (Figure 4).

Under the goal of coordinated improvement, optimizing parental combinations becomes critical. High-biomass genotypes typically exhibit strong stalk growth, whereas high-sugar genotypes excel in sucrose accumulation and quality. Hybridizing these types can expand recombination variation in progeny. However, relying solely on empirical parental selection is inefficient; current approaches increasingly emphasize scientifically guided design based on genetic background, trait complementarity, and combining ability. Studies have shown that specific

combining ability (SCA) may contribute more to yield variation than general combining ability (GCA), indicating that identifying superior hybrid combinations is more important than selecting individual elite parents.

In practice, parental design should focus on NTrait-based complementarity, such as crossing high-biomass but moderate-sugar genotypes with high-sugar parents to develop ideotypes with strong source-large sink-optimal maturity. Successful cases demonstrate that systematic parental design combined with multi-trait selection can overcome traditional trade-offs and achieve simultaneous improvements in yield and sugar content (Wu et al., 2024; Liu et al., 2025). Additionally, a comprehensive multi-trait evaluation system should be established during progeny selection, integrating early-stage elimination with multi-environment validation in later stages, thereby shifting conventional breeding from experience-driven to target-oriented design.

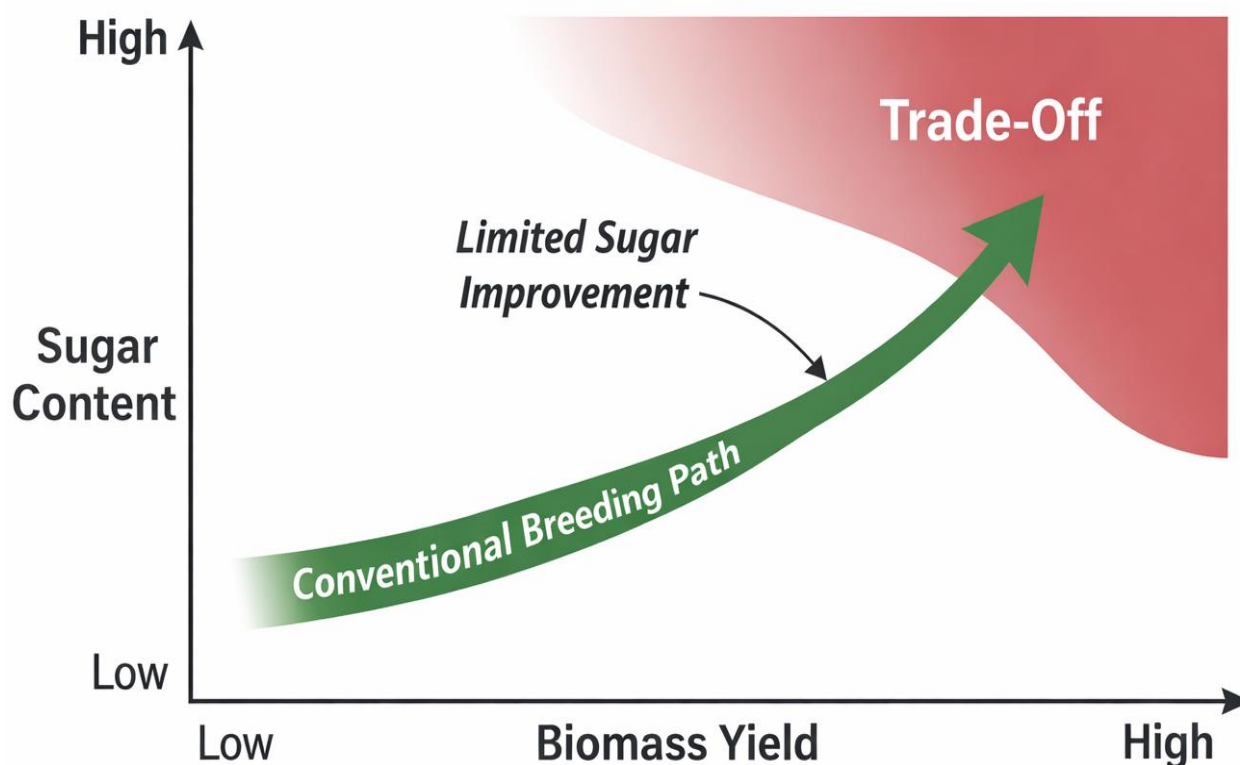


Figure 4 Conceptual relationship between biomass yield and sugar content in conventional breeding

Image caption: Limited improvement in sugar content despite yield gains

6.2 Applications of marker-assisted Selection, genomic selection, and gene editing

Advances in molecular breeding technologies have significantly improved sugarcane breeding efficiency. Marker-assisted selection (MAS) enables early-generation screening based on genotypic information and has been successfully applied to relatively simple traits such as disease resistance—for example, the use of Bru1-associated markers in rust resistance breeding (Lu et al., 2024). However, for complex quantitative traits such as yield and sugar content, which are controlled by multiple genes and environment interactions, the explanatory power of single markers is limited, and MAS is mainly used as a supplementary tool.

With the development of high-density SNP markers and GWAS, an increasing number of loci associated with yield and sugar traits have been identified, providing a foundation for pyramiding favorable alleles (Eltaher et al., 2025). In contrast, genomic selection (GS) is more suitable for complex traits, as it uses genome-wide markers to predict breeding values and capture the combined effects of numerous small-effect loci. Studies have shown that GS can improve selection accuracy, shorten breeding cycles, and provide stable predictions across environments, making it a key tool for future coordinated improvement in sugarcane.

Future trends involve integrating GS with high-throughput phenotyping, environmental data, and machine learning approaches to build a genotype-phenotype-environment predictive framework, thereby improving the understanding of G×E×M interactions and enhancing selection efficiency (Amaresh et al., 2025). Meanwhile, gene editing technologies such as CRISPR/Cas offer new opportunities for precisely regulating genes involved in sucrose metabolism, cell wall synthesis, and stress responses, with the potential to optimize carbon allocation and mitigate the physiological trade-offs between high yield and high sugar (Amaresh et al., 2025; Brant et al., 2025). Although the polyploid genome of sugarcane presents challenges, these technologies are rapidly advancing.

6.3 Integrated breeding approaches for coordinated high yield and high sugar

Because sugarcane yield and sugar content are complex traits, no single technology can achieve major breakthroughs; therefore, future improvement requires an integrated breeding system combining multiple approaches. Within the breeder's equation framework, it is necessary to simultaneously increase genetic variation, selection accuracy, and selection intensity while shortening breeding cycles. This relies on genome-wide data mining, multi-environment phenotyping, and modeling of G×E×M interactions (Amaresh et al., 2025). Thus, coordinated improvement should be conducted under a unified NTrait framework, rather than focusing on single traits independently.

In terms of germplasm resources, expanding the genetic base and strengthening pre-breeding are essential. Introducing wild species and diverse ecotypes can help identify genes associated with high biomass, high sugar content, and stress resistance (Eltaher et al., 2025). A continuous strategy of germplasm expansion-pre-breeding-targeted improvement can provide richer genetic resources for ideotype development. At the same time, breeding objectives should shift from single-trait selection to multi-trait ideotype design, incorporating NTrait components such as tillering, root system architecture, canopy structure, and carbon allocation efficiency to improve indirect selection efficiency.

At the implementation level, a hierarchical breeding pipeline should be established, integrating molecular prediction-phenotypic validation-multi-environment calibration. Early generations can be rapidly screened using molecular markers and GS; intermediate stages can evaluate key traits using high-throughput phenotyping and physiological indicators; and final stages can validate yield, sugar content, and stability through multi-environment trials (Amaresh et al., 2025). In addition, strategies such as recurrent genomic selection (RGS) and reciprocal recurrent genomic selection (RRGS) can be incorporated to accumulate favorable alleles while exploiting heterosis, thereby improving both parental development and hybrid performance and accelerating the coordinated breeding of high-yield and high-sugar sugarcane.

7 Effects of Agronomic Management on the Coordinated Improvement of Sugarcane Yield and Sugar Content

7.1 Regulation of stalk growth and sugar accumulation by water and fertilizer management

Water and nutrient management directly influence the balance between biomass formation and sucrose accumulation in sugarcane by regulating root uptake, canopy structure, and photosynthesis. Adequate water supply during early growth promotes germination, tillering, and rapid stalk elongation, thereby increasing millable cane number and single stalk weight and laying the foundation for high yield. However, excessive water supply in later stages may delay maturation, stimulate vegetative growth, and dilute sucrose concentration, reducing sugar accumulation efficiency. Studies on integrated water-fertilizer management indicate that water and nitrogen jointly promote population establishment and individual growth, but high water and high fertilizer inputs do not necessarily result in high sugar content, reflecting the trade-off between biomass and sugar concentration. Therefore, optimizing the timing and amount of water supply is critical for balancing yield and quality.

In terms of nutrient management, nitrogen, phosphorus, potassium, and micronutrients play distinct roles at different growth stages. Nitrogen promotes leaf area expansion and vegetative growth, but excessive application can delay maturity and reduce sugar content. Phosphorus supports root development and early population establishment, while potassium plays a key role in sucrose transport and synthesis. Recent studies have shown that combined application of potassium with micronutrients such as boron and zinc can improve both yield and sugar

quality (Manzoor et al., 2023). In addition, fertilizer source, placement, and timing are important factors, and synchronizing nutrient supply with crop demand can significantly enhance nutrient use efficiency. Overall, stage-specific and precise water and nutrient management-promoting biomass accumulation in early stages and moderately restricting water and nitrogen in later stages-can redirect carbon flow toward sucrose storage and achieve coordinated improvement of yield and sugar content.

7.2 Effects of planting density and population structure on yield and quality

Planting density and spatial configuration influence sugarcane yield and quality by regulating canopy structure and resource use efficiency. Moderate increases in planting density can enhance millable cane number, leaf area index, and light interception, thereby improving canopy photosynthesis and dry matter accumulation (Joseph et al., 2024). However, excessive density intensifies competition among plants, reduces light penetration and ventilation, inhibits individual stalk development, and lowers sucrose accumulation efficiency. Therefore, optimal density should achieve a dynamic balance between increasing population size and maintaining individual plant quality, rather than simply maximizing or minimizing density.

From a long-term production perspective, moderate density is generally more favorable for maintaining population stability across both plant cane and ratoon crops. Although low density may promote individual plant growth, it is less conducive to sustained high yield. Spatial arrangements, such as row spacing and double-row planting, also affect the coordination between yield and quality, with different configurations favoring either biomass production or sugar accumulation (Joseph et al., 2024). In addition, varietal characteristics and belowground conditions can modify density effects, as plants may compensate through adjustments in stalk number and individual stalk weight. An ideal population should have a moderate leaf area index, good light penetration and ventilation, and uniform stalk distribution to balance photosynthetic efficiency and sugar accumulation, thereby achieving both high yield and high sugar content.

7.3 Importance of growth stage regulation and timely harvest for high yield and high sugar formation

The formation of sugarcane yield and sugar content is highly dependent on developmental stages. The tillering and elongation phases determine population size and biomass foundation, while the maturation phase governs sucrose accumulation and quality improvement. By regulating planting time, water and nutrient supply, and irrigation withdrawal, it is possible to coordinate the timing of peak biomass formation and peak sugar accumulation. Moderate restriction of water and nitrogen during maturation can suppress excessive vegetative growth and promote carbon allocation to sucrose storage. In contrast, continuous high water and nutrient supply may increase biomass but often reduces sugar concentration. Therefore, proper regulation of the maturation process is essential for achieving synchronized improvements in yield and quality.

Planting and harvesting windows are also closely related to varietal maturity types and regional climatic conditions. Appropriate planting time is critical for achieving high yield, whereas delayed planting often results in yield reduction. Different maturity types require different optimal harvest times, and moderately delayed harvesting generally improves quality traits such as Brix, purity, and CCS. Moreover, harvest season and scheduling significantly affect sugar yield, and optimized harvest planning can greatly enhance overall production efficiency and economic returns (Gebrehiwot et al., 2025). Therefore, timely harvesting should be determined based on variety characteristics, environmental conditions, and processing capacity, ensuring coordination between production and processing to maximize the potential for high yield and high sugar content.

8 Future Research Directions and Development Trends

8.1 Multi-omics integration for deciphering mechanisms of high yield and high sugar traits

With advances in genomics, transcriptomics, and metabolomics, the coordinated improvement of yield and sugar content in sugarcane increasingly relies on multi-omics integration to systematically elucidate the continuum from genetic variation-physiological processes-agronomic performance. Since yield and sugar content are complex quantitative traits involving multiple layers such as carbon fixation, sucrose metabolism, source-sink partitioning, and hormonal regulation, single-omics approaches are insufficient to fully explain their formation. Therefore, integrating genomic, transcriptomic, proteomic, metabolomic, and phenomic data has become a key strategy for

uncovering trait mechanisms. Studies have shown that multi-omics analyses can identify differentially expressed genes, metabolites, and key pathways associated with high yield and high sugar, mainly involving carbon metabolism, secondary metabolism, and hormone signaling networks (Li et al., 2024). Further integration with co-expression and metabolic network analyses enables the identification of key modules and hub genes related to sugar content, fiber, and yield, providing targets for NTrait marker development and molecular breeding.

In the regulation of sucrose accumulation, multi-omics approaches have moved beyond transcript-level analysis toward integrated protein-metabolite-phenotype systems. Evidence suggests that enzymes, transporters, and regulatory factors involved in sucrose metabolism act coordinately in time and space, with an expanding number of candidate proteins highlighting the importance of photosynthesis and primary carbon metabolism in high sugar formation (Fan et al., 2025). This indicates that future research should focus more on the integrated regulation of functional proteins and metabolic pathways. In addition, multi-omics studies reveal dynamic changes in carbon allocation during development, shifting from early growth and structural formation to later sugar storage and stabilization, providing insights into the regulation of maturation and source-sink relationships. In the future, integrating pan-omics with machine learning frameworks is expected to enable precise identification of key regulatory modules and trait prediction, advancing sugarcane breeding from association analysis to predictive design.

8.2 Integration of high-throughput phenotyping, smart breeding, and digital agriculture

In sugarcane breeding, low efficiency and limited accuracy of phenotyping have long been major bottlenecks for studying complex traits. Traditional field measurements are labor-intensive and subject to human error, making them unsuitable for large-scale population evaluation. Therefore, high-throughput phenotyping (HTP) technologies have emerged as a key breakthrough for improving breeding efficiency and precision. In recent years, UAV-based systems, multispectral/hyperspectral imaging, LiDAR, and field sensors have enabled rapid acquisition of key traits such as canopy structure, biomass, water status, and photosynthesis-related parameters. These traits are not only closely related to yield but can also serve as intermediate indicators in genomic selection models, transforming phenotypic data from static end-point measurements into dynamic traits across the entire growth cycle.

Future research should expand the scope of HTP to include NTrait indicators such as tillering dynamics, stalk number changes, early growth vigor, canopy temperature, and sugar accumulation processes, and evaluate their genetic relationships with final yield and sugar content (Amaresh et al., 2025). This will improve prediction accuracy for complex traits and enable early selection of superior genotypes. At the same time, integration of phenotypic, genomic, and environmental data is driving the development of smart breeding. Under the “Breeding 4.0” framework, machine learning-assisted genomic selection models can more accurately predict breeding values across environments, optimizing parental selection and breeding strategies. In addition, digital agriculture technologies integrating remote sensing, environmental monitoring, and management data can enable precise regulation of water and fertilizer use, pest control, and harvest timing. More advanced developments include the construction of digital twin breeding systems, which simulate breeding and management strategies in virtual environments and optimize decisions in real time (Wang et al., 2024a). Combined with data sharing and blockchain technologies, such systems will significantly enhance collaborative breeding and supply chain management efficiency.

8.3 Breeding directions for multi-objective coordinated improvement

With the diversification of the sugarcane industry toward sugar production, bioenergy, and biomaterials, future breeding objectives are shifting from single traits to multi-trait optimization. Ideal varieties should simultaneously possess high yield, high sugar content, strong stress resistance, wide adaptability, and suitability for mechanized harvesting (Lu et al., 2024; Wang et al., 2025). Thus, breeding targets are evolving into a comprehensive system encompassing yield, sugar content, stress resistance, mechanization, and industrial adaptability. In ideotype design, a series of NTrait intermediate traits play key roles, including optimal canopy structure, appropriate leaf angle, deep root systems, stay-green ability, and high single stalk weight. These traits contribute to efficient light use,

balanced source-sink relationships, and enhanced stress tolerance, and should be incorporated into multi-trait selection frameworks.

Multi-omics studies further reveal that certain key regulatory factors can simultaneously influence sucrose accumulation, cell wall composition, and stress responses, providing opportunities for coordinated improvement of high sugar-high biomass-high resilience. For mechanization requirements, ideal varieties should also exhibit uniform plant architecture, strong lodging resistance, and synchronous maturity, traits that have begun to be elucidated at molecular and metabolic levels (Li et al., 2024). In terms of stress resistance, greater use of wild germplasm resources is needed to broaden the genetic base, combined with molecular markers, genomic selection, and gene editing technologies to achieve coordinated improvement of stress tolerance, yield, and sugar content (Lu et al., 2024). At the same time, future varieties should also meet the needs of processing and biorefinery applications, with cell wall structures optimized for both sugar extraction and bioenergy conversion (Wang et al., 2025), thereby promoting the transition of sugarcane from a single-purpose crop to a multifunctional industrial resource.

9 Conclusion

Sugarcane yield and sugar content are the two core traits determining the economic value of raw cane and sugar yield per unit area. Their formation is not governed by a single factor but results from the coordinated interaction of agronomic traits, physiological processes, and molecular regulatory networks. At the agronomic level, traits such as tiller number, millable cane number, single stalk weight, plant height, and stalk diameter jointly determine cane yield, while sucrose content, maturity, and juice quality directly influence sugar levels. At the physiological level, photosynthetic efficiency, dry matter accumulation, source-sink relationships, and assimilate transport and partitioning are key processes linking biomass production and sugar accumulation. From a genetic perspective, these traits are typical complex quantitative traits controlled by multiple genes with significant non-additive effects. Their essence lies in the dynamic balance of carbon allocation between biomass and sugar, rather than a fixed antagonistic relationship.

The realization of high yield and high sugar content in sugarcane depends on the synergistic interaction of genotype \times environment \times management (G \times E \times M). Relying solely on genetic improvement or agronomic practices is insufficient to achieve optimal performance. In breeding, it is necessary to optimize parental combinations, broaden the genetic base, and apply multi-trait selection strategies, combined with molecular markers, genomic selection, and gene editing technologies to identify genotypes with both high biomass and strong sugar accumulation potential. In agronomic management, optimizing variety selection, nutrient supply, and input levels-particularly the balance of nitrogen, potassium, magnesium, and calcium-is essential to promote tillering, population structure formation, and sucrose accumulation. Studies indicate that under suitable environmental conditions and appropriate management, high biomass and high sugar content can coexist, highlighting the importance of an integrated genotype-environment-management framework for stabilizing sugar yield.

For the high-quality development of the sugarcane industry, future efforts should focus on advancing coordinated improvement of yield and sugar content at theoretical, resource, and technological levels. Theoretically, an NTrait-based framework should integrate outcome traits (e.g., tillering, stalk number, single stalk weight, sugar content, and fiber) with mechanistic traits such as carbon allocation, nutrient use efficiency, and maturation regulation to enable precise ideotype design. In terms of resources, pan-genomics, whole-genome resequencing, and GWAS should be used to identify key loci and favorable haplotypes. Technologically, integrating genomic selection, rapid breeding approaches, and CRISPR-based gene editing will improve the efficiency of complex trait improvement. Meanwhile, incorporating high-throughput phenotyping, digital agriculture, and artificial intelligence into breeding and management, along with strengthening germplasm innovation and international collaboration, will facilitate the development of a modern sugarcane breeding system that integrates theoretical models, molecular tools, and region-specific applications, thereby supporting the sustained improvement of high yield and high sugar content and the sustainable development of the sugarcane industry.

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Conflict of Interest Disclosure

The authors affirm 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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