Horticulture Research, 2026, **13**: uhag108
https://doi.org/10.1093/hr/uhag108
Article

Horticulture Research OXFORD

Evolution and diversification of PAL-mediated salicylic acid biosynthesis in Rosaceae

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Abstract

Salicylic acid (SA) is a central phytohormone in plant immunity and stress responses, yet the evolutionary dynamics of its phenylalanine ammonia-lyase (PAL)-mediated biosynthetic route remain poorly understood despite recent biochemical advances. As the original source of SA, *Spiraea* (Rosaceae) holds historical and evolutionary significance for studying SA biosynthesis. Here, we generated a chromosome-level genome assembly of *Spiraea chinensis* and integrated comparative genomics, transcriptomic, and targeted metabolite profiling to investigate the evolutionary diversification of SA biosynthesis across Rosaceae. Phylogenomics places *S. chinensis* in the subfamily Amygdaloideae, diverging from other genera ~57.8 Mya. Extensive chromosome fission–fusion events and lineage-specific whole-genome duplication (WGD) have driven karyotype diversification across Rosaceae. Comparative analyses revealed the PAL-mediated route as the dominant SA biosynthetic pathway across Rosaceae, with WGD-driven expansion in Amygdaloideae and combined WGD- and small-scale duplication (SSD)-derived origins in Rosoideae. WGD-derived PAL-route genes largely retained synteny and stable high expression, whereas lineage-specific SSD-derived paralogs exhibited reduced synteny and variable expression, consistent with post-duplication regulatory divergence and subfunctionalization. Transcriptome analyses revealed pronounced tissue-specific expression of PAL-route genes across Rosaceae, and UPLC–MS/MS profiling further demonstrated differential SA accumulation in *S. chinensis*, with the highest levels in branches (606–1038 ng/g FW), followed by leaves (183–432 ng/g FW) and flowers (42–56 ng/g FW), supporting active SA biosynthesis in both vegetative and reproductive tissues. Collectively, our results establish the PAL-mediated pathway as the primary and evolutionarily conserved route of SA biosynthesis in Rosaceae and demonstrate how genome dynamics and regulatory diversification jointly drive the evolutionary innovation within this pathway.

Introduction

Salicylic acid (SA) is a central signaling molecule in plants, playing indispensable roles in defense against biotic stresses—particularly through the activation of systemic acquired resistance—as well as in modulating responses to diverse abiotic stressors [1–5]. In response to pathogen infection, SA orchestrates immune activation by promoting defense gene expression and hypersensitive responses, whereas under abiotic stresses such as heat, drought, and salinity, SA contributes to stress acclimation by modulating redox balance and cellular homeostasis [1, 3, 6, 7]. Beyond its canonical defensive role, accumulating evidence indicates that SA functions as a dose- and context-dependent integrator of plant growth, development, and environmental adaptation, coordinating extensive transcriptional reprogramming, redox homeostasis, and hormonal crosstalk [6, 8]. Through interactions with other phytohormones, such as jasmonic acid, ethylene, auxin, and abscisic acid, SA reprioritizes resource allocation between growth and defense, providing a mechanistic basis for growth–defense tradeoffs and shaping plant fitness under fluctuating environments [8–11]. Recent advances using genetically encoded SA sensors further revealed

Received: 29 September 2025. **Accepted:** 18 March 2026. **Published:** 2 April 2026. **Corrected and Typeset:** 1 July 2026
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temperature was maintained at 40°C, with a flow rate of 0.2 ml/min and an injection volume of 1 μl. The gradient elution program was as follows: 0–8 min, 25% to 30% B; 8.1–11 min, hold at 90% B; and 11.1–15 min, hold at 25% B. Mass spectrometry was performed in negative ion mode with the following parameters: ion source temperature, 250°C; sheath gas flow, 35 arb; auxiliary gas flow, 10 arb; ion transfer tube temperature, 275°C; and spray voltage, 2.5 kV. Quantification was conducted in multiple reaction monitoring mode using the transition m/z 137.1 → 65.3, with a collision energy of 27.0 eV and an RF lens voltage of 75 V.

Acknowledgments

This work was supported by the Guangdong Flagship Project of Basic and Applied Basic Research (2023B0303050001), the National Natural Science Foundation of China (32500199), and the Guangzhou Youth Doctor 'Sailing' Project (2025A04J0277). We thank Shao-Hua Zeng, Ting Wang, Peng-Chong Zhang, Ding-Ding Shi, and Shu Wang for their valuable help and suggestions on this manuscript.

Author contributions

X.-F.W. and H.-F.Y. designed and supervised the research; Q.-L.Z, T.-J.L., Y.-B.W., and X.-F.W. analyzed data and wrote the manuscript; other authors contributed to data analysis and manuscript revision.

Data availability

The raw sequencing data, including Illumina short reads, HiFi reads, Hi-C reads, and RNA short reads, have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC), China National Center for Bioinformation (CNCB) under BioProject PRJCA046475 with the accession number CRA030184 [122, 123]. The final genome assembly and annotation files are available in the Genome Warehouse (GWH) at NGDC under the same BioProject (accession SAMC5926829) [124], and have also been deposited in the Figshare (https://doi.org/10.6084/m9.figshare.30143092). In addition, the genome assembly has been deposited in the National Center for Biotechnology Information (NCBI) under the BioProject PRJNA1330000 with accession number JBRBAJ000000000.

Conflicts of interest statement

The authors declare no conflict of interests.

Supplementary material

Supplementary material is available at *Horticulture Research* online.

References

1. Corina Vlot A, Dempsey DA, Klessig DF. Salicylic acid, a multifaceted hormone to combat disease. *Annu Rev Phytopathol*. 2009;47:177–206
2. Arif Y, Sami F, Siddiqui H. *et al.* Salicylic acid in relation to other phytohormones in plant: a study towards physiology and signal transduction under challenging environment. *Environ Exp Bot*. 2020;175:104040
3. Ahmad F, Singh A, Kamal A. Salicylic acid-mediated defense mechanisms to abiotic stress tolerance. In: Khan MIR, Reddy PS, Ferrante A, Khan NA, eds. *Plant Signaling Molecules: Role and Regulation Under Stressful Environments*. Cambridge, UK: Woodhead Publishing, 2019,355–69
4. Gaffney T, Friedrich L, Vernooij B. *et al.* Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*. 1993;261:754–6
5. Delaney TP, Uknes S, Vernooij B. *et al.* A central role of salicylic acid in plant disease resistance. *Science*. 1994;266:1247–50
6. Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. *J Exp Bot*. 2011;62:3321–38
7. Miura K, Tada Y. Regulation of water, salinity, and cold stress responses by salicylic acid. *Front Plant Sci*. 2014;5:4
8. Singh D, Dhiman VK, Pandey H. *et al.* Crosstalk between salicylic acid and auxins, cytokinins and gibberellins under biotic stress. In: Aftab T, ed. *Auxins, Cytokinins and Gibberellins Signaling in Plants. Signaling and Communication in Plants*. Springer: Cham, 2022,249–62
9. Hou S, Tsuda K. Salicylic acid and jasmonic acid crosstalk in plant immunity. *Essays Biochem*. 2022;66:647–56
10. Zhang P, Jackson E, Li X. *et al.* Salicylic acid and jasmonic acid in plant immunity. *Hortic Res*. 2025;12:uhaf082
11. Huot B, Yao J, Montgomery BL. *et al.* Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Mol Plant*. 2014;7:1267–87
12. Tang B, Lu J, Leontovyčová H. *et al.* SALICYLIC ACID SENSOR1 reveals the propagation of an SA hormone surge during plant pathogen advance. *Science*. 2025;390:188–94
13. Wang Z, Yuan Y, Dong R. *et al.* Salicylic acid regulates biosynthesis of floral fragrance (E)-β-farnesene via NPR3-WRKY1 module in chrysanthemum. *Mol Hortic*. 2025;5:1–22
14. Rekhter D, Lüdke D, Ding Y. *et al.* Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. *Science*. 2019;365:498–502
15. Wildermuth MC, Dewdney J, Wu G. *et al.* Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*. 2001;414:562–5
16. Widhalm JR, Dudareva N. A familiar ring to it: biosynthesis of plant benzoic acids. *Mol Plant*. 2015;8:83–97
17. Peng Y, Yang J, Li X. *et al.* Salicylic acid: biosynthesis and signaling. *Annu Rev Plant Biol*. 2021;72:761–91
18. Liu Y, Xu L, Wu M. *et al.* Three-step biosynthesis of salicylic acid from benzoyl-CoA in plants. *Nature*. 2025;645:201–7
19. Tian L, Feussner I. Hidden route to salicylic acid has finally been uncovered. *Plant Commun*. 2025;6:101496
20. Dempsey DA, Vlot AC, Wildermuth MC. *et al.* Salicylic acid biosynthesis and metabolism. *Arabidopsis Book*. 2011;9:e0156
21. Qualley AV, Widhalm JR, Adebesin F. *et al.* Completion of the core β-oxidative pathway of benzoic acid biosynthesis in plants. *Proc Natl Acad Sci USA*. 2012;109:16383–8

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22. Van Moerkercke A, Schauvinhold I, Pichersky E. *et al.* A plant thiolase involved in benzoic acid biosynthesis and volatile benzenoid production. *Plant J*. 2009;60:292–302
23. Wang Y, Miao H, Qiu J. *et al.* Species- and organ-specific contribution of peroxisomal cinnamate: CoA ligases to benzoic and salicylic acid biosynthesis. *Plant Cell*. 2025;37:koae329
24. Colquhoun TA, Marciniak DM, Wedde AE. *et al.* A peroxisomally localized acyl-activating enzyme is required for volatile benzenoid formation in a *petunia* × *hybrida* cv. 'Mitchell Diploid' flower. *J Exp Bot*. 2012;63:4821–33
25. Klempien A, Kaminaga Y, Qualley A. *et al.* Contribution of CoA ligases to benzenoid biosynthesis in petunia flowers. *Plant Cell*. 2012;24:2015–30
26. MacDonald MJ, D'Cunha GB. A modern view of phenylalanine ammonia lyase. *Biochem Cell Biol*. 2007;85:273–82
27. Mahdi JG. Medicinal potential of willow: a chemical perspective of aspirin discovery. *J Saudi Chem Soc*. 2010;14:317–22
28. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res*. 2003;110:255–8
29. Zhang Z, Fan L, Yang J. *et al.* Alkaloid polymorphism and ITS sequence variation in the *Spiraea japonica* complex (Rosaceae) in China: traces of the biological effects of the Himalaya-Tibet Plateau uplift. *Am J Bot*. 2006;93:762–9
30. Potter D, Eriksson T, Evans RC. *et al.* Phylogeny and classification of Rosaceae. *Plant Syst Evol*. 2007;266:5–43
31. Yu T, Lu L. Spiraea. In: Yu TT, ed. *Flora Reipublicae Popularis Sinicae, Tomus 36*. Science Press: Beijing, 1974,1–66
32. Huh M-K. Genetic diversity and relationship of genus *Spiraea* by random amplified polymorphic DNA markers. *J Life Sci*. 2010;20:983–90
33. Poliakova TA. Adaptive strategies and genetic stability of species from the *Spiraea* genus (Rosaceae) in natural population systems. *Biol Bull Rev*. 2022;12:S96–107
34. Yu SX, Gadagkar SR, Potter D. *et al.* Phylogeny of *Spiraea* (Rosaceae) based on plastid and nuclear molecular data: implications for morphological character evolution and systematics. *Perspect Plant Ecol Evol Syst*. 2018;34:109–19
35. Laczkó L, Jordán S, Póliska S. *et al.* The draft genome of *Spiraea crenata* L. (Rosaceae)—the first complete genome in tribe Spiraeeae. *Sci Data*. 2024;11:219
36. Xiang Y, Huang CH, Hu Y. *et al.* Evolution of Rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication. *Mol Biol Evol*. 2017;34:262–81
37. Zong D, Liu H, Gan P. *et al.* Chromosomal-scale genomes of two Rosa species provide insights into genome evolution and ascorbate accumulation. *Plant J*. 2024;117:1264–80
38. Wang Y, Guan J, Zhang Q. Chromosome-scale genome, together with transcriptome and metabolome, provides insights into the evolution and anthocyanin biosynthesis of *Rubus rosaefolius* Sm. (Rosaceae). *Hortic Res*. 2024;11:uhae064
39. Li L, Li M, Wu J. *et al.* Genome-wide identification and comparative evolutionary analysis of sorbitol metabolism pathway genes in four Rosaceae species and three model plants. *BMC Plant Biol*. 2022;22:341
40. Zhang F, Wang Y, Lin Y. *et al.* Haplotype-resolved genome assembly provides insights into evolutionary history of the *Actinidia arguta* tetraploid. *Mol Hortic*. 2024;4:4
41. Daccord N, Celton JM, Linsmith G. *et al.* High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. *Nat Genet*. 2017;49:1099–106
42. Zhang SY, Yan HF, Wei L. *et al.* Plastid genome and its phylogenetic implications of Asiatic *Spiraea* (Rosaceae). *BMC Plant Biol*. 2024;24:23
43. Zhang SD, Jin JJ, Chen SY. *et al.* Diversification of Rosaceae since the Late Cretaceous based on plastid phylogenomics. *New Phytol*. 2017;214:1355–67
44. Zhang L, Tao H, Zhang J. *et al.* 5-aminolevulinic acid activates the MdWRKY71-MdMADS1 module to enhance anthocyanin biosynthesis in apple. *Mol Hortic*. 2025;5:1–18
45. Duan X, Wang K, Tang R. *et al.* Recent advances in biosynthesis and regulation of strawberry anthocyanins. *Hortic Res*. 2025;12:uhaf135
46. Lu LD, Alexander C. Spiraea. In: Wu ZY, Raven PH, Hong DY, eds. *Flora of China*. Vol. 9. Missouri Botanical Garden Press: Beijing/St. Louis, 2003,47–73
47. Dickson EE, Arumuganathan K, Kresovich S. *et al.* Nuclear DNA content variation within the Rosaceae. *Am J Bot*. 1992;79:1081–6
48. Siljak-Yakovlev S, Pustahija F, Šolić EM. *et al.* Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. *Adv Sci Lett*. 2010;3:190–213
49. Jung S, Lee T, Cheng CH. *et al.* 15 years of GDR: new data and functionality in the genome database for Rosaceae. *Nucleic Acids Res*. 2019;47:D1137–45
50. Byung-Yun S, Kim T-J, Kim CH. A biosystematic study on polyploid populations of the genus *Spiraea* (Rosaceae) in Korea. *J Plant Biol*. 1997;40:291–7
51. Ou S, Chen J, Jiang N. Assessing genome assembly quality using the LTR Assembly Index (LAI). *Nucleic Acids Res*. 2018;46:e126
52. Lv S, Wang Z, Yang X. *et al.* Transcriptional profiling of rice treated with MoHrip1 reveal the function of protein elicitor in enhancement of disease resistance and plant growth. *Front Plant Sci*. 2016;7:1818
53. Jia X, Wang L, Zhao H. *et al.* The origin and evolution of salicylic acid signaling and biosynthesis in plants. *Mol Plant*. 2023;16:245–59
54. Zou Z, Fan Q, Zhou X. *et al.* Biochemical pathways of salicylic acid derived from l-phenylalanine in plants with different basal SA levels. *J Agric Food Chem*. 2024;72:2898–910
55. Li G, Wang L, Yang J. *et al.* A high-quality genome assembly highlights rye genomic characteristics and agronomically important genes. *Nat Genet*. 2021;53:574–84
56. Han X, Zhang Y, Zhang Q. *et al.* Two haplotype-resolved, gap-free genome assemblies for *Actinidia latifolia* and *Actinidia chinensis* shed light on the regulatory mechanisms of vitamin C and sucrose metabolism in kiwifruit. *Mol Plant*. 2023;16:452–70
57. Huang HR, Liu X, Arshad R. *et al.* Telomere-to-telomere haplotype-resolved reference genome reveals subgenome divergence and disease resistance in triploid Cavendish banana. *Hortic Res*. 2023;10:uhad153
58. Shi X, Cao S, Wang X. *et al.* The complete reference genome for grapevine (*Vitis vinifera* L.) genetics and breeding. *Hortic Res*. 2023;10:uhad061

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59. Xiao TW, Liu X, Fu N. *et al.* Chromosome-level genome assemblies of *Musa ornata* and *Musa velutina* provide insights into pericarp dehiscence and anthocyanin biosynthesis in banana. *Hortic Res*. 2024;11:uhae079
60. Almeida-Silva F, Van De Peer Y. Whole-genome duplications and the long-term evolution of gene regulatory networks in angiosperms. *Mol Biol Evol*. 2023;40:msad141
61. Qiao X, Li Q, Yin H. *et al.* Gene duplication and evolution in recurring polyploidization-diploidization cycles in plants. *Genome Biol*. 2019;20:38
62. Luo Y, Liu M, Cao J. *et al.* The role of salicylic acid in plant flower development. *For Res*. 2022;2:0
63. Martínez C, Pons E, Prats G. *et al.* Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant J*. 2004;37:209–17
64. Liu M, Li W, Zhao G. *et al.* New insights of salicylic acid into stamen abortion of female flowers in tung tree (*Vernicia fordii*). *Front Genet*. 2019;10:316
65. Feng B, Zhang C, Chen T. *et al.* Salicylic acid reverses pollen abortion of rice caused by heat stress. *BMC Plant Biol*. 2018;18:245
66. Shah K, An N, Kamanova S. *et al.* Regulation of flowering time by improving leaf health markers and expansion by salicylic acid treatment: a new approach to induce flowering in *Malus domestica*. *Front Plant Sci*. 2021;12:655974
67. Shah K, Wang M, Li X. *et al.* Transcriptome analysis reveals dual action of salicylic acid application in the induction of flowering in *Malus domestica*. *Plant Sci*. 2022;324:111433
68. Zhang X, Liu CJ. Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. *Mol Plant*. 2015;8:17–27
69. Doležel J, Bartoš J. Plant DNA flow cytometry and estimation of nuclear genome size. *Ann Bot*. 2005;95:99–110
70. Marçais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics*. 2011;27:764–70
71. Weib CL, Pais M, Cano LM. *et al.* nQuire: a statistical framework for ploidy estimation using next generation sequencing. *BMC Bioinform*. 2018;19:122
72. Ranallo-Benavidez TR, Jaron KS, Schatz MC. GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat Commun*. 2020;11:1432
73. Zhang H, Zhao X, Ding X. *et al.* Preparation of megabase-size DNA from plant nuclei. *Plant J*. 1995;7:175–84
74. Cheng H, Concepcion GT, Feng X. *et al.* Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. *Nat Methods*. 2021;18:170–5
75. Wick RR, Schultz MB, Zobel J. *et al.* Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics*. 2015;31:3350–2
76. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv: 1303.3997v2[q-bioGN]. 2013
77. Dudchenko O, Batra SS, Omer AD. *et al.* De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science*. 2017;356:92–5
78. Nadalin F, Vezzi F, Policriti A. GapFiller: a de novo assembly approach to fill the gap within paired reads. *BMC Bioinform*. 2012;13:S8
79. Li H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*. 2018;34:3094–100
80. Manni M, Berkeley MR, Seppey M. *et al.* BUSCO: assessing genomic data quality and beyond. *Curr Protoc*. 2021;1:e323
81. Ou S, Su W, Liao Y. *et al.* Benchmarking transposable element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome Biol*. 2019;20:275
82. Rhie A, Walenz BP, Koren S. *et al.* Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies. *Genome Biol*. 2020;21:245
83. Flynn JM, Hubley R, Goubert C. *et al.* RepeatModeler2 for automated genomic discovery of transposable element families. *Proc Natl Acad Sci USA*. 2020;117:9451–7
84. Bao W, Kojima KK, Kohany O. Repbase Update, a database of repetitive elements in eukaryotic genomes. *Mob DNA*. 2015;6:11
85. Tarailo-Graovac M, Chen N. Using RepeatMasker to identify repetitive elements in genomic sequences. *Curr Protoc Bioinforma*. 2009;25:4.10.1–4.10.14
86. Ellinghaus D, Kurtz S, Willhoeft U. LTRharvest, an efficient and flexible software for de novo detection of LTR retrotransposons. *BMC Bioinform*. 2008;9:18
87. Ou S, Jiang N. LTR_retriever: a highly accurate and sensitive program for identification of long terminal repeat retrotransposons. *Plant Physiol*. 2018;176:1410–22
88. Su W, Gu X, Peterson T. TIR-learner, a new ensemble method for TIR transposable element annotation, provides evidence for abundant new transposable elements in the maize genome. *Mol Plant*. 2019;12:447–60
89. Xiong W, He L, Lai J. *et al.* HelitronScanner uncovers a large overlooked cache of Helitron transposons in many plant genomes. *Proc Natl Acad Sci USA*. 2014;111:10263–8
90. Kim D, Paggi JM, Park C. *et al.* Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol*. 2019;37:907–15
91. Gabriel L, Hoff KJ, Brůna T. *et al.* TSEBRA: transcript selector for BRAKER. *BMC Bioinform*. 2021;22:566
92. Brůna T, Lomsadze A, Borodovsky M. GeneMark-EP+: eukaryotic gene prediction with self-training in the space of genes and proteins. *NAR Genomics Bioinforma*. 2020;2:lqaa026
93. Stanke M, Keller O, Gunduz I. *et al.* AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res*. 2006;34:W435–9
94. Nawrocki EP, Eddy SR. Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics*. 2013;29:2933–5
95. Kalvari I, Nawrocki EP, Ontiveros-Palacios N. *et al.* Rfam 14: expanded coverage of metagenomic, viral and microRNA families. *Nucleic Acids Res*. 2021;49:D192–200
96. Cantalapiedra CP, Hernandez-Plaza A, Letunic I. *et al.* eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol*. 2021;38:5825–9
97. Huerta-Cepas J, Szklarczyk D, Heller D. *et al.* EggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res*. 2019;47:D309–14
98. Törönen P, Medlar A, Holm L. PANNZER2: a rapid functional annotation web server. *Nucleic Acids Res*. 2018;46:W84–8

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99. Bolger M, Schwacke R, Usadel B. MapMan visualization of RNA-seq data using Mercator4 functional annotations. *Methods Mol Biol*. 2021;2354:195–212
100. Jones P, Binns D, Chang HY. *et al.* InterProScan 5: genome-scale protein function classification. *Bioinformatics*. 2014;30:1236–40
101. Ashburner M, Ball CA, Blake JA. *et al.* Gene ontology: tool for the unification of biology. *Nat Genet*. 2000;25:25–9
102. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28:27–30
103. Zheng Y, Jiao C, Sun H. *et al.* iTAK: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. *Mol Plant*. 2016;9:1667–70
104. Wang XF, Liu TJ, Feng T. *et al.* A telomere-to-telomere genome assembly of *Camellia nitidissima*. *Sci Data*. 2025;12:815
105. Wang ZH, Wang XF, Lu T. *et al.* Reshuffling of the ancestral core-eudicot genome shaped chromatin topology and epigenetic modification in *Panax*. *Nat Commun*. 2022;13:1902
106. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol*. 2015;16:157
107. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol*. 2019;20:238
108. Ge SX, Jung D, Jung D. *et al.* ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*. 2020;36:2628–9
109. Kozlov AM, Darriba D, Flouri T. *et al.* RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*. 2019;35:4453–5
110. Smith SA, O'Meara BC. TreePL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics*. 2012;28:2689–90
111. Wang Y, Tang H, DeBarry JD. *et al.* MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res*. 2012;40:e49
112. Tang H, Krishnakumar V, Zeng X. *et al.* JCVI: a versatile toolkit for comparative genomics analysis. *iMeta*. 2024;3:e211
113. Wang D, Zhang Y, Zhang Z. *et al.* KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genomics Proteomics Bioinforma*. 2010;8:77–80
114. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013;30:772–80
115. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*. 2009;25:1972–3
116. Morel B, Kozlov AM, Stamatakis A. *et al.* GeneRax: a tool for species-tree-aware maximum likelihood-based gene family tree inference under gene duplication, transfer, and loss. *Mol Biol Evol*. 2020;37:2763–74
117. Ødum MT, Teufel F, Thumuluri V. *et al.* DeepLoc 2.1: multi-label membrane protein type prediction using protein language models. *Nucleic Acids Res*. 2024;52:W215–20
118. Chen C, Wu Y, Li J. *et al.* TBtools-II: a 'one for all, all for one' bioinformatics platform for biological big-data mining. *Mol Plant*. 2023;16:1733–42
119. Dobin A, Davis CA, Schlesinger F. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29:15–21
120. Liao Y, Smyth GK, Shi W. FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 2014;30:923–30
121. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15:550
122. Bao Y. *et al.* Database resources of the national genomics data center, China National Center for Bioinformation in 2024. *Nucleic Acids Res*. 2024;52:D18–32
123. Chen T, Chen X, Zhang S. *et al.* The genome sequence archive family: toward explosive data growth and diverse data types. *Genomics Proteomics Bioinforma*. 2021;19:578–83
124. Chen M, Ma Y, Wu S. *et al.* Genome warehouse: a public repository housing genome-scale data. *Genomics Proteomics Bioinforma*. 2021;19:584–9

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Horticulture Research, 2026, 13, 1–18
https://doi.org/10.1093/hr/uhag108
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