

Climate drives plasticity and genetic adaptation in apple trees

Running Title: Climate-driven responses in apples

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34 **Summary (200 words)**

- 35 • Perennial crops can respond to climate change through phenotypic plasticity and genetic
36 adaptation, yet how these processes interact and are shaped by domestication and population
37 history remains poorly understood in long-lived species.
- 38 • We combined controlled reciprocal climate experiments with phenotypic, transcriptomic,
39 and genomic analyses in cultivated apple (*Malus domestica*) and its wild relatives. We grew
40 1,248 seedlings from four wild apple populations under four simulated European climates
41 alongside *M. domestica*. Early survival and twelve phenotypic traits were quantified, and
42 gene expression was analyzed in 96 individuals using RNA sequencing, together with DNA
43 sequencing from a previous study.
- 44 • Early seedling survival revealed strong population-specific responses to climate. Phenotypic
45 and transcriptional responses to climate were widespread and largely conserved, with a core
46 set of climate-responsive genes enriched for stress- and nutrient-related functions and
47 evolving under strong purifying selection. Population-specific regulatory responses were
48 limited and largely reflected genetic divergence. Domestication was associated with altered
49 expression patterns and reduced deleterious mutation load.
- 50 • These results show that climate responses in apple seedlings reflect a balance between
51 conserved plastic responses and population-specific genetic constraints shaped by
52 evolutionary history and domestication, with implications for predicting climate resilience in
53 perennial crops.

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55 **Keywords:** Phenotypic plasticity, local adaptation, gene expression, genomic-environment
56 association, deleterious mutations, climate resilience, perennial fruit trees, apple

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58 **Introduction**

59 Rapid anthropogenic climate change is placing unprecedented pressure on both natural and
60 cultivated plant populations (Jump & Peñuelas, 2005). While past climatic shifts, such as the
61 Pleistocene glacial–interglacial cycles, shaped species distributions over millennia,
62 contemporary warming is occurring at an unprecedented pace (Hewitt, 1999, 2004; Williams
63 *et al.*, 2008; Corlett & Westcott, 2013). Whether perennial species can adapt fast enough to
64 survive this acceleration is a critical question in evolutionary ecology, with implications for
65 biodiversity conservation and food security (Thomas *et al.*, 2004; Rellstab *et al.*, 2021;
66 Capblancq *et al.*, 2023; Rezaei *et al.*, 2023; Cortés, 2024; Alseekh *et al.*, 2025).

67 Plants can respond to environmental change through local adaptation, which is genetic
68 differentiation that increases fitness in native environments, and/or through phenotypic
69 plasticity, in which a single genotype can produce different phenotypes depending on
70 environmental conditions (Kudoh, 2016; Fox *et al.*, 2019; Anderson & Song, 2020; Satake *et al.*,
71 2022). While many studies have focused on morphological traits through common-garden
72 or reciprocal transplant experiments (Savolainen *et al.*, 2013; de Villemereuil *et al.*, 2016;
73 Sork, 2018; Benito Garzón *et al.*, 2019; Schwinning *et al.*, 2022), the underlying
74 physiological and molecular mechanisms, particularly in long-lived species, remain poorly
75 understood. Perennial plants must simultaneously buffer short-term environmental variability
76 and maintain fitness over decades, implying a complex interplay between plastic responses
77 and adaptive differentiation.

78 From an evolutionary genomic perspective, how phenotypic plasticity and local
79 adaptation are encoded, regulated, and constrained is an active area of research, in particular
80 in long-lived perennial species (Savolainen *et al.*, 2013; Lobo *et al.*, 2018; Honjo & Kudoh,
81 2019; Anderson *et al.*, 2021; Kremer *et al.*, 2025). Unlike annuals, perennials must repeatedly
82 cope with environmental variability over decades, implying a complex interplay between
83 plastic responses and adaptive differentiation. This balance is likely further complicated by
84 domestication, which can reshaped selection regimes, demographic histories, and regulatory
85 architectures in crop species. Domestication can alter gene expression diversity, modify the
86 strength and targets of purifying and positive selection, and reorganize regulatory networks,
87 potentially canalizing responses to environmental cues (Swinnen *et al.*, 2016; Burban *et al.*,
88 2021; Andersson & Purugganan, 2022). At the same time, demographic processes associated
89 with perennial domestication including clonal propagation, gene flow, and selective sweeps
90 (Gaut *et al.*, 2015) can influence the accumulation and distribution of deleterious variation
91 across the genome (Gaut *et al.*, 2018; Fiscus *et al.*, 2025; Zhang *et al.*, 2025; Chen *et al.*,

2025). Whether these processes ultimately constrain plasticity and adaptive potential, or instead preserve environmentally responsive pathways, remains an open question. Addressing this question also requires comparison with wild relatives, which persisted through past climatic oscillations and may retain greater plasticity or local adaptation potential (Zhang *et al.*, 2017). Understanding how domestication has reshaped the balance between plasticity, adaptation, and genomic constraint in perennial crops, therefore, remains a central challenge (Bosse *et al.*, 2019; Gao *et al.*, 2023; Kremer *et al.*, 2025).

Trees offer an ideal model for addressing these questions due to their long lifespan, wide geographic distribution, and high levels of genetic and phenotypic diversity (Hampe & Petit, 2005; Bouillé & Bousquet, 2005; Kremer *et al.*, 2012). Sequencing technologies have enabled integrative approaches that examine phenotypic plasticity, gene expression, and genome–environment associations, allowing the dissection of the molecular basis of climate responses in natural populations (Capblancq *et al.*, 2023; Kremer *et al.*, 2025; Verrico *et al.*, 2025). Among trees, fruit crops are particularly informative but largely understudied, as they have undergone domestication and thus provide a unique framework to test how human-mediated selection has shaped plasticity and local adaptation in long-lived perennials (Cornille *et al.*, 2015; Gaut *et al.*, 2015; Besnard *et al.*, 2018). In this context, the cultivated apple (*Malus domestica* Borkh.) and its wild relatives constitute a robust system for investigating plasticity and adaptation in both natural and artificial environments. *Malus domestica* originated from the Central Asian wild apple *Malus sieversii* Ledeb., with additional contributions from *M. orientalis* Uglitzk. and *M. sylvestris* Mill. during its westward spread along the Silk Routes from Asia to Europe (Cornille *et al.*, 2012, 2014). Today, cultivated apples are grown across a wide range of temperate environments, and their wild relatives occupy ecologically distinct habitats. Previous studies using microsatellite markers revealed genetic differentiation among European *M. sylvestris* populations resulting from the last past glacial maximum (Cornille *et al.*, 2015), pinpointing potential for local adaptation or plasticity to climatic conditions (Chen *et al.*, 2023). *Malus orientalis*, primarily distributed in the Caucasus, occupies a distinct ecological niche and may retain traits associated with stress tolerance (Volk *et al.*, 2015; Bina *et al.*, 2022). Yet, how phenotypic plasticity and (local) adaptation interact to shape climate responses across wild and cultivated apples remains poorly understood.

Here, we leverage the well-characterized domestication history and population genetic structure of the wild and cultivated apples (Cornille *et al.*, 2012, 2013, 2014, 2015, 2019; Chen *et al.*, 2023, 2025) to conduct an integrative assessment of early-stage climate responses

in *M. domestica*, *M. orientalis*, and *M. sylvestris*. We grew seedlings from five apple populations (Fig. 1a) in climate-controlled chambers (ecotrons) simulating four contrasting European climates (Fig. 1b), and measured survival and 12 physiological and morphological traits (Fig. 1b). We further sequenced the transcriptome (RNA-seq) on a subset of individuals and analyzed genomic divergence, genotype–environment associations (GEAs), and patterns of deleterious mutation load (Fig. 1b). This study addresses four central questions: (1) Do phenotypic and molecular traits primarily reflect plasticity or local adaptation across climates? (2) Are there shared, climate-responsive genes consistently expressed across populations, suggesting a conserved molecular basis for plasticity? (3) Do gene expression responses vary across populations, indicating population-specific plasticity or local adaptation? (4) How do gene expression patterns relate to past population history and genomic constraints such as mutational load?

By integrating phenotypic, transcriptomic, and genomic data, we identify the molecular mechanisms that underpin climate resilience in a perennial crop. We reveal that gene expression plasticity is widespread and, in some cases, overlaps with genomic signals of selection, suggesting that plastic responses themselves may be targets of adaptive evolution in both wild and cultivated apples. Beyond basic science results, our findings also have important implications for conservation and climate-resilient breeding, as we identified candidates associated with climate responses in both cultivated and wild relatives of an emblematic fruit tree.

Materials and Methods

Experimental design and climate conditions

Seeds from five apple populations were used: *Malus sylvestris* from Romania, France, and Denmark (Cornille *et al.*, 2015); *M. orientalis* from Armenia, known for its stress tolerance (Volk *et al.*, 2015); and cultivated apple (*M. domestica*) from controlled cultivar crosses (Table S1; Fig. 1a). For each population, 9–12 seeds per mother tree were stratified for three months at 4°C in moist sand: vermiculite in equal volumes and later sown into climate-controlled ecotron chambers (Roy *et al.*, 2021). Climate conditions from four native sites (France, Denmark, Romania, and Armenia) were simulated for one of two years: 2019 for Armenia and Denmark, and 2021 for Romania and France (Fig.1, Figs. S1–S3; Supporting Text S1). Climate parameters were based on average meteorological records from 1985 to 2014 (<https://code.mpimet.mpg.de/projects/cdi/wiki>), and daily daylength data (<https://ptaff.ca/>, Supporting Text S1).

In total, approximately 60–70 seedlings per population were sown, grown, and monitored in Jiffy pellets under each simulated climate, resulting in ~1,270 seedlings sown across all population × climate combinations (Tables S1-S2) and monitored for two months (61 days). Trays were rotated daily, and seedlings were irrigated once a week. Stratification and sowing dates differed by year. Among those, genomic DNA from surviving wild seedlings on day 61 (last day of the experiment) was genotyped using 12 simple sequence repeat (SSR) markers, and a hybrid index (*Pdom*) was computed with the introgress R package (Gompert & Buerkle, 2010), Table S1, Supporting Text S1).

Survival, growth, and physiological trait measurements

Growth traits (height, leaf number) were measured every 2–3 days. The absolute (AGR) and relative (RGR) growth rates were calculated using previously reported formulas (Briggs *et al.*, 1920; Radford, 1967). The photosynthetic traits chlorophyll (Chl) and flavonoids (Flav), and the nitrogen balance index (NBI) were measured on day 61 using a Dualex® instrument (Force-A, France) on 13 seedlings per population per simulated climate ($N = 260$ in total, Fig. 1a). Dualex exploits the fluorescence and absorption properties of leaves to assess physiological state (Supporting Text S1). Destructive sampling of 5–19 seedlings per population allowed measurements of leaf area, and carbon and nitrogen content were determined by dry combustion using a CN auto-analyzer (Flash EA 1112 series, Therm Finnigan). Root/Shoot ratio, and C/N ratios for stems, roots, and leaves were then calculated.

At the end of the experiment, the survival rates of the seedlings were recorded (Table S2, Supporting Text S1). Seedling survival at day 61 was analyzed as a binary trait using binomial models that accounted for population, climatic condition, and their interaction, with mother identity included to avoid pseudoreplication (Table S2; Supporting Text S1).

In total, for surviving seedlings, 12 traits were analyzed across four categories: growth (AGR height, AGR leaf, RGR height), photosynthetic (Chl, Flav, NBI), stoichiometric (C/N of stem, leaf, and roots), and morphological (SLA, root/shoot, leaf area, Table S1). Subsampling and trait overlap ensured consistency across analyses below (Tables S1, S3).

Trait correction and multivariate analysis

Year effects were tested using a linear mixed model (lme4 (Bates *et al.*, 2015)) (Eqn 1; Supporting Text S1). Growth, photosynthetic, stoichiometric, and morphological trait values were corrected using best linear unbiased predictions (BLUPs) from random-effects models

(Eqns 2, 3; Supporting Text S1). Corrected traits were used in a correlation analysis with the corrplot package in R (Wei & Simko, 2024) and in a principal component analysis (PCA) with the FactoMineR package in R (Lê *et al.*, 2008).

Local adaptation and plasticity

Local adaptation was assessed using linear mixed-effect models with population, climate, and their interaction as fixed effects, and year and mother tree as random effects (Eqn 4; Supporting Text S1). Model selection was based on AIC.

Climate-induced phenotypic plasticity within populations was quantified using random-slope regression mixed models (RRMMs, Eqn 5 (Schielzeth & Nakagawa, 2013; Gómez *et al.*, 2025)), with climate treated as a fixed effect and mother as a random effect.

Phenotypic stability of populations across climates was evaluated using Finlay–Wilkinson regressions (FWR, (Finlay & Wilkinson, 1963)), applied to wild populations only, with slopes close to one indicating stable trait expression across environments (Supporting Text S1).

RNA extraction and gene expression analysis

Leaves from 100 seedlings (20 per population) were sampled on day 61 (Table S3, Fig. 1a). Leaves were chosen for transcriptomic analyses as they integrate climatic signals related to photosynthesis, water balance, and metabolic regulation. Total RNA was extracted using NucleoSpin (Macherey-Nagel) with PVP40 and CTAB added, and quality-checked with a Nanodrop and Bioanalyzer. RNA-seq libraries were prepared with a Novogene PT044 kit and sequenced on a NovaSeq 6000 instrument as paired-end 150-bp reads.

Quality of raw reads was assessed with FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and then reads with a mean phred score lower than 15 were filtered using Fastp with a trimming of the first 10 bases due to a lower overall quality, and SortMeRNA was used to remove remaining ribosomal RNA with default parameters (Kopylova *et al.*, 2012; Chen *et al.*, 2018). After filtering, 96 samples passed QC. Filtered reads were mapped to the GDDH13 reference transcriptome (Daccord *et al.*, 2017) using STAR (Dobin *et al.*, 2013) and quantified with Salmon (Patro *et al.*, 2017). Differentially expressed genes (DEGs) were identified using DiCoExpress (Lambert *et al.*, 2020) and edgeR (Robinson *et al.*, 2010) with an FDR cutoff of 0.05, after trimmed mean of M values (TMM) normalization (Robinson & Oshlack, 2010) and filtering (counts per million [CPM] > 5). Generalized linear models (GLMs) were tested for population, climate,

and interaction effects (Eqn 6; Supporting Text S1). To better understand how population history, climate, and G×E interactions shape gene expression, the complete list of DEGs identified into three components: population effects (population DEGs, reflecting evolutionary history), climate effects (climate-responsive DEGs, reflecting plastic responses), and their interaction (reflecting local adaptation or plasticity) (Supporting information Text S1). UpSetR plots (Conway *et al.*, 2017) were drawn to visualize overlaps among categories.

Gene ontology (GO) term enrichment and co-expression network analysis

GO term enrichment analysis was performed using the R package *clusterProfiler* (v4.6.0) (Xu *et al.*, 2024) with a custom OrgDb from the GDDH13 reference genome. A Benjamini–Hochberg false discovery rate (FDR) correction (Benjamini & Hochberg, 1995) was applied. Weighted Gene Co-expression Network Analysis (WGCNA (Langfelder & Horvath, 2008)) was used to identify gene modules correlated with phenotypic traits and to intersect them with lists of DEGs. Enrichment analysis was performed on modules containing more than 50 DEGs.

Genetic divergence estimated using RNA sequencing data

Single-nucleotide polymorphisms (SNPs) were called from RNA-seq reads using GATK HaplotypeCaller (Auwera & O’Connor, 2020) and annotated with SnpEff and SnpSift (Cingolani *et al.*, 2012). In total, 1 million exon-wide SNPs were identified from the RNA-seq data (Supporting Text S1), including 468,714 synonymous SNPs.

To quantify neutral population genomic differentiation, pairwise genetic differentiation (F_{ST}) among populations was estimated using Pixy (Korunes & Samuk, 2021). Analyses were restricted to 468,714 synonymous and unlinked SNPs and invariant sites to minimize the influence of selection and linkage and avoid biases in measurement, following the default pixy behavior, providing robust estimates of background genomic differentiation among populations.

To investigate the genomic basis of differential gene expression, gene-level absolute divergence D_{XY} was estimated to identify highly divergent genes. The average number of nucleotide differences per site (D_{XY}) was first calculated per exon using Pixy (Korunes & Samuk, 2021). Gene-level D_{XY} values were obtained by aggregating the Pixy metrics count_diff and count_comparison across all exons within a gene. Absolute measures of divergence, such as D_{XY} , were preferred over relative metrics like F_{ST} , as they are sensitive to within-population genetic diversity and can be biased by local variation in recombination rate

or linked selection (Charlesworth, 1998). Gene-level D_{XY} values were standardized using Z-scores, and genes with Z-scores greater than 2 were classified as highly divergent outliers (Supporting Text S1). The overlap between these genetically divergent genes and DEGs was then assessed to evaluate the contribution of genetic divergence to population-level differences in gene expression.

Genome-environment association analyses (GEA) in *M. sylvestris* and overlap with DEGs

To investigate the genomic basis of local climate adaptation in European wild apple, leveraging its extensive geographic sampling, we focused subsequent analyses solely on *M. sylvestris*.

A total of 5.5 million genome-wide SNPs from 64 *M. sylvestris* individuals (Chen *et al.*, 2025) were analyzed (Supporting Texts S1 and S2, Table S4). After quality filtering and linkage disequilibrium (LD) pruning using a threshold of 0.2 (PLINK, (Purcell *et al.*, 2007)), 26,009 SNPs were retained for population structure inferences with fastStructure (Raj *et al.*, 2014). LD pruning was applied only for population structure analyses; all 5.5 million SNPs were retained for GEA analyses using LFMM (Frichot *et al.*, 2013) and Samβada (Duruz *et al.*, 2019). Six uncorrelated bioclimatic variables (BIO1, BIO2, BIO3, BIO4, BIO8, BIO12) were selected for downstream analyses (Supporting Text S2). Significant climate-associated SNPs were annotated to nearby genes; GEA genes detected by both methods were retained. As we investigated the genomic basis of local climate adaptation in the European wild apple, leveraging its extensive geographic sampling

We performed an additional differential gene expression analysis restricted to *M. sylvestris* RNA-seq data and redefined core climate-responsive genes as those consistently differentially expressed across populations in response to climate, reflecting conserved molecular plasticity. Population-specific DEGs were defined as genes differentially expressed across all climatic conditions among populations.

Finally, population-specific and climate-responsive DEGs were intersected with (i) genes associated with climate variables identified by GEA (Supporting Text S2) and (ii) outlier genes detected using D_{XY} . This integrative framework allowed us to identify robust candidate genes underlying local adaptation, supported by concordant signals of regulatory variation, climate-associated genomic variation, and gene divergence.

Genetic diversity, gene expression, and selection efficiency

Gene expression variation, molecular diversity, and deleterious mutation load were compared between cultivated apple (*M. domestica*) and its wild relatives (*M. sylvestris* and *M. orientalis*). Analyses focused on three gene categories: (i) dom–wild DEGs, (ii) core climate-responsive DEGs, and (iii) background expressed genes (Sauvage *et al.*, 2017). To assess the impact of domestication on molecular diversity, the 1,086,764 SNPs called from RNA-seq data (see above “Genetic divergence estimated using RNA sequencing data” and Supporting Text S1) were used to estimate nucleotide diversity at nonsynonymous (πN) and synonymous (πS) sites with Pixy (Korunes & Samuk, 2021). We modeled the log-ratio of diversity between cultivated and wild apples as a function of gene category for both πN and πS (Supporting Text S1, Eqns 7–8), thereby distinguishing genome-wide effects of domestication from gene-category-specific deviations. Differences in purifying selection efficiency were further evaluated by modeling $\pi N/\pi S$ across populations and gene categories, including their interaction (Supporting Text S1, Eqn 9).

Derived deleterious alleles were already annotated on whole-genome SNPs from Chen *et al.* (2025), annotated using SIFT 4G (Vaser *et al.*, 2016). Only individuals from populations in our study, were kept (Table S4). For each individual, the average number of deleterious mutations per site was calculated for each gene category and analyzed as a function of population, gene category, genotype, and their interactions (Supporting Text S1, Eqn 10). To account for unequal gene category sizes, all analyses were repeated using random subsampling across multiple proportions of each gene category. Full methodological details are provided in Supporting Text S1.

Results

Climate shapes both adaptive survival and phenotypic plasticity in young apple seedlings

Out of 1,248 seedlings sown across four simulated climate conditions (France, Denmark, Romania, and Armenia, Fig. 1a), 894 survived and were phenotyped for 12 traits (Tables S1 and S2). Among these, we genotyped 710 wild seedlings (*M. orientalis* and *M. sylvestris*) using SSR markers to estimate P_{dom} , their hybrid index with *M. domestica*. We observed a wide range of admixture levels ($0.009 < P_{dom} < 0.94$; Supporting Text S1). In addition to genotyping, total RNA was successfully extracted from 96 seedlings for RNA-seq analysis

(Table S3; Supporting Text S1, Fig. 1b), representing all population and climate combinations.

Local adaptation and population-specific survival patterns

Seedling survival at day 61 varied markedly among populations and across simulated climatic conditions (Table S2a, Fig. 2). Overall survival proportions were high for *M. domestica* and *M. orientalis* across all environments, whereas the three *M. sylvestris* populations showed more variable and generally lower survival rates (Table S2a, Fig. 2). Model comparison based on Akaike's Information Criterion supported the mother-aggregated binomial model as the best-supported framework to analyze survival variation (Table S2b).

Fixed-effect estimates from this model revealed significant population effects and a significant population $\times$ climate interaction (Table S2c), indicating that population differences in survival depended on climatic conditions. Post hoc Tukey comparisons confirmed pronounced population-specific differences within climates (Table S2d). Under French climatic conditions, the French *M. sylvestris* population exhibited significantly higher survival than both the Danish and Romanian *M. sylvestris* populations (Table S2d), whereas no significant differences were observed between the French *M. sylvestris* population and either *M. domestica* or *M. orientalis*. By contrast, the Danish *M. sylvestris* population did not show enhanced survival under Danish conditions, as no significant pairwise differences were observed between this population and other *M. sylvestris* populations under these conditions (Table S2d). Across all climatic conditions, the Romanian *M. sylvestris* population consistently showed lower survival than *M. domestica*, *M. orientalis*, and the French *M. sylvestris* population (Tables S2a,d), suggesting reduced early-life fitness. In contrast, *M. domestica* and *M. orientalis* maintained high and relatively stable survival probabilities across climates, with few significant differences between them (Tables S2a, d), consistent with broad environmental tolerance during early seedling establishment.

Overall, early seedling survival revealed population-specific responses to climate. The French *M. sylvestris* population showed enhanced survival in its native climate, consistent with local adaptation, whereas the Romanian *M. sylvestris* population exhibited consistently low survival across all environments, suggesting reduced early-life fitness. In contrast, *M. domestica* and *M. orientalis* maintained high and stable survival across climates, indicating broad environmental tolerance during early establishment.

Climate is the dominant driver of phenotypic variation

Early seedling growth and physiology, corrected for year effects, were predominantly influenced by climate rather than population origin (Table 1, Tables S5 and S6). Significant population $\times$ climate interactions were detected only for three traits: chlorophyll content, relative growth rate in height, and absolute growth rate in leaf number, suggesting that most trait variation was shaped by main climate effects (Table 1; Fig. S4). For example, *M. domestica* adopted a growth-oriented strategy, with the largest leaf area (Fig. S4), while Romanian *M. sylvestris* displayed a more stress-related response, with higher flavonoid accumulation. However, these population-level patterns were often overridden by climate. The French climate promoted vigorous aerial growth, while the Armenian climate induced the strongest physiological stress, including elevated flavonoid levels. Traits related to biomass allocation (e.g., Root/Shoot) and tissue composition (C/N ratios) also varied with climate, but followed different geographic patterns. Despite strong climate effects detected in univariate and mixed-effects models, multivariate ordinations revealed substantial overlap among populations and climatic treatments, indicating that climate-driven phenotypic responses occurred largely within a continuous phenotypic space rather than forming discrete clusters (Fig. S5). This apparent discrepancy reflects the fact that climate-driven trait variation was largely parallel across populations, resulting in shared plastic shifts rather than discrete phenotypic clustering. Thus, while climate significantly altered trait means and survival probabilities, these changes occurred within a largely continuous phenotypic space, particularly at the early seedling stage.

The plasticity analysis, based on random-slope regression mixed models (RRMMs, (Gómez *et al.*, 2025)) for each population and trait, confirmed that climate-induced plasticity is both widespread and variable among populations (Table S7a). Some traits, such as absolute growth rate (AGR) of height, chlorophyll content (Chl), and the root-to-shoot ratio, exhibited statistically significant shifts across climates in most populations, indicating broadly conserved plastic responses. In contrast, traits such as C/N ratios and specific leaf area (SLA) showed more population-specific patterns of plasticity (Table S7a). Notably, *M. domestica* exhibited significant plasticity for several traits, suggesting that domestication has not fully constrained its capacity for environmental responsiveness. The direction and magnitude of these traits shift across simulated climates are detailed in the estimated marginal means (Table S7b) and in pairwise Tukey post hoc comparisons (Table S7c), which clarify which specific environmental contrasts (e.g., Armenia vs. France) drive the observed differences. To identify which climate condition induced the strongest trait shifts, we examined the fixed-effect estimates from the RRMMs (Table S7b). The Armenian climate consistently elicited

stress-related responses across populations, including increased flavonoid accumulation and elevated NBI values, confirming its relative harshness. In contrast, the French climate promoted vigorous shoot growth and larger leaf area, highlighting its favorable conditions for biomass accumulation. Among wild populations, Romanian *M. sylvestris* (MSylRO) showed significant plasticity across most traits, indicating strong environmental responsiveness.

Finlay–Wilkinson regressions revealed that most wild populations had slopes close to 1 (Table 2; Figs. S6–S9), indicating stable trait expression across climates. The exception was Romanian *M. sylvestris*, which exhibited steeper slopes and higher variance, highlighting greater sensitivity to environmental changes.

In summary, phenotypic plasticity was widespread but trait- and population-specific. While most wild populations showed stable trait expression across climates, Romanian *M. sylvestris* stood out for its low stability and poor performance. *Malus domestica* exhibited substantial plasticity across traits. The Armenian climate, characterized by a hot, harsh environment, consistently triggered stress-related responses, including increased flavonoid levels and NBI. These patterns prompted us to further investigate the molecular and genomic bases underlying such plasticity and climate responses.

Decoupling adaptation, plasticity, and their genomic signatures in apple seedlings

Limited differential gene expression between populations mostly reflects neutral genomic divergence among populations

The number of population DEGs, defined as genes differentially expressed across populations and consistently across all simulated climate conditions, was substantially lower than the number of DEGs associated with climate effects (Fig. 3a,b). *Malus orientalis* seedlings exhibited the highest number of population-specific DEGs (Fig. 3b), consistent with their pronounced transcriptomic divergence and supported by their strong neutral genomic differentiation (Fig. 3c). Indeed, pairwise DEG counts between *M. orientalis* and *M. sylvestris* populations followed a clear geographic gradient, with Danish *M. sylvestris* showing the greatest number of DEGs relative to *M. orientalis*, followed by the French and Romanian populations (Fig. 3c). This pattern closely mirrored neutral genomic differentiation, as indicated by a strong correlation between pairwise F_{ST} values and the number of DEGs (Spearman’s $\rho = 0.95$, $p < 2.2 \times 10^{-16}$). Together, these results suggest that population-level expression divergence largely reflects underlying neutral genetic divergence rather than adaptive differentiation.

The *M. domestica* population displayed only seven population DEGs, clearly distinguishing it from the three wild populations (Fig. 3b). The smallest number of DEGs was observed between *M. domestica* and Romanian *M. sylvestris* (Fig. 3c; Fig. S10). The few DEGs separating *M. domestica* from wild populations nonetheless included notable candidates: *MD06G1132200*, a homolog of *Arabidopsis thaliana* INDUCER OF CBF EXPRESSION 1 (*ICE1*), involved in flowering time regulation under cold condition (Lee *et al.*, 2015), and *MD15G1374500* in *M. orientalis*, a homolog of HOMEBOX PROTEIN 16 (*ATHB 16*), a potential regulator of flowering and leaf expansion in response to blue light (Wang *et al.*, 2003) (Tables S8 and S9).

Population-DEGs overlapped with D_{XY} outlier genes, especially in *M. orientalis* (33 overlapping genes out of 117 DEGs specific to *M. orientalis*), indicating their role in adaptive divergence among populations (Table S8).

Climate induces a strong and modular transcriptomic response

In contrast to the population effects above, climate exerted a significant influence on gene expression, with 1,810 DEGs consistently responsive to climate across all populations (Fig. 3a,d, Table S8). This core set of climate-responsive DEGs ("core climate DEGs") highlights the conserved nature of transcriptional plasticity in response to environmental variation. Notably, the simulated Armenian climate conditions induced the most distinct transcriptomic signature, with 68 DEGs unique to this environment (Fig. 3d), compared to 5, 1, and 0 for Denmark, Romania, and France, respectively (Table S8).

GO term enrichment analysis revealed that these genes are significantly enriched in functional terms related to nutrient starvation responses, cell wall biosynthesis, seed development, and phosphate metabolism (Fig. 3e). Thirty-five of these DEGs were also linked to flowering-time regulation (Tables S8 and S9), pointing to environmentally responsive regulation of developmental timing. A co-expression network analysis with WGCNA identified 18 modules, of which six (yellow, tan, black, green, blue, purple) were enriched for core-climate DEGs (Fig. S11). The yellow, green, and black modules were all enriched for DEGs associated with distinct GO terms (Figs. S12 and S13), linking plastic gene expression to coordinated physiological responses, including photosynthesis, nutrient metabolism, and structural remodeling.

Genotype×climate interactions and population-specific gene expression and adaptation

To identify population-specific transcriptomic plasticity or local adaptation, we analyzed DEGs exhibiting significant population×climate interactions, resulting in 1,542 DEGs across all populations (Fig. 3f, Table S10). In wild populations, we focused on contrasts between native simulated climate conditions (“home”) and other climate conditions (“away”). For *M. domestica*, we tested all simulated climate conditions separately.

In *M. orientalis*, 390 genes showed consistent expression responses matching the native Armenian climate, with expression levels systematically higher or lower in the home population when grown under its native climatic conditions (Fig. 3f). Two candidate genes were expressed at lower levels under all simulated climate conditions, except in Armenia, and overlapped with D_{XY} outlier genes, suggesting strong local regulatory adaptation: *MD01G1231900* (encoding a Peptidase family C78 domain protein) and *MD02G1245800* (encoding a protein related to the wheat rust resistance kinase Lr10) (Table S10).

The Danish population of *M. sylvestris* exhibited 98 climate-aligned DEGs (Fig. 3f, Table S10), followed by 55 in the French and 34 in the Romanian populations. Most DEGs showed limited overlap between populations, but some candidates stood out (Table S10). We identified one gene, *MD11G1246600* (homologous to *At1g16930* of *Arabidopsis*, encoding an F-box protein related to F-BOX AND LEUCINE RICH REPEAT DOMAINS CONTAINING PROTEIN [FBD]), as a GEA candidate, a D_{XY} outlier, and a climate-aligned DEG in both the French and Danish populations (Fig. 3c). This finding supports its role as a robust candidate for local adaptation.

In the *M. domestica* population, climate-specific DEGs were most numerous under the simulated Armenian (561) and Danish (541) climate conditions, with minimal overlap (nine shared genes), suggesting largely distinct plastic responses (Table S10). The gene *MD12G1045900* (Fig. 3g), homologous to *Arabidopsis ATP-binding cassette G22* (*ABCG22*), which is involved in stomatal opening regulation, showed expression shifts in *M. domestica* but not in wild populations, suggesting domestication-driven rewiring of stress-responsive plasticity.

Genomic basis of climate adaptation in *M. sylvestris*

After removing collinear variables (Pearson’s correlation coefficient $r > 0.7$) (Fig. S14), the overlap at each SNP between the two GEA methods was modest (0.07%) (Figs. S15 and S16), as expected (Chen *et al.*, 2021). Still, the convergence at the gene level was stronger

after mapping SNPs to regions encompassing genes and to ± 2 kb upstream and downstream (8% overlap at the gene level compared to 0.07% at the SNP level). We detected the strongest GEA signals for BIO12 (precipitation), followed by temperature-related variables (BIO1, BIO2, BIO3, BIO4, BIO8) (Fig. 4a, top, Table S11), suggesting that both precipitation and temperature gradients have shaped the genomic architecture of adaptation to climate in *M. sylvestris*.

Genes identified by the GEA and overlapping with DEGs included both climate-responsive DEGs shared across the three wild populations (*core climate DEGs of M. sylvestris*) and population-specific DEGs (Fig. 4a, middle and bottom). The overlap between GEA hits and climate DEGs was extreme for BIO12 (annual precipitation, Table S11), reinforcing the potential adaptive significance of precipitation-responsive gene expression.

Focusing on candidate genes showing climate-aligned expression patterns within populations (i.e., consistent upregulation or downregulation in the home climate), we identified 165, 125, and 119 genes in the Danish, French, and Romanian *M. sylvestris* populations, respectively (Fig. 4b, Table S12). Of these, 10 (Denmark), 2 (France), and 6 (Romania) were also D_{XY} outliers, indicating concordant transcriptomic and genomic divergence and suitable candidates for local adaptive divergence.

Comparison across populations revealed no shared candidates for local adaptation between France and Romania. In contrast, six genes were shared between the French and Danish populations, and five between the Danish and Romanian populations. One gene, *MD11G1246600*, stood out as a strong candidate (Fig. 4c). Indeed, it showed climate-aligned expression in both Denmark and France, was a D_{XY} outlier in both Denmark vs Romania and France vs Romania comparisons, and exhibited a significant GEA signal. Functional annotation identified a similarity (e-value = $2e-41$) to the Arabidopsis *At1g16930* gene, which encodes an F-box/FBD/LRR-repeat protein involved in protein turnover and environmental signaling, supporting a potential role in local climate adaptation.

Overall, more population-specific DEGs overlapped with D_{XY} outlier genes (i.e., genomic divergence) than other DEGs. By contrast, core climate DEGs overlapped more frequently with GEA candidates (see Results: Genomic basis of climate adaptation in *M. sylvestris* below), suggesting that transcriptomic plasticity sometimes reflects adaptive responses to environmental gradients (i.e., adaptive plasticity). Genes with significant $G \times E$ effects overlapping with D_{XY} or GEA outliers provide compelling evidence for local adaptation or maladaptation.

Gene expression divergence and selective constraint in cultivated apple and its wild relatives

We showed the consequences of domestication on gene expression divergence, molecular diversity, and mutation load (Tables S13 and S14). Notably, *M. domestica* displayed the lowest average $\log(\pi N/\pi S)$ among all populations (Fig. 5a; Table S15), suggesting globally stronger purifying selection in the cultivated apple. Core climate DEGs exhibited significantly lower $\log(\pi N/\pi S)$ values than did background genes, indicating stronger purifying selection acting on genes involved in plastic responses to climate. By contrast, dom-wild DEGs showed significantly higher $\log(\pi N/\pi S)$ values, suggesting relaxed constraint on genes with expression patterns having diverged during domestication (Fig. 5b, Table S16).

Patterns of nucleotide diversity supported these findings. πN did not differ significantly between core climate DEGs and background genes, indicating that climate-relevant genes did not show a change in diversity indicative of selective sweeps. By contrast, DEGs between the cultivated apple and wild apple populations (dom-wild DEGs) (Table S8) exhibited markedly lower πN in *M. domestica* relative to the wild populations, suggesting preferential purging of deleterious variants during domestication (Fig. 5c, Table S17). Synonymous diversity (πS) was also lower in dom-wild DEGs than in background genes, though to a lesser extent (Fig. 5d, Table S17), consistent with a smaller effective population size or linked selection.

Regarding deleterious mutation load, gene category and populations both had significant main effects ($p < 2e-16$) (Table S14), with dom-wild DEGs showing the highest load, followed by background genes and core climate DEGs, which carried the fewest deleterious mutations (Fig. 5e; Table S18). Across populations, *M. domestica* consistently exhibited the lowest deleterious mutation burden ($p < 0.001$), suggesting enhanced purging during domestication. Importantly, this pattern was genotype-dependent: the lower mutational load of the *M. domestica* population was most evident in individuals with homozygous genotypes. At the same time, the Romanian *M. sylvestris* population had the highest proportion of heterozygous individuals (Fig. 5f). These results highlight how domestication is associated with lower synonymous and non-synonymous diversity and the purging of deleterious alleles, particularly in genes underlying expression divergence. By contrast, genes involved in core climate responses show lower mutation load and stronger evolutionary constraints, supporting their conserved function across apple lineages.

Discussion

This study provides the first integrative evidence that both plasticity and genetic adaptation contribute to climate responses in a perennial fruit tree. By combining simulated climate experiments with transcriptomic and genomic analyses in *M. domestica* and its wild relatives, we dissect the molecular, phenotypic, and evolutionary mechanisms that allow these taxa to respond to climate variability. Our results reveal a dual strategy of climate response that combines broadly conserved phenotypic plasticity with lineage-specific patterns of local adaptation and genomic constraint. We show that plasticity in gene expression is a pervasive and evolutionarily conserved feature across wild and cultivated apple populations, likely contributing to resilience under variable conditions. At the same time, population-specific regulatory responses and genotype–climate associations highlight the role of local adaptation in *M. sylvestris*. Moreover, variation in the burden of deleterious mutations is reduced in cultivated apples and elevated in some wild populations, suggesting that demographic history and selection during domestication further shape the evolutionary potential of each lineage. Together, these findings underscore that a complex interplay between plasticity, adaptation, and genetic load governs climate response in long-lived crops. They provide a refined framework for predicting resilience trajectories and for identifying both conserved and lineage-specific targets for climate-resilient breeding and conservation strategies.

In perennial species, phenotypic plasticity is increasingly recognized as a key mechanism for persistence under environmental uncertainty (Liepe *et al.*, 2022; Kremer *et al.*, 2025; Maury & Plomion, 2025). Species that endured the climatic oscillations of the Pliocene–Pleistocene transition often evolved life-history traits that buffer against variability, including robust plastic responses (Kremer *et al.*, 2014; Caignard *et al.*, 2024). Our study builds on this framework by providing both phenotypic and molecular evidence of such plasticity in fruit trees. In wild apples, which persisted through glacial cycles (Cornille *et al.*, 2013, 2015), we show that phenotypic responses to climate were widespread but trait-specific. Growth-related traits, such as shoot elongation, exhibited consistent plastic responses across climates, whereas physiological traits associated with stress, including flavonoid accumulation, primarily reflected climate-induced stress exposure (i.e., in the Armenian climate). Other traits showed more population-specific responses. Most wild populations displayed relatively stable trait expression across climates, consistent with robust, and in some cases potentially adaptive, plasticity. A notable exception was the Romanian *M. sylvestris* population, which exhibited low phenotypic stability, high trait variance, and poor performance across all simulated climates, suggesting maladaptation or demographic

constraints. In contrast, *M. domestica* exhibited high plasticity across multiple traits, consistent with its maintained genetic diversity (Cornille et al., 2012; Migicovsky et al., 2021), indicating that domestication has not fully constrained environmental responsiveness, although the adaptive value of this plasticity remains to be established. Among climates, the Armenian simulation, representing a hot and dry environment, consistently triggered strong physiological stress responses, including elevated flavonoid and NBI levels, confirming its environmental harshness and reinforcing the use of flavonoids as ecophysiological indicators of stress. Collectively, these results illustrate how trait-specific plasticity, climatic harshness, and population history interact to shape resilience in both wild and cultivated apples.

At the molecular level, we identified 1,810 core genes with consistent expression shifts across climates and populations. These genes are enriched in stress- and nutrient-related pathways and are under strong purifying selection, suggesting they form a conserved molecular toolkit for climate responsiveness paralleling findings in other trees (Bräutigam et al., 2013; Maury & Plomion, 2025). In addition, we detected lineage-specific responses: *M. sylvestris* from France showed high survival in its native climate and harbored candidate genes with overlapping signatures of local adaptation, including climate-aligned expression and genomic differentiation. In contrast, Romanian *M. sylvestris* exhibited elevated heterozygous mutation load and low performance, which may reflect poor adaptation. These contrasting trajectories underscore the local evolutionary histories in shaping adaptive capacity. However, compared to the pronounced plasticity response observed at both the phenotypic and molecular levels, signatures of local adaptation were relatively weak, suggesting that plasticity may be the dominant climate-response mechanism in juvenile apple trees, as previously observed in forest species (Liepe et al., 2022). This pattern is further supported by survival data that reflect population-level variation in early-life fitness across different climates. In particular, the French *M. sylvestris* population showed significantly higher survival under French conditions, supporting local adaptation. Conversely, Romanian *M. sylvestris* consistently exhibited the lowest survival rates across all climates, in line with its reduced phenotypic stability and elevated mutation load, suggesting maladaptation or demographic constraints. By contrast, both *M. domestica* and *M. orientalis* showed high and stable survival across climates, indicating broad environmental tolerance during early establishment. These findings reinforce the idea that while plasticity is widespread and conserved, lineage-specific local adaptation also shapes early survival and resilience. We note that part of the observed variation in early survival may also reflect population differences in vernalisation sensitivity, as stratification conditions were standardized and may

have been more optimal for some lineages than others. In particular, the consistently high survival of *M. domestica* and *M. orientalis* across all simulated climates may partly reflect reduced vernalisation constraints or broader developmental tolerance, consistent with domestication history and adaptation to heterogeneous environments. Importantly, the climate-specific survival advantage of the French *M. sylvestris* population supports a contribution of local adaptation beyond vernalisation effects.

Our findings also support the hypothesis that adaptive plasticity is at play in the apple. The climate-responsive genes we identified are both evolutionarily conserved and under purifying selection features consistent with a model in which plasticity is maintained by natural or artificial selection. Furthermore, population-specific DEGs were enriched for genotype–environment association signals, reinforcing the idea that plastic responses can confer fitness advantages and thus be adaptive (Ghalambor *et al.*, 2007). This idea of plasticity as a trait under selection is increasingly recognized in forest trees, where common-garden experiments reveal genotype $\times$ environment interactions and nonzero reaction-norm slopes for key traits (Rehfeldt *et al.*, 2002; Chambel *et al.*, 2005; Marchi *et al.*, 2022; Leites & Benito Garzón, 2023). However, formally testing the adaptive plasticity remains challenging in perennial species, where lifetime fitness is difficult to measure due to long generation times (McGraw & Caswell, 1996). While early vegetative traits, such as shoot growth and flavonoid accumulation, can serve as proxies for physiological stress responses, they may not fully predict reproductive success. Integrating long-term field trials with metrics such as fruit production, carbon assimilation, or survival under recurrent stress could offer more robust proxies for adaptive plasticity (Nicotra *et al.*, 2010; Hartmann *et al.*, 2020). Taken together, these findings underscore that while our results provide strong molecular and phenotypic evidence of plasticity, its adaptive value will require further validation across the full life cycle of trees.

Our study also contributes to understanding how domestication influences climate responses. *Malus domestica* maintained broad stable performance across climates. Its gene expression profile was distinct from wild relatives, and its climate-responsive genes showed reduced non-synonymous diversity and mutation load. These patterns suggest that artificial selection may have purged deleterious variants from exonic sequences and a canalization or stabilizing selection of key regulatory loci in a cultivated context (Gaut *et al.*, 2018). At the same time, we identified genes that distinguish *M. domestica* from wild apples and are under relaxed selection, suggesting domestication-driven expression divergence. Such rewiring of

transcriptional networks has also been observed in tomato and cacao (Sauvage *et al.*, 2017; Hämälä *et al.*, 2020), and may have significant consequences for stress resilience.

Overall, our findings support the synergistic contribution of plastic and genetic effects to adaptive evolution in fruit trees. In forest species, covariation between plasticity and selection suggests that plastic responses can pave the way for genetic change, a pattern consistent with the Baldwin effect (Crispo, 2008; Kremer *et al.*, 2014). Studies of tree species, such as *Quercus suber* and *Abies alba*, have shown that variation in traits, including drought tolerance, shoot growth, and tree-ring patterns, reflects both plastic and adaptive responses to environmental heterogeneity (Lanner, 2002; Ramírez-Valiente *et al.*, 2010; Fréjaville *et al.*, 2019). In our system, genes exhibiting both plastic expression and signatures of selection provide candidates for such synergistic evolution. These insights align with empirical evidence from multisite trials in forest trees, where genetic and plastic contributions often reinforce each other and jointly enhance resilience (Maes *et al.*, 2019; Alexandre *et al.*, 2020; Caignard *et al.*, 2024).

Despite the depth of our dataset, our controlled experiments involved necessary compromises, including trade-offs in sampling depth and replication across mother lines. We accounted for year effects in our statistical models, but further validation in long-term field trials and across developmental stages is needed. Still, this study provides a foundational framework for future work. It highlights the power of integrating phenotypic, transcriptomic, and genomic data to unravel the evolutionary dynamics of climate response in perennial crops.

In conclusion, we provide evidence that both plasticity and genetic adaptation contribute to climate resilience in fruit trees, and that these responses may be conserved or lineage-specific. These findings open new avenues for conserving wild germplasm and for breeding strategies to enhance resilience in cultivated perennial crops. Understanding and harnessing this interplay between plasticity and adaptation, such as epigenomic variation, will be key to ensuring the persistence and productivity of fruit trees under global change (Maury & Plomion, 2025).

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Competing interests

The authors declare no competing interests.

Author contributions

AMC initiated the study; AMC and SB conceived the study and secured the funding; AMC, AV, CR, ND, SB, SC, AH, AR, TK, TMU, AR, GMV, and GA collected samples and performed the experiments. RD, XC, AMC, JS, ND, MC, and KA analyzed the data. RD, XC, AMC, NCS, EM, and SB interpreted the results. AMC, RD, JS, and XC wrote the paper with critical input from co-authors. All authors revised and approved the final manuscript.

Data availability

All scripts are available at https://github.com/CornilleEclecticLab/Apple-climate_change_Ecotron; the SSR and the SNP vcf data are available on Zenodo: <https://doi.org/10.5281/zenodo.18186528>. All raw RNA-seq data have been deposited in the NCBI Sequence Read Archive (SRA) under accession PRJNA1399139. All DNA-seq data generated in this study have been deposited in the NCBI under BioProject accession number PRJNA1252109.

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- 996 **Supporting Information**
- 997 **Fig. S1.** Simulated temperature profiles across ecotron climate chambers.
 998 **Fig. S2.** Simulated relative humidity profiles across ecotron climate chambers.
 999 **Fig. S3.** Simulated day length across ecotron climate chambers.
 1000 **Fig. S4.** Combined residual trait means from linear mixed models across simulated ecotron
 1001 climates
 1002 **Fig. S5.** Principal component analysis (PCA) of phenotypic trait variation among apple
 1003 seedlings.
 1004 **Fig. S6.** Finlay–Wilkinson regression plots for growth traits in wild apple populations across
 1005 simulated climates.
 1006 **Fig. S7.** Finlay–Wilkinson regression plots for carbon uptake traits in wild apple populations
 1007 across simulated climates.
 1008 **Fig. S8.** Finlay–Wilkinson regression plots for carbon/nitrogen ratio in wild apple
 1009 populations.
 1010 **Fig. S9.** Finlay–Wilkinson regression plots for dry matter and leaf area traits in wild apple
 1011 populations.
 1012 **Fig. S10.** Principal component analysis (PCA) of normalized gene expression across
 1013 populations and climate conditions.
 1014 **Fig. S11.** Proportion of differentially expressed genes (DEGs) within WGCNA modules in
 1015 response to climate or population.
 1016 **Fig. S12.** Gene ontology term enrichment analysis across WGCNA modules associated with
 1017 climate-responsive differentially expressed genes (DEGs).
 1018 **Fig. S13.** Correlation between the expression levels of genes within each WGCNA co-
 1019 expression module and early phenotypic traits in apple seedlings.
 1020 **Fig. S14.** Pairwise Pearson’s correlation coefficients among 55 environmental variables from
 1021 WorldClim2.
 1022 **Fig. S15.** Manhattan plot of genotype×environment associations (GEAs) in *Malus sylvestris*
 1023 across six bioclimatic variables based on statistics from LFMM2.
 1024 **Fig. S16.** Manhattan plot of genotype×environment associations (GEAs) in *Malus sylvestris*
 1025 based on G-score statistics from Samβada.
 1026
 1027 **Table S1.** Seedling provenance and experimental use across climate simulations.
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 1029 tests.
 1030 **Table S3.** Number of seedlings measured for each trait across populations, climate
 1031 simulations, and experimental years.
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 1033 study.
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1036	ecotron experiment.
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1038	regression mixed models (RRMMs).
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1044	expression test.
1045	Table S13. Linear mixed model results for nucleotide diversity statistics obtained from
1046	single-nucleotide polymorphism (SNP) calling of RNA-seq data.
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1048	the dataset from Chen et al. 2025.
1049	Table S15. Estimated marginal means and pairwise contrasts of log-transformed pN/pS ratios
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1052	ratios between apple populations.
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1054	ratios between domesticated and wild apple populations.
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1056	per combination of population and genotype, and per gene type.
1057	
1058	Figures

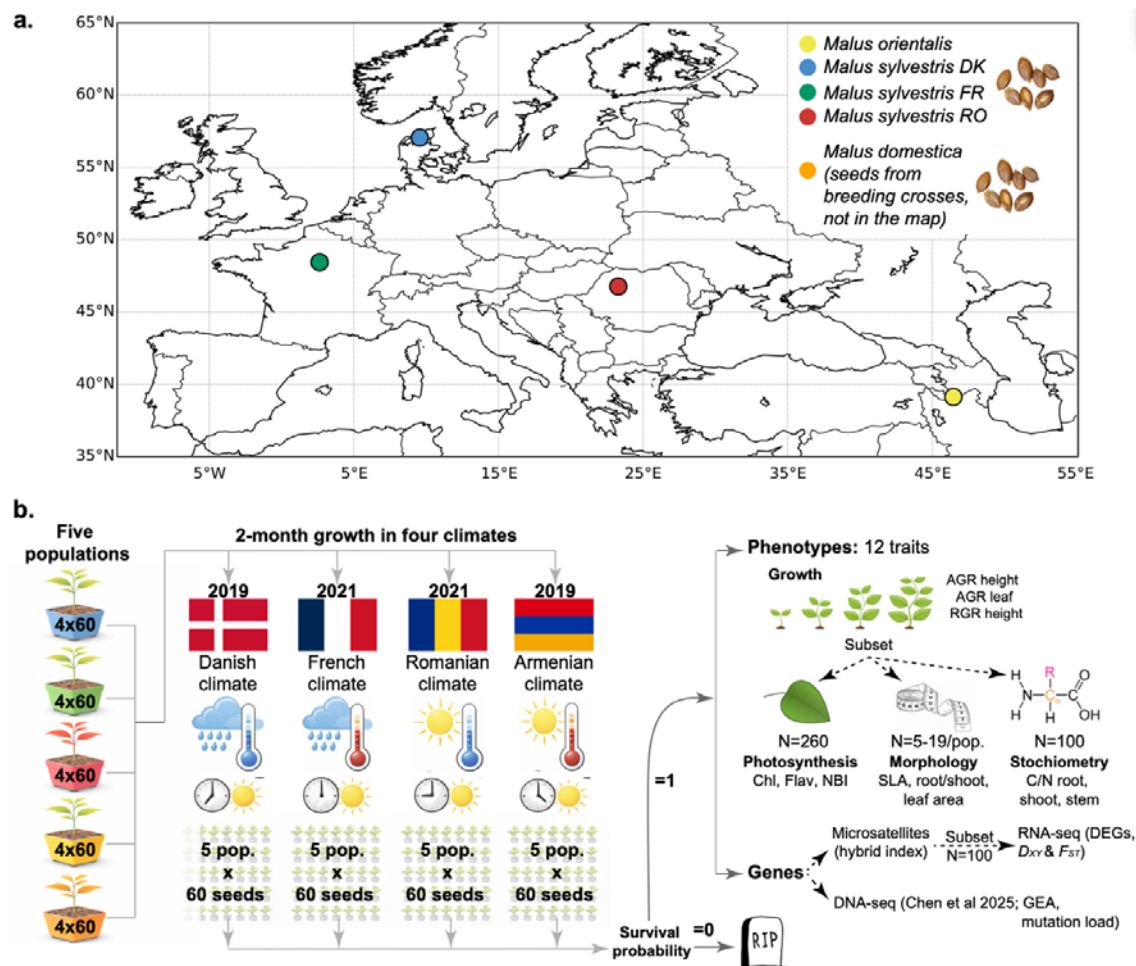


Figure 1. Experimental design of the reciprocal climate chamber experiment and overview of phenotypic and molecular measurements in wild and cultivated apple seedlings. **a.** Geographic origin of the apple populations studied: Wild Caucasian apple (*M. orientalis*), and three European wild apple populations of *M. sylvestris* from Denmark (DK), France (FR), Romania (RO). The cultivated apple (*Malus domestica*) seeds originated from controlled breeding crosses and are not represented on the map. **b.** Overview of the experimental design and associated phenotypic and molecular data. Seedling survival was monitored over the 2-month experiment. Twelve phenotypic traits were measured across four functional categories: growth (absolute and relative growth rates of height and leaf number), photosynthesis (chlorophyll, flavonoids, nitrogen balance index; $N = 260$), morphology (specific leaf area, root/shoot ratio, leaf area; destructively sampled subset), and stoichiometry (C/N ratios of roots, shoots, and stems; $N = 100$). Genetic data included microsatellite genotyping of wild seedlings to estimate hybrid index, whole-genome resequencing of *M. sylvestris* populations (Chen et al. 2025) for genome–environment association mutation load and, and RNA sequencing ($N = 100$) to characterize differential

1075 gene expression (DEGs), gene divergence (D_{XY} and F_{ST}). Dashed arrows indicate subset
1076 relationships among datasets.

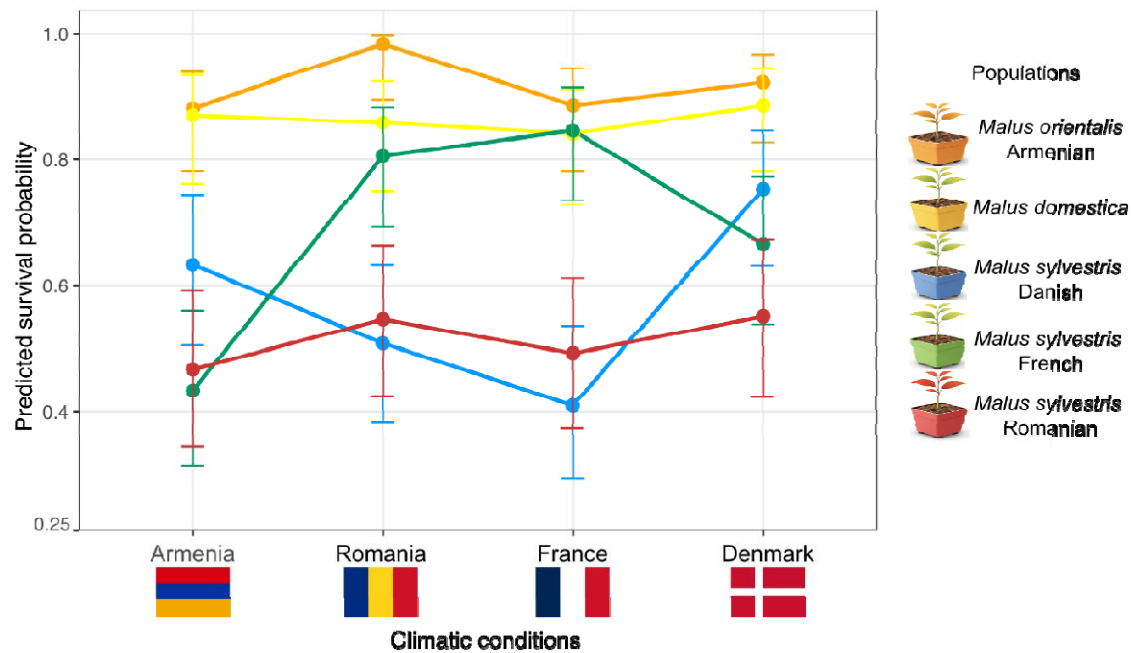


Figure 2. Climate drives early-stage survival in apple seedlings. Population-specific survival responses across climatic conditions. Predicted survival probabilities ($\pm 95\%$ CI) at day 61 for five populations (*M. domestica*, *M. orientalis*, and three *M. sylvestris* populations from France, Denmark, and Romania) across four simulated climatic conditions. Estimates are derived from the mother-aggregated binomial model (Table S2 and Supporting Information S1).

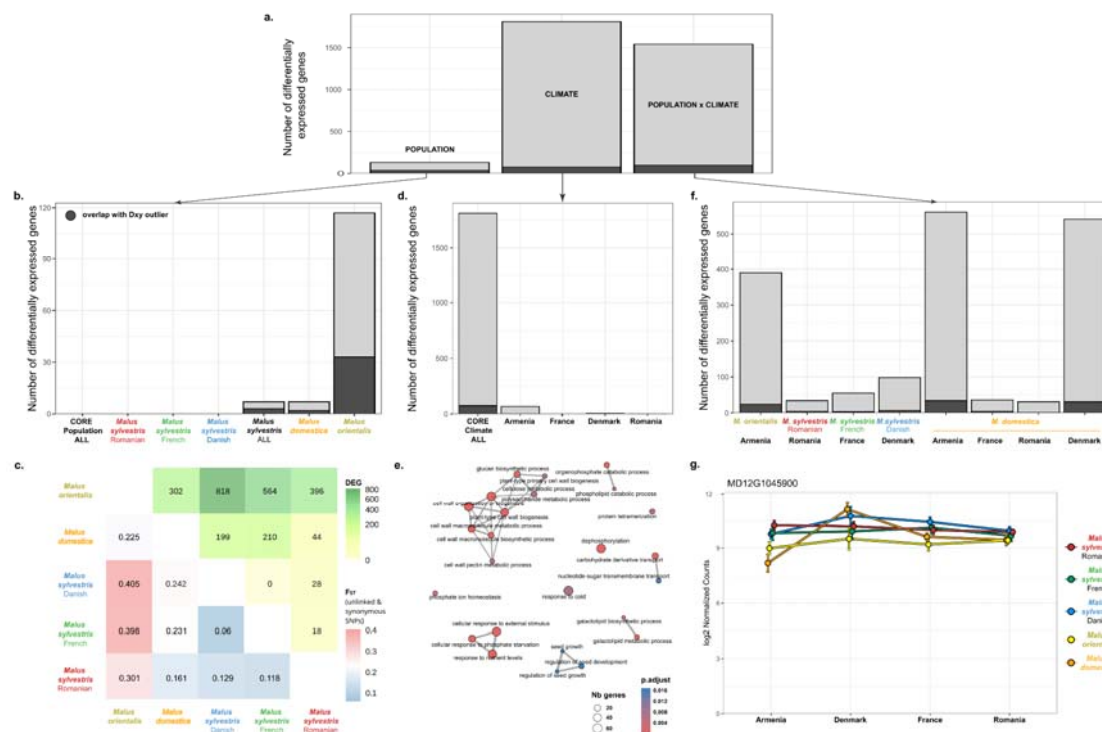


Figure 3. Population and climate effects on gene expression, and underlying adaptive variation in wild and cultivated apples. (a) Number of differentially expressed genes (DEGs) attributed to the fixed effects of population, climate, or their interaction. Dark gray indicates the proportion of DEGs that overlap with D_{XY} outliers, highlighting genomic divergence. (b) Number of population-specific DEGs per taxon, with overlaps to D_{XY} outliers indicated in dark gray. (c) Heatmap of pairwise population F_{ST} (bottom triangle, computed using 468,714 synonymous and unlinked SNPs) and number of population-specific DEGs (top triangle) between populations. (d) Number of DEGs showing a consistent response to climate across all populations (core climate DEGs), and number of DEGs specific to each climate (Armenia, France, Denmark, Romania). Dark gray indicates the proportion of DEGs that overlap with D_{XY} outliers. (e) Gene Ontology (GO) term enrichment map of the 1,810 core-climate DEGs, showing significant functional enrichment in nutrient response, starvation, cell wall biogenesis, and regulation of seed development. Node size reflects the number of DEGs associated with each GO term; color reflects adjusted p -values. (f) Number of DEGs showing a population-specific response to climate (population $\times$ climate interaction), per taxon and climate. Dark gray bars denote overlap with D_{XY} outliers, suggesting possible local adaptation. (g) Expression profile of MD12G1045900 across all simulated climate conditions and populations.

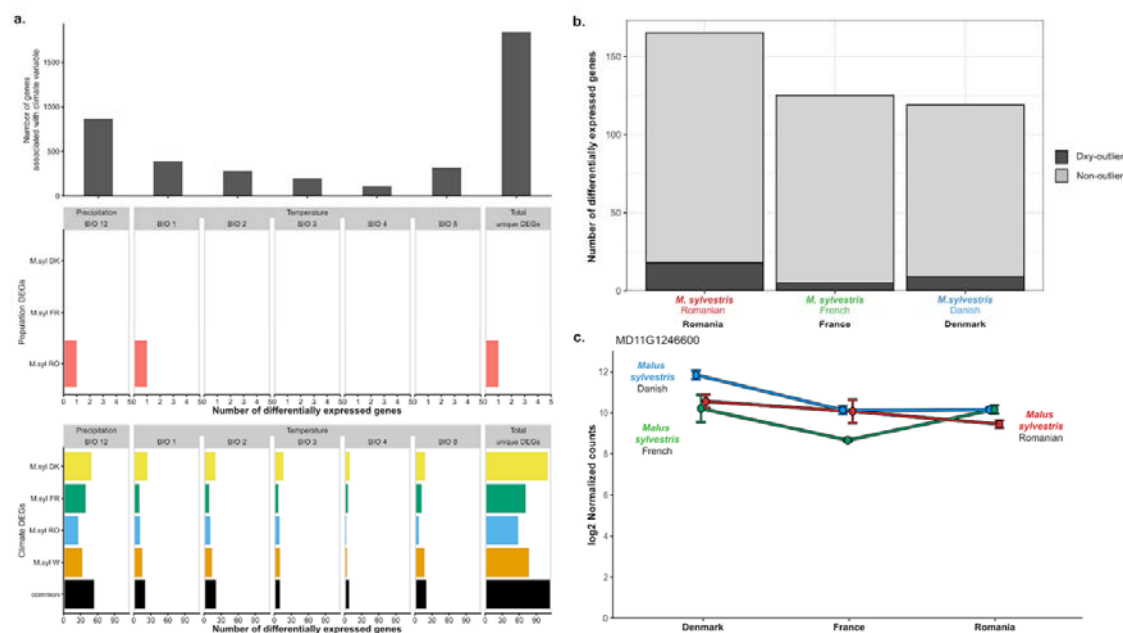


Figure 4. Genomic basis of climate adaptation in *Malus sylvestris*. (a) Intersection between gene×environment associations (GEAs) and differential expression analysis across bioclimatic variables and differentially expressed gene (DEG) categories in *M. sylvestris*. Top panel, number of genes significantly associated with each climatic variable (bioclim; $p < 0.001$) based on redundancy GEA. The strongest associations were detected for precipitation-related variable BIO12, followed by temperature-related variables. Middle panel, proportion of population-specific DEGs in *M. sylvestris* overlapping with genes associated with each bioclim variable. Bottom panel, proportion of climate DEGs overlapping with GEA-identified genes for each bioclim variable. (b) Number of DEGs showing a potential local adaptation pattern. Dark gray bars show the proportion of D_{XY} outliers. (c) Plot of log₂ normalized expression profile of MD11G1246600 for each population and climate. Values are shown as log₂-normalized counts. Each point represents the mean $\pm$ standard error. Colors represent the population.

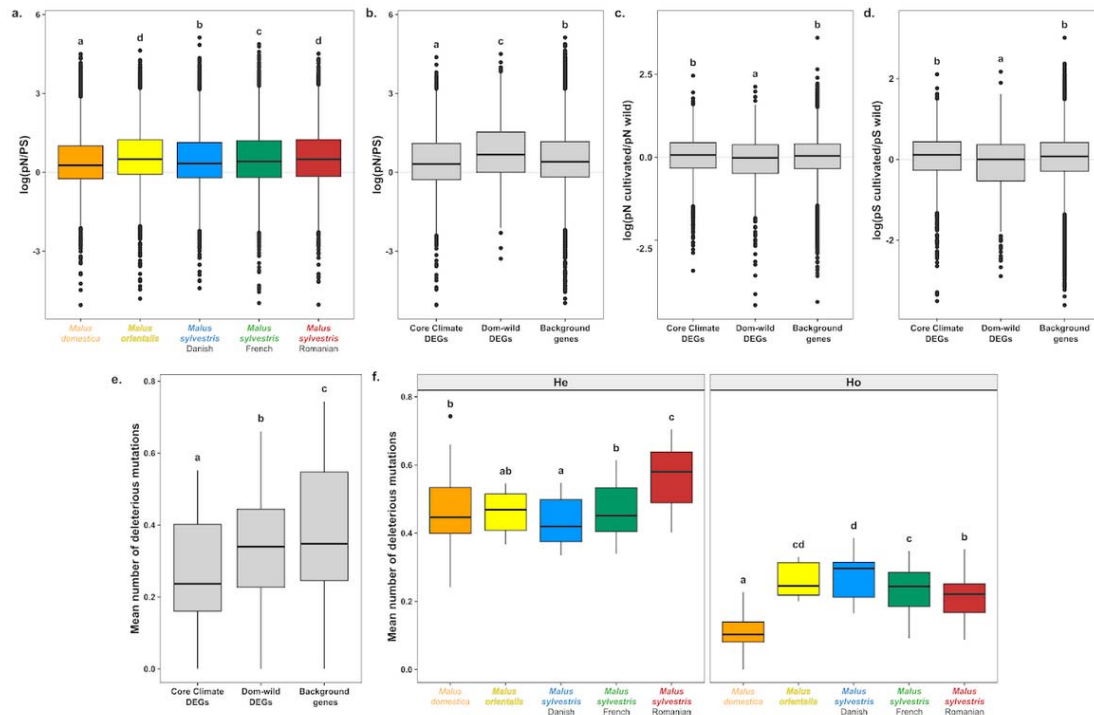


Figure 5. Patterns of coding sequence evolution and deleterious mutation load across gene categories and apple populations.

(a, b) Ratio of nonsynonymous to synonymous polymorphisms ($\log[\pi N/\pi S]$) across (a) five apple populations (color-coded) or (b) three gene categories, i.e., background genes, core climate-responsive differentially expressed genes (DEGs) (Core climate DEGs), and DEGs distinguishing between domesticated and wild apples (Dom-wild DEGs). (c, d) Log-transformed ratios of nucleotide diversity between domesticated and wild populations, shown for (c) nonsynonymous (πN) or (d) synonymous (πS) polymorphisms, across the three gene categories. (e, f) Accumulation of deleterious mutations per gene across populations and gene categories: (e) mean number of deleterious mutations per individual per gene for each gene category, separated by genotype at the deleterious allele (heterozygous, He ; homozygous, Ho). Different lowercase letters indicate significant differences, as determined by Tukey's pairwise comparisons following a linear model ($p < 0.05$). (f) mean number of deleterious mutations per individual per gene across the five apple populations.

1132 Tables

1133 **Table 1. Effects of population, climate condition, and their interaction on the variation of the 12 phenotypic traits in apple seedlings**
 1134 **(Linear mixed model).** A total of 894 seedlings were grown across four ecotron climate chambers representing simulated climate conditions for
 1135 Armenia, Denmark, France, and Romania, with experiments conducted in two phases: 2019 (Armenia and Denmark conditions) and 2021
 1136 (France and Romania conditions). Seedlings came from five population groups: cultivated *Malus domestica*, wild *M. orientalis* (Armenia), and
 1137 *M. sylvestris* from Denmark, France, and Romania. The most appropriate model of residual variance structure is indicated as either HomVar
 1138 (homogeneous variance) or HetVarC (heterogeneous variance by condition). *F*-values and associated degrees of freedom (Df, denDF) are
 1139 reported for each fixed effect (population, condition, and their interaction). Statistically significant results are marked: $p < 0.05^*$, $< 0.01^{**}$, $<$
 1140 0.001^{***} . Model-based residual variance corrections are listed in the final column.

Variable	N	Population				Condition				Population : Condition				Model c hosen	Weight model correction
		Df	denDF	F value	Pr(>F)	D f	denD F	F value	Pr(>F)	Df	denDF	F valu e	Pr(>F)		
AGR height	893	4	48	11.131	<0.0001**	3	301	80.956	<0.0001* **	—				HetVarC	Condition
RGR height	872	4	48	25.175	<0.0001**	3	283	995.479	<0.0001* **	12	283	2.25 1	0.0099**	HetVarC	Condition
AGR leaf	705	4	48	4.317	0.0046**	3	215	27.249	<0.0001* **	12	215	8.43 2	<0.0001* **	HetVarC	Condition
Chloroph yll	256	4	48	6.001	0.0005***	3	30	16.167	<0.0001* **	12	30	2.72 1	0.0129*	HomVar	—

Flavonoids	256	4	48	4.309	0.0047	3	42	13.782	<0.0001***	–	HetVarC	Condition
NBI	250	4	48	4.1752	0.0056**	3	41	15.529	<0.0001***	–	HetVarC	Condition
C/N roots	100	4	38	0.467	0.7594	3	13	9.199	0.0016**	–	HomVar	–
C/N leaves	99	4	37	1.6789	0.1756	3	13	18.228	0.0001**	–	HomVar	–
C/N stem	99	4	37	0.6957	0.5998	3	13	14.878	0.0002**	–	HomVar	–
Root/shoot	248	4	47	2.7661	0.0381*	3	56	24.996	<0.0001***	–	HomVar	–
leaf area	283	4	48	12.6189	<0.0001**	3	57	27.168	<0.0001***	–	HomVar	–
SLA	283	4	48	2.494	0.552	3	58	7.776	0.0002**	–	HetVarC	Condition

1141

1142 AGR height, absolute growth rate of seedling height (cm/day); RGR height, relative growth rate of height, standardized by initial size; AGR leaf,
1143 absolute growth rate for number of leaves; Chlorophyll, leaf chlorophyll content, as proxy for photosynthetic capacity; Flavonoids, leaf
1144 flavonoid content, an indicator of stress response; NBI , nitrogen balance index, calculated as the ratio of chlorophyll to flavonoids; C/N roots,
1145 C/N leaves, C/N stem , carbon-to-nitrogen ratio in root, leaf, and stem tissues, respectively; Root/shoot , biomass allocation ratio between root
1146 and shoot; leaf area, total surface area of leaves per plant; SLA , specific leaf area (cm²/g), indicator of leaf morphology and growth strategy.

1147 **Table 2. Stability of early-stage phenotypic traits in wild apple populations assessed using Finlay–Wilkinson regression.** Regression
1148 intercepts and slopes ($\pm$ standard errors) from Finlay–Wilkinson regressions applied to early developmental traits of wild apple populations,
1149 including *Malus orientalis* (Armenia) and *M. sylvestris* from Denmark, France, and Romania. *Malus domestica* was excluded from this analysis
1150 due to insufficient replication of maternal genotypes across climate conditions. For each trait (excluding survival), values were first averaged per
1151 mother tree and then regressed against the mean trait performance of all individuals within each simulated climate condition (Armenia,
1152 Denmark, France, and Romania). The regression intercept represents the average genotypic performance across environments, while the slope
1153 quantifies phenotypic plasticity, expressed as sensitivity to environmental quality. A slope close to 1 indicates average responsiveness and stable
1154 trait expression across climates; slopes significantly greater than 1 indicate above-average plasticity and heightened environmental sensitivity,
1155 whereas slopes lower than 1 indicate reduced responsiveness or canalized trait expression.

Species	<i>M. orientalis</i>		<i>M. sylvestris</i> (Denmark)		<i>M. sylvestris</i> (France)		<i>M. sylvestris</i> (Romania)	
Trait	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
AGR height	0.105 $\pm$ 3.78	0.981 $\pm$ 0.57	1.387 $\pm$ 4.528	0.732 $\pm$ 0.755	1.057 $\pm$ 7.477	0.927 $\pm$ 1.298	5.323 $\pm$ 9.558	0.219 $\pm$ 1.283
RGR height	0 $\pm$ 0.003	1.006 $\pm$ 0.106	0.001 $\pm$ 0.005	0.989 $\pm$ 0.218	– 0.005 $\pm$ 0.023	1.237 $\pm$ 1.106	0.001 $\pm$ 0.002	0.978 $\pm$ 0.129
AGR leaf	– 0.021 $\pm$ 0.088	1.075 $\pm$ 0.358	0.006 $\pm$ 0.35	1 $\pm$ 1.655	– 0.007 $\pm$ 0.274	1.023 $\pm$ 1.132	– 0.076 $\pm$ 0.221	1.362 $\pm$ 0.94
Chlorophyll	– 0.12 $\pm$ 6.517	1.006 $\pm$ 0.336	3.776 $\pm$ 25.856	0.852 $\pm$ 1.174	0.237 $\pm$ 8.611	0.992 $\pm$ 0.371	1.436 $\pm$ 5.33	0.936 $\pm$ 0.281
Flavonoids	– 0.102 $\pm$ 2.432	1.069 $\pm$ 1.633	0.66 $\pm$ 1.87	0.516 $\pm$ 1.259	0.04 $\pm$ 0.74	0.98 $\pm$ 0.505	– 0.353 $\pm$ 1.883	1.22 $\pm$ 1.134

NBI	- 0.051 ± 35.911	1.003 ± 1.455	6.433 ± 84.518	0.731 ± 3.582	- 9.157 ± 62.667	1.365 ± 2.463	5.958 ± 47.952	0.736 ± 2.088
C/N stem	- 32.835 ± 72.217	1.344 ± 0.771	- 4.736 ± 17.57	1.167 ± 0.23	7.27 ± 15.239	0.897 ± 0.234	2.817 ± 81.187	1.057 ± 1.102
C/N leaf	- 5.62 ± 28.099	1.081 ± 0.615	- 0.155 ± 19.367	1.003 ± 0.469	3.878 ± 8.857	0.899 ± 0.297	9.056 ± 36.618	0.806 ± 1.075
C/N root	- 1.219 ± 25.472	1.032 ± 0.385	- 4.098 ± 4.68	1.082 ± 0.054	- 7.421 ± 24.333	1.108 ± 0.378	23.655 ± 61.743	0.636 ± 1.136
Root/Shoot	- 0.132 ± 0.222	1.289 ± 0.574	0.153 ± 0.339	0.706 ± 0.415	0.076 ± 0.489	0.864 ± 0.75	0.07 ± 0.131	0.863 ± 0.241
Leaf area	0.862 ± 7.567	0.893 ± 1.136	1.06 ± 5.442	0.868 ± 0.849	0.027 ± 2.782	0.957 ± 0.661	3.713 ± 3.053	0.531 ± 0.437
SLA	11.838 ± 129.041	0.884 ± 1.235	18.29 ± 56.4	0.833 ± 0.642	39.405 ± 142.132	0.626 ± 1.291	20.367 ± 28.644	0.84 ± 0.25

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1157 AGR height, absolute growth rate of seedling height (cm/day); RGR height, relative growth rate of height, standardized by initial size; AGR leaf,
1158 absolute growth rate for number of leaves; Chlorophyll, leaf chlorophyll content, as proxy for photosynthetic capacity; Flavonoids, leaf
1159 flavonoid content, an indicator of stress response; NBI, nitrogen balance index, calculated as the ratio of chlorophyll to flavonoids; C/N stem,
1160 C/N leaf, C/N root: carbon-to-nitrogen ratio in stem, leaf, and root tissues, respectively; Root/Shoot: biomass allocation ratio between root and
1161 shoot; Leaf area: total surface area of all leaves per plant; SLA, specific leaf area (cm²/g), indicator of leaf thickness and growth strategy.

