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Genome-wide assessment of *Quercus pedunculiflora* differentiation within the *Quercus robur* (Fagaceae) species complex—implications for forest management in Central-Eastern Europe

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ABSTRACT

Climate change is expected to increase drought stress across large parts of Central Europe, raising the question of whether southern oak populations may represent valuable genetic resources for future climate-adaptive forest management. In this exploratory study, we investigated genomic relationships among *Quercus robur* (pedunculate oak), *Q. pedunculiflora* (greyish oak), and morphologically transitional individuals sampled from Bulgaria, Romania, and Hungary using low-coverage whole-genome resequencing. Genome-wide analyses revealed weak but consistent nuclear genomic differentiation within the *Q. robur* complex, consistent with the recognition of *Q. pedunculiflora* as a differentiated lineage. Within the limitations of the present sampling design, this pattern is compatible with subspecific rank rather than treatment as a separate species or a simple ecological variant of *Q. robur*. No genomic evidence was found for the occurrence of *Q. pedunculiflora* in the sampled Hungarian material, including individuals previously assigned to this taxon based on morphology. Phylogenomic analyses indicated heterogeneous chromosome-level signals, while morphologically transitional individuals did not form a separate genomic lineage. Analyses of 21 previously identified drought-responsive candidate genes showed substantial overlap in normalised mapped dosage between taxa and geographic groups, with no statistically supported differences. Together, these results provide no evidence that systematic dosage differences in the examined drought-responsive candidate genes underline the observed genomic differentiation. From an applied perspective, Balkan oak populations represent candidates for further evaluation in provenance and assisted gene-flow trials, rather than genetically demonstrated sources of drought-adaptive variation for immediate operational deployment.

1. Introduction

Oaks (the genus *Quercus* L.) comprise approximately 435 species across the Northern Hemisphere and represent one of the most ecologically and economically important forest tree groups (Aldrich and Cavender-Bares, 2011; Hipp, 2024; Kremer and Hipp, 2020; Menitsky, 1984; Nixon, 1993). Species delimitation within the genus is challenging due to extensive phenotypic plasticity, frequent hybridisation, and ongoing gene flow, resulting in weak genetic differentiation among closely related taxa (Gailing and Curtu, 2014; Gerber et al., 2014; Hipp et al., 2020; Leroy et al., 2020; Semerikova, 2025). Oaks are therefore often considered a paradigmatic syngameon, in which species boundaries remain permeable despite persistent lineage cohesion (Hardin, 1975; Hipp et al., 2019; Hipp, 2024; Ribicoff et al., 2025). The syngameon may contribute to their evolutionary success by combining substantial lineage divergence with episodic inter-lineage gene flow, thereby facilitating adaptation across heterogeneous environments (Cannon and Petit, 2020; Hipp et al., 2020; Hipp, 2024; Kremer and Hipp, 2020; Leroy et al., 2020).

Advances in next-generation sequencing have enabled high-resolution genomic analyses of oak evolution (Hipp et al., 2014, 2019; Leroy et al., 2020; McVay et al., 2017; Morales-Saldaña et al., 2024). Additionally, studies indicate that genomic differentiation is heterogeneous: most regions remain weakly differentiated and relatively

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permeable to gene flow, whereas a smaller subset of loci shows strong divergence, consistent with the action of selection (Guichoux et al., 2013; Scotti-Saintagne et al., 2004). The availability of chromosome-scale genome assemblies for several oak species has further improved our understanding of genome evolution and phylogenomic discordance within the genus. For example, studies on *Quercus mongolica* (Ai et al., 2022), *Q. gilva* (Zhou et al., 2022) and North-American white oaks (Ribicoff et al., 2025) have demonstrated that different genomic regions may retain distinct evolutionary histories as a result of incomplete lineage sorting and historical introgression. Moreover, whole-genome screening approaches have proven effective in identifying near-diagnostic genetic markers and clarifying species boundaries within both the American (Hipp et al., 2019; Ribicoff et al., 2025) and the European white oak complex (Kremer et al., 2024).

Quercus robur L., a key European member of the white oak species complex, is a dominant lowland tree species in Europe, with high ecological and economic importance and exceptional associated biodiversity, and is widely regarded as a keystone species supporting a large number of dependent organisms (Csóka and Ambrus, 2016; Hung et al., 2025; Mölder et al., 2019). However, these ecosystems are increasingly threatened by climate change, especially towards the southern margin, particularly by drought (Aitken et al., 2008). In Central-Eastern Europe, including Hungary, the long-term persistence of lowland and drought-exposed *Q. robur* stands in the southern part of its distribution range is increasingly uncertain, prompting the search for alternative or complementary taxa suitable for future conditions (Bartha et al., 2018). Identifying genetically suitable source populations has therefore become a key objective in climate-adaptive forest management (Benke et al., 2022). In this context, populations originating from south-eastern Europe, particularly from the Balkan Peninsula, have gained increasing attention as potential sources of adaptive genetic variation due to their exposure to warmer and drier climatic conditions. However, the introduction of more southerly lineages should be approached cautiously, as a large-scale population genetic study of Turkey oak (*Quercus cerris*) revealed multiple genetic groups across Central and Southeast Europe, indicating that long-distance transfers of reproductive material may result in admixture between genetically differentiated groups (Lados et al., 2026). Genetic assessment of potential source populations should therefore precede their consideration for forest management applications.

Quercus pedunculiflora K. Koch is a taxon closely related to *Q. robur*. Their taxonomic relationship is confusing: *Q. pedunculiflora* has sometimes been treated as an ecotype (Doniță et al., 2003; Menitsky, 1971) or as a subspecies (Böhlmann, 2015; Menitsky, 1984) of *Q. robur*, but it is more often regarded as a separate species (Apostol et al., 2017; Georgescu and Morariu, 1948; Koch, 1849; Mátyás, 1967; Schwarz, 1937). *Q. pedunculiflora* is morphologically very similar to *Q. robur*, but certain leaf indumentum characteristics (Apostol, 2019; Enescu, 2017; Hegedüs et al., 2025) and a single subtle but diagnostic morphological character, namely the apex of the acorn's cupule scales (Georgescu and Morariu, 1948; Hegedüs and Bartha, 2025), distinguish the two taxa. Genetic evidence indicates very low differentiation, an absence of diagnostic alleles, and extensive sharing of chloroplast haplotypes between these taxa, suggesting incomplete lineage sorting and ongoing gene flow (Curtu et al., 2011). These patterns are consistent with an early stage of divergence within the European white oak complex (Curtu et al., 2007). In such systems, we expect divergence to affect only part of the genome, while extensive ancestral polymorphism and ongoing introgression maintain broad genetic similarity between taxa.

More pronounced is the difference in habitat preference. The literature describes *Q. pedunculiflora* as a taxon adapted to warmer and drier environments in south-eastern Europe and western Asia (Apostol, 2019; Enescu, 2017), where its distribution partly overlaps with that of *Q. robur*. Its range spans the north-eastern part of the Balkan Peninsula, the lowland regions around the Caucasus, and parts of Asia Minor, extending as far southeast as north-western Iran (Fig. 1), where it occupies sites with lower water availability than *Q. robur* (Georgescu and Morariu, 1948; Schwarz, 1937), and the two show mutual ecological vicariance in sympatry (Yilmaz and Yilmaz, 2016). The northwestern range limit of *Q. pedunculiflora* lies directly adjacent to the Pannonian Basin, where oaks are major components of similar lowland habitats (Bohn et al., 2000–2003). Given the morphological similarity and habitat availability, its occurrence in Hungary has been suggested but remains unconfirmed (Gencsi and Vancsura, 1992; Pintér et al., 2025). As distinct oak lineages reflect multiple colonisation routes in Central and Southeastern Europe (Bordács et al., 2002), the presence of

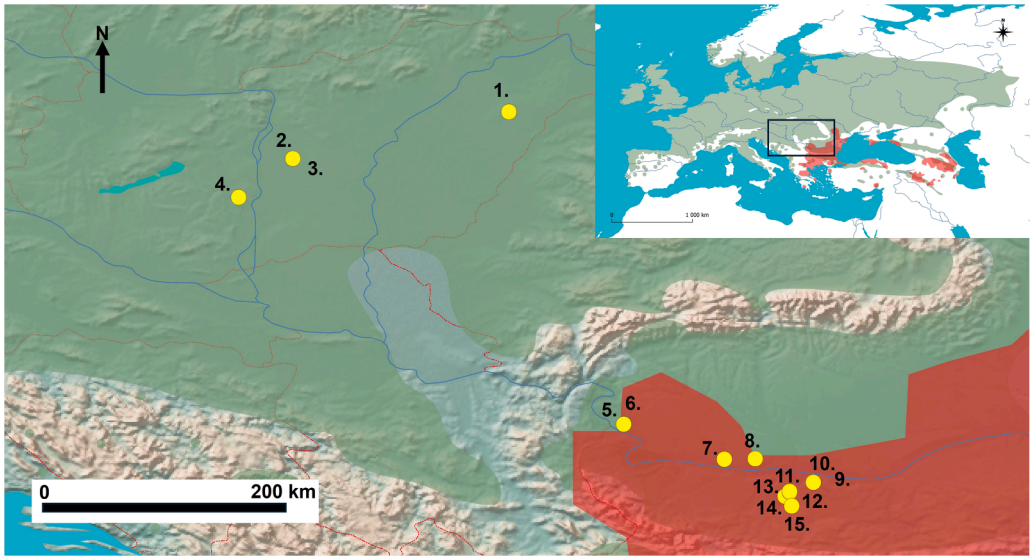


Fig. 1. Sampling locations and distribution ranges of the studied *Quercus* taxa. Yellow dots indicate the sampling locations included in this study. The green shaded area represents the distribution range of *Quercus robur* based on EUFORGEN data, whereas the red shaded area indicates the reported distribution range of *Quercus pedunculiflora* based on Jalas and Souminen (1976) and Menitsky (1984). Some peripheral occurrences, particularly in N Serbia, remain insufficiently confirmed and should be regarded as tentative. The inset map highlights the overall distribution of the studied taxa in Europe and adjacent regions. Localities on the map: 1. HU: Debrecen: Nagyerdő: Medve-sarok; 2–3. HU: Kunpeszér; 4. HU: Bikács 65/C; 5–6. RO: Poiana Gruii; 7. RO: Piscu Sadovei; 8. RO: Câmpu Părului; 9–10. BG: Muselievo; 11–12. BG: Komarevo to Podem roadside; 13. BG: Riben; 14. BG: Bivolare: Vit river plain; 15. BG: Pleven: public park.

Q. pedunculiflora in the Pannonian Basin as a relict is also plausible, including tentative reports from the northern Banat region of Serbia (Josifović, 1970).

Climate change is progressing rapidly and is accompanied by an increasing frequency of extreme weather events (IPCC, 2021). In Central-Eastern Europe, projections indicate that summers will become particularly hot and dry (Bartholy et al., 2014). One possible silvicultural response to these changes is the increased use of tree species that are better adapted to drought, potentially replacing species that are less resilient under future climatic conditions (Keenan, 2015). In this context, *Q. robur* often occurs near the xeric edge of its ecological tolerance and is therefore particularly susceptible to prolonged drought periods (Bartha et al., 2018; Mátyás et al., 2018). However, rather than replacing indigenous tree species, climate-smart and ecologically sensitive forestry may employ assisted gene flow to mitigate the effects of climate change while maintaining native forest components (Aitken et al., 2008; Aitken and Whitlock, 2013; Kurz et al., 2023). Because long-lived species such as forest trees may be especially vulnerable to rapid climatic change owing to their relatively inflexible climatic niches (Aitken and Bemmels, 2015; Tischenko et al., 2026), populations of *Q. pedunculiflora* from warmer and drier parts of its range may represent potential sources for experimental assisted gene flow involving *Q. robur* (Hegedüs et al., 2025).

Nevertheless, because the presence of *Q. pedunculiflora* cannot be fully excluded in the Pannonian Basin, in the present study, we aimed to genomically characterise Hungarian and northern Balkan individuals of *Q. robur* and *Q. pedunculiflora* using low-coverage whole-genome resequencing to assess genomic differentiation within the complex and to investigate the genomic identity of the sampled Hungarian individuals. Genomic approaches are especially valuable because they enable genetic variation potentially associated with adaptive traits to be investigated at the level of genes and genomic regions (Leroy et al., 2020; Wang et al., 2025). In forest trees, drought adaptation is typically polygenic and involves coordinated physiological responses (Kremer and Le Corre, 2012; Müller and Gailing, 2019). Among candidate genes, dehydrins play a key role in stress responses and variation at these loci has previously been investigated in Romanian oak species. Variation at such loci can show species-specific patterns even where overall genomic differentiation is low (Crăciunesc et al., 2015). In this study, we applied whole-genome sequencing to individuals of *Q. robur, Q. pedunculiflora*, and morphologically intermediate individuals from Bulgaria, Romania, and Hungary. Our specific aims were to (i) reconstruct phylogenetic relationships using plastid and nuclear genomic data, (ii) explore how genetic structure relates to geographic origin and taxonomic assignment, and (iii) analyse variation in previously identified drought-responsive candidate genes. This was all done to support forestry management with actual recommendations on the potential need for assisted gene flow, facilitating drought-responsive gene transfer from *Q. pedunculiflora* to Pannonian *Q. robur*.

2. Methods

2.1. Plant material, sampling design and DNA extraction

Building upon our previous leaf morphometric study (Appendix A), we revisited 14 individual oak trees that had previously been examined for diagnostic leaf traits and collected dormant bud samples from these individuals in autumn 2025. In addition, one specimen was collected from the Bikács forest (Hungary), a mixed oak stand located at the xeric limit of forest-steppe vegetation, where *Quercus pedunculiflora*-like morphotypes have previously been reported, likely reflecting hybridisation and introgression processes (Pintér et al., 2025). In total, 15 individual trees were sampled across multiple localities in Bulgaria, Romania and Hungary (Fig. 1). Sampling sites comprised isolated trees, roadside individuals, urban park trees, and small local stands rather than demographically defined populations. Accordingly, throughout this study, we refer to sampling sites or trees rather than populations (Table 1) and to regional genetic structure rather than population structure. The genomic sampling was therefore designed as an exploratory comparison of individuals representing contrasting morphotypes and geographic origins rather than as a representative population-genetic survey. Regarding specific nomenclature during this paper, we follow a pragmatic framework in which *Q. pedunculiflora* is treated as part of the *Q. robur* complex, while recognising that its precise rank remains debated. Although *Q. pedunculiflora* has traditionally been treated as a separate species, previous morphological and genetic evidence have also supported its interpretation as *Q. robur* subsp. *pedunculiflora* (Menitsky, 1984).

Individuals showing intermediate leaf morphology were classified as morphologically transitional (*Quercus* "transitional"). These individuals exhibited simple uniseriate trichomes and stellate trichomes on the abaxial leaf surface, while *Q. robur* only displays the former and *Q. pedunculiflora* additionally the fasciculate type of trichome. For each individual, approximately 10–15 dormant buds were collected and stored in silica gel until DNA extraction. Genomic DNA was extracted following the cetyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987) at the laboratory of the Evolutionary Genomics Research Group, University of Debrecen. The extracted DNA was purified using AMPure XP magnetic beads (Beckman Coulter Inc., Brea, CA, U.S.A.). DNA concentration and quality were assessed using a NanoDrop Lite Plus spectrophotometer (Thermo Scientific Inc., Waltham, MA, U.S.A.) and a Qubit 3 fluorometer (Thermo Scientific Inc.), and DNA integrity was verified by agarose gel electrophoresis. High-quality DNA extracts were sent for whole-genome shotgun sequencing provided by a commercially available service provider (Novogene Europe GmbH, Munich, Germany).

2.2. Sequencing and raw data quality filtering

Whole-genome sequencing libraries were prepared and sequenced using Illumina paired-end 150 bp technology. Sequencing generated a total of approximately 201.4 Gb of raw data across all samples, with an average output of ca 10 × genome coverage per individual. Initial quality assessment of raw reads was performed using FastQC v0.12.1 (Andrews, 2010). Reads containing adapter contamination, >10% ambiguous bases (N), or >50% low-quality bases ($Q \leq 5$) were removed by using fastp v1.0.1 (Chen et al., 2018). Clean reads exhibited consistently high quality, with Q20 values exceeding 98% across all samples and low estimated sequencing error rates.

2.3. Plastome assembly, annotation and phylogenetic analysis

Plastome genomes were assembled from quality-filtered reads using GetOrganelle v1.7.7.1 (Jin et al., 2020). Reference-guided assembly was performed using an annotated and curated *Quercus robur* plastome (GenBank acc. nr.: NC_046388) following the default settings recommended by the author of the tool. For plastome-based phylogenomic analyses, two outgroup taxa were included to provide a stable rooting framework. The resulting phylogenomic tree was rooted using *Quercus cerris* L. (OY770020) as a representative of subgenus *Cerris* (Denk et al., 2017), while the American *Quercus lobata* Née (CM012305; Sork et al., 2016) was included as a close outgroup from subgenus *Quercus* section *Quercus* to improve resolution of deeper relationships within the genus.

Circular plastomes were verified, and their annotation was performed using PGA v1.0 (Qu et al., 2019) using the above *Quercus robur* plastome (NC_046388) as a reference. Where required, the assembled circular plastomes were reverse-complemented and reoriented to a consistent starting position based on the *rps*12 gene. To avoid redundancy due to inverted repeat regions, one copy of the inverted repeat (IR) was masked based on annotation-derived coordinates. The restarted and oriented whole-plastome (excl. one copy of the IR) sequences were aligned using MAFFT v7.525 (Katoh and Standley, 2013). To account for

Table 1
Geographic origin, taxonomic assignment, and sampling details of the studied Quercus individuals. Taxonomic assignment follows the morphometric results published in Hegedüs et al. (2025), except sample K5169, which is based on expert opinion (S. Bordács). Numbers after the location refer to location numbers on Fig. 1.

ID code	Taxon	Location	Coordinate (N)	Coordinate (E)	Altitude (m)	Sampling date
K5169	*Quercus pedunculiflora*	HU: Bikács 65/C (4)	46.676530	18.738688	153	07.10.2025
K5171	*Quercus robur*	BG: Bivolare: Vit river plain (14)	43.493448	24.552942	51	11.10.2025
K5173	*Quercus robur*	BG: Riben (13)	43.537267	24.603578	36	11.10.2025
K5175	*Quercus* "transitional"	BG: Komarevo to Podem roadside (11)	43.545115	24.601795	45	11.10.2025
K5177	*Quercus pedunculiflora*	BG: Komarevo to Podem roadside (12)	43.545060	24.601782	45	11.10.2025
K5179	*Quercus robur*	BG: Muselievo (9)	43.641560	24.853717	31	11.10.2025
K5181	*Quercus pedunculiflora*	BG: Muselievo (10)	43.641698	24.853683	31	11.10.2025
K5183	*Quercus robur*	BG: Pleven: public park (15)	43.390664	24.623192	122	11.10.2025
K5185	*Quercus robur*	RO: Piscu Sadovei (7)	43.887762	23.906685	42	12.10.2025
K5187	*Quercus pedunculiflora*	RO: Poiana Gruii (6)	44.263565	22.837422	103	12.10.2025
K5189	*Quercus* "transitional"	RO: Poiana Gruii (5)	44.261126	22.835696	94	12.10.2025
K5191	*Quercus pedunculiflora*	RO: Câmpu Părului (8)	43.893405	24.235120	110	12.10.2025
K5193	*Quercus robur*	HU: Kunpeszér (3)	47.087965	19.315031	104	23.10.2025
K5195	*Quercus robur*	HU: Kunpeszér (2)	47.088022	19.315073	104	23.10.2025
K5197	*Quercus robur*	HU: Debrecen: Nagyerdő: Medve-sarok (1)	47.586198	21.613947	126	23.10.2025

the phylogenetic noise introduced by highly variable intergenic spacers, we curated the whole-plastome alignment to remove poorly aligned regions using BMGE v1.12 (Criscuolo and Gribaldo, 2010) using default settings. Maximum likelihood phylogenetic inference was performed using IQ-TREE v2.4.0 (Minh et al., 2020) with model selection (MFP) on an unpartitioned matrix. The robustness of the phylogenetic tree was assessed in 100 non-parametric bootstrap replicates (Felsenstein, 1985) and was mapped onto the best-scoring tree.

2.4. Genome-wide SNP calling and population genomic analyses

Quality-filtered reads were mapped onto the Quercus robur reference genome (dhQueRobu3.1; Bordénès et al., 2016; Plomion et al., 2018) available in GenBank (GCA_932,294,415.1) using BWA-MEM2 v2.2.1 (Li and Durbin, 2009; Vasimuddin et al., 2019) using default settings. Variants were called jointly across all samples using FreeBayes v1.3.10 (Garrison and Marth, 2012) for each chromosome (*N.B.* cytoplasmic regions excluded) separately. In each variant file containing raw calls, bcftools v1.22 (Danecek et al., 2011) was used to retain high-quality sites (QUAL $\geq$ 30) and to remove genotypes with low read depth (DP $<$ 3). Variants were normalised, and duplicate positions were removed. Only biallelic sites with minor allele frequency (MAF $\geq$ 0.05) and a maximum of 30% missing data per site were retained. Additionally, indels were excluded, and only SNPs were used. To reduce linkage among loci, linkage disequilibrium (LD) pruning was performed in PLINK v1.9.0-b.8 (Chang et al., 2015) using a sliding window approach (50 SNPs, step size 5, r^2 threshold = 0.25). The resulting LD-pruned dataset was used for downstream analyses.

For phylogenomic analyses, chromosome-level VCF files containing filtered SNPs were converted to alignment format using vcf2phylip v2.9 (Ortiz, 2019), and maximum likelihood (ML) trees were inferred with IQ-TREE, with branch support estimation based on 1000 ultrafast bootstrap (UFboot) and Shimodaira–Hasegawa-like approximate likelihood-ratio test (SH-aLRT) tests, to assess genomic heterogeneity in phylogenetic signal. Because the matrices contained variable sites only, we used ascertainment-bias correction (+ASC) to correct likelihood calculations for the absence of invariant sites. The best-fitting substitution model was selected with ModelFinder using the -m MFP+ASC option. Gene concordance factor (gCF) analyses were used to quantify the proportion of chromosome trees supporting each bipartition. A summary tree was constructed based on concordance patterns, and node support was evaluated using UFboot and SH-aLRT statistics. This analysis was regarded as a chromosome-level consensus (as "chromosome concordance factors"), not as a formal multi-species coalescence analysis. In addition to the chromosome-level inference, we concatenated the chromosome-level VCF files using bcftools and executed an ML tree reconstruction in IQ-TREE using the same settings as above. This analysis is regarded as the representation of the tree that best fits all characters combined, assuming a single underlying topology. A branch was considered to be supported in the ML-tree analyses if both SH-aLRT $\geq$ 80% and UFboot $\geq$ 95% were true.

The concatenated dataset was used in further analyses of regional genetic structure, which was explored using principal component analysis (PCA) as implemented in PLINK. In this exploratory analysis and the phylogenomic analyses above, two individuals from the locality Muselievo (Table 1) proved to be highly outlying from the dataset (see Results). These, regarded as most probably representing a non-indigenous genepool, were excluded from further analyses. The remaining 13 samples were subject to a model-based k-means clustering using ADMIXTURE v1.3.0 (Alexander et al., 2009). Analyses were performed on a reduced SNP dataset (~50,000 loci) selected for high discriminatory power by defining the two taxa found in our phylogenomic analyses (see Results) and calculating F_{ST} between these groups using VCFtools. A similar approach was found useful in discriminating North-American species of the white oak syngameon (Hipp et al., 2019). The most likely number of clusters (K) was determined using cross-validation error, and results were visualised as individual ancestry proportions.

Finally, we wanted to visualise the syngameon genomic landscape of differences between the two taxa we recovered. To achieve this goal, we calculated the mean F_{ST} in 50-kb sliding windows with a 10-kb step, retaining windows containing at least five SNPs. The resulting values were visualised in R v4.5.1 (R Core Team, 2025) using the package ggplot2 v4.0.1 (Wickham, 2016). The broad low-F_{ST} regions were interpreted as shared genomic background/gene flow, and narrow high-F_{ST} peaks as candidate islands of divergence according to the syngameon model. In addition, we tested whether predefined drought-responsive candidate loci coincided with regions of strongest genomic differentiation. To achieve this goal, we intersected the genomic coordinates of the 21 candidate loci (including 2-kb flanking regions) with the top 1% of 50-kb sliding windows (10-kb step) ranked by mean F_{ST}, using BEDTools v2.31.1 (Quinlan and Hall, 2010).

2.5. Candidate-gene dosage analysis

To explore possible differences in relative copy-number signal among drought-related candidate genes, a targeted mapping-based dosage analysis was performed. A total of 21 candidate genes, previously associated with drought tolerance and abiotic stress responses (Homolka et al., 2012; Trudić et al., 2021), were selected for analysis (Table 2). Candidate genomic regions were identified on the *Quercus robur* chromosome-level reference genome using translated sequence similarity searches. A local BLAST database was constructed using *makeblastdb*, and sequence similarity searches were performed with *tblastx* using the BLAST+ suite v2.17.0+ (Camacho et al., 2009). For

Table 2
Candidate drought-responsive genes analysed in this study. Functional annotations and accession numbers are based on previously identified drought-associated loci in oak species (Homolka et al., 2012[1]; Trudić et al., 2021[2]). For each candidate, the table lists the functional annotation, accession number of the source sequence used for candidate identification, and the reference oak species from which that sequence was originally reported. Fold-change values summarize the comparison of normalised mapped dosage between *Quercus pedunculiflora* and *Q. robur*, expressed as the ratio of group means ("fold mean") and group medians ("fold median"), where values >1 indicate higher relative dosage in *Q. pedunculiflora* and values <1 indicate higher relative dosage in *Q. robur*. Statistical support for per-gene differences was assessed using Wilcoxon rank-sum tests on per-sample normalised depth values, and the final column reports Benjamini–Hochberg-adjusted P-values.

	Functional annotation	Accession number	Reference species	fold mean	fold median	p-value Wilcox	p-value BH adj.
ARF16[1]	Auxin responsive factor 16	FP033974	*Q. robur*	1.18	1.25	0.52	0.72
BMY7[1]	Beta-amylase 1	FP061723	*Q. robur*	1.07	1.07	0.62	0.72
DHN2[1]	Dehydrin 2	FP045825	*Q. robur*	1.11	1.14	0.43	0.72
ERD8[1]	Heat shock cognate protein	FP041070	*Q. robur*	0.98	0.99	0.94	0.94
GLP3A[1]	Germin-like protein	FP024682	*Q. robur*	0.99	1.03	0.62	0.72
LEA14[1]	Desiccation protectant protein	FP049285	*Q. robur*	0.94	0.95	0.35	0.72
LTP[1]	Proline-rich protein	FP031479	*Q. robur*	1.07	1.04	0.22	0.72
PER6[1]4	Peroxidase 64	FP051623	*Q. robur*	1.14	1.03	0.43	0.72
PIP1E[1]	Aquaporin PIP1–2	FP040796	*Q. robur*	1.35	1.36	0.22	0.72
RD26[1]	Responsive to desiccation 26	FN712698	*Q. robur*	1.20	1.09	0.52	0.72
ATDI21[2]	Drought-induced gene 21	CR627776	*Q. petraea*	1.10	1.02	0.43	0.72
AWMP[2]	AWPM-19-like membrane protein	XM_031067968	*Q. lobata*	0.98	1.03	0.62	0.72
GASA[2]	GASA/Snakin family protein	XM_031099810	*Q. lobata*	1.16	1.07	0.35	0.72
GLOBULIN[2]	Basic 7S globulin	XM_024060619	*Q. suber*	1.07	0.99	0.52	0.72
GST[2]	Glutathione-S-transferase	XM_031105862	*Q. lobata*	1.17	1.65	0.94	0.94
LEA[2]	Late embryogenesis abundant protein	XM_031068332	*Q. lobata*	1.11	1.03	0.43	0.72
RD22[2]	Dehydration-responsive protein	FP041436	*Q. petraea*	1.19	1.06	0.35	0.72
SAG21[2]	Senescence associated gene	XM_031092942	*Q. lobata*	1.07	1.17	0.72	0.80
WRKY53[2]	WRKY transcription factor	FN727229	*Q. petraea*	1.09	1.07	0.52	0.72
XERO2[2]	Dehydrin Xero2	AM711636	*Q. petraea*	1.11	1.00	0.35	0.72
XET2[2]	Xyloglucan endotransglucosylase	XM_031074399	*Q. lobata*	1.08	1.08	0.43	0.72

each candidate gene, the best-scoring genomic hit was retained and extended by 2 kb on both sides to include flanking regions. These intervals were extracted and compiled into a reduced "mini-reference" containing one sequence per candidate locus.

The filtered reads from all samples were then mapped against this mini-reference using BWA-MEM2. The resulting alignments were processed as BAM files and used to estimate read depth across each candidate locus using samtools v1.22.1 (Danecek et al., 2011). For each sample, per-base coverage across the full length of each mini-reference sequence was calculated with samtools depth using the -a option, so that uncovered positions were also included in the output. Mean depth per candidate gene region was then calculated by averaging coverage across all nucleotide positions belonging to that candidate in each sample.

Because sequencing depth differed among samples, candidate-specific depth values were normalised by sample-wide background coverage estimated from the original BAM files mapped to the full *Q. robur* reference genome. For each sample, mean genomic background depth was obtained from the whole-genome alignment and used as an external denominator. Thus, the final response variable was a normalised depth value for each candidate gene in each sample, calculated as candidate mean depth divided by the corresponding sample-wide genomic background depth. These normalised values were interpreted as relative mapped dosage signals rather than absolute copy-number estimates, because short-read mapping to homologous gene-family regions can reflect both true copy number and sequence similarity.

These data were imported into R, where data handling and visualisation were performed using the tidyverse v 2.0.0 (Wickham et al., 2019), and plots were generated using ggplot2. Differences between the two ancestry groups identified by the ADMIXTURE analysis (Fig. 5), which broadly corresponded to the a priori taxonomic assignments to *Quercus robur* and *Q. pedunculiflora*, were evaluated separately for each candidate gene using Wilcoxon rank-sum tests applied to per-sample normalised depth values. P-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure. For each gene, summary statistics were calculated, including the number of samples per group, mean, median, standard deviation, fold difference, raw P-value, and FDR-adjusted P-value.

To assess overall differences across the full candidate gene set, linear mixed-effects models were fitted using the packages lme4 v1.1.38 (Bates et al., 2015) and lmerTest v3.1.3 (Kuznetsova et al., 2017). The primary model used log-transformed normalised depth as the response variable, with taxon (or geographic group, see below) included as a fixed effect, and candidate gene identity and sample identity specified as random intercepts to account for repeated measurements and baseline differences among genes. Model comparison was performed using likelihood-ratio tests between models with and without the focal fixed effect. Fixed-effect estimates were interpreted on the log scale and exponentiated to express multiplicative differences in relative dosage between groups.

Because candidate genes differed substantially in absolute depth scale, an additional complementary analysis was performed using values standardized within each gene. For this, log-transformed normalised depth values were z-transformed separately for each candidate gene, and mixed-effects models were refitted using the standardized response. In these within-gene standardised analyses, sample identity was retained as a random effect, whereas the between-gene baseline effect was already removed by the standardization procedure. This second modelling approach was used to test whether one group showed a consistent directional shift across genes after removing locus-specific scale differences.

Geographic analyses were performed analogously by grouping samples into two regional categories, Hungary and Balkans (Romania + Bulgaria), and fitting the same per-gene and global mixed-effects models with region as the focal explanatory variable. Boxplots of normalised depth by group were generated for each candidate gene to visualize overlap, dispersion, and directional trends among groups.

Finally, genetic structure within drought-responsive candidate regions was assessed using PCA. For each candidate gene, the best tblastx hit against the *Q. robur* reference genome was retained, converted to a BED interval with ±2 kb flanking sequence, and used to extract SNPs from the filtered joint genome-wide, concatenated VCF. The resulting candidate-region SNP dataset was used to perform PCA in PLINK, and the first principal components were used to visualize differentiation among individuals in the candidate genomic regions.

3. Results

3.1. Plastome phylogeny

The maximum likelihood phylogeny inferred from the BMGE-curated whole-plastome alignment revealed limited phylogenetic structuring among the sampled individuals (Fig. 2). The tree was rooted using *Quercus cerris*, with *Q. lobata* included as an additional outgroup.

Within the ingroup, individuals assigned *a priori* to *Q. robur, Q. pedunculiflora*, and morphologically transitional forms did not form clearly separated, reciprocally monophyletic clades. Instead, samples were interspersed across the tree, with several well-supported local groupings containing mixed taxonomic assignments.

Hungarian *Q. robur* individuals formed a fully supported subclade; however, this grouping also included the individual form Bikács that has previously been assigned to *Q. pedunculiflora*. This sample consistently clustered with Hungarian *Q. robur* rather than with Balkan *Q. pedunculiflora* individuals.

Balkan samples of *Q. pedunculiflora* and morphologically "*transitional*" individuals were distributed across multiple parts of the tree rather than forming a single distinct lineage. Overall, plastome variation did not support a clear phylogenetic separation between *Q. robur* and *Q. pedunculiflora*, indicating shared maternal lineages among the studied individuals.

3.2. Nuclear phylogenomics and genome-wide population structure

Phylogenetic analyses based on genome-wide SNP data revealed a structured genomic difference between the sampled individuals. The summary tree derived from chromosome-level maximum likelihood trees (Fig. 3A) showed generally high branch support values, but low gene concordance across loci, indicating substantial topological discordance among chromosomes.

Despite this heterogeneity, consistent grouping patterns were observed. Individuals assigned to *Q. robur* and *Q. pedunculiflora* formed reciprocally monophyletic clades, if we disregard two outliers: a *Q. pedunculiflora* from Bikács, Hungary, which was allegedly associated with this taxon (see Introduction), and a *Q. robur* from Muselievo, Bulgaria. The phylogenetic placement of an additional individual of *Q. robur* (K5183) from Pleven, Bulgaria, was inconsistent: on the consensus tree, it was clustered with another *Q. robur* from Bulgaria within a clade of *Q. robur* from the sympatric area of the studied species (Fig. 3A); whereas on the summary (concatenated) tree (Fig. 3B) it was nested within the *Q. pedunculiflora* clade clustered with samples from Poina Gruii, Romania. Within *Q. robur*, two geographically structured groups were apparent, corresponding to Hungarian and Balkan samples, whereas *Q. pedunculiflora* individuals formed a more cohesive cluster across analyses.

Morphologically transitional individuals, defined by the presence of simple-uniseriate and stellate trichomes but the absence of fasciculate trichomes on the abaxial leaf surface (Hegedüs et al., 2025), did not form a separate cluster. Instead, these individuals were consistently grouped with *Q. pedunculiflora* samples across chromosome-level trees and in the summary tree (Fig. 3). No transitional individual was placed within the main *Q. robur* cluster.

These patterns were further supported by genome-wide population structure analyses. Principal component analysis (Fig. 4) revealed three main groupings corresponding to Hungarian *Q. robur*, Balkan *Q. robur* and *Q. pedunculiflora*, plus two individuals from Muselievo, Bulgaria. The first two principal components explained 8.8% and 8.2% of the total variance, respectively. Transitional individuals were positioned within or adjacent to the *Q. pedunculiflora* cluster rather than occupying intermediate positions. Within the Balkan oak group, the *Q. robur* from Romania and Bulgaria occupied a slightly transitional position to the Hungarian *Q. robur* group.

In the admixture analysis (Fig. 5), with the Muselievo outliers excluded, cross-validation analysis across K = 1–5 identified K = 2 as the best-supported ancestry model, with the lowest cross-validation error (Fig. 5A). At K = 2, the two major genomic ancestry components broadly corresponded to the *a priori* taxonomic assignments: individuals assigned to *Q. pedunculiflora* and the transitional samples were characterised almost entirely by one component, whereas *Q. robur* individuals were dominated by the other (Fig. 5B). The two Balkan *Q. robur* individuals from Bivolare and Pleven showed a minor contribution from the *Q. pedunculiflora*-associated component. In contrast, individuals assigned to *Q. pedunculiflora* were characterised by a distinct ancestry component, and transitional individuals showed assignment patterns similar to that group. The inclusion of the two Muselievo individuals in the ADMIXTURE analysis did not alter the broad ancestry pattern observed in the dataset, although both Muselievo samples showed strongly mixed ancestry proportions and remained distinct from the other individuals (Fig. S1).

At K = 3, however, an additional ancestry component differentiated the Balkan *Q. robur* samples from the Hungarian *Q. robur*, demonstrating

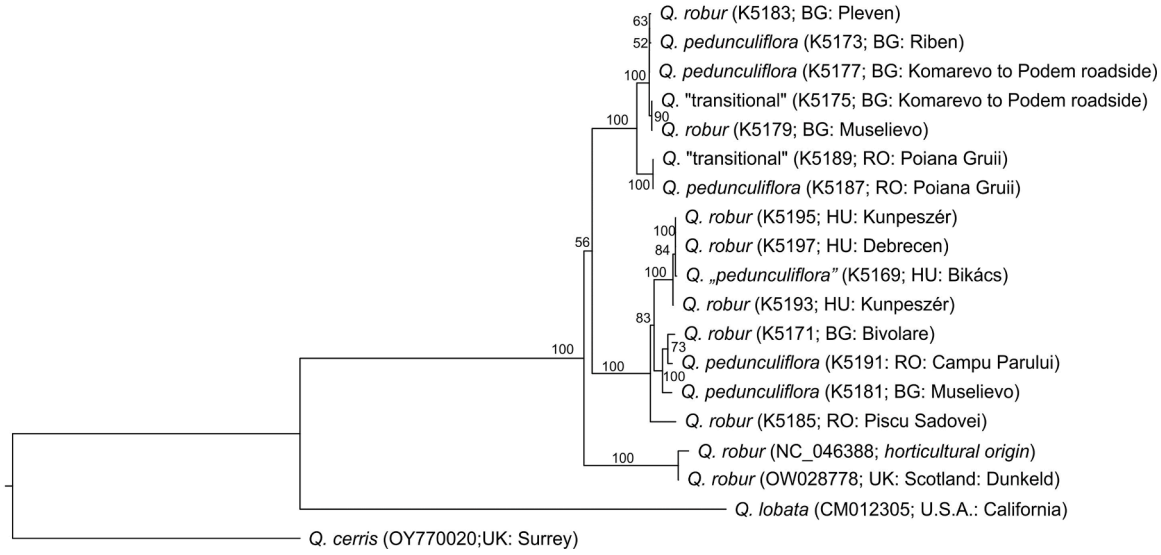


Fig. 2. Maximum-likelihood plastome phylogeny inferred by IQ-TREE from the BMGE-curated whole-plastome alignment, with 100 bootstrap replicates. The tree was rooted on *Quercus cerris* as outgroup. Bootstrap values are shown at the corresponding nodes.

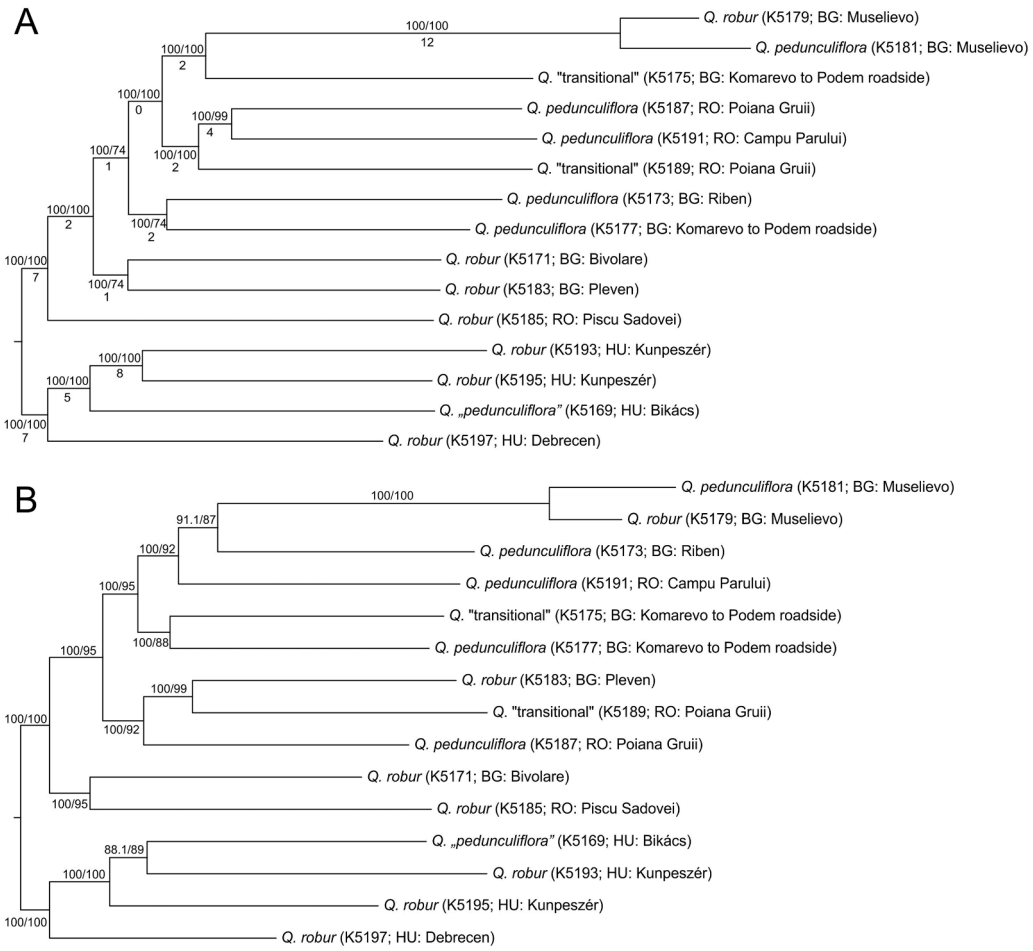


Fig. 3. Phylogenomic relationship between the studied samples as depicted by phylogenetic trees of the nuclear genome displayed as a phylogram. A.) Summary tree based on gene concordance factor analysis across 12 chromosome-level maximum-likelihood trees. Values at nodes indicate ultrafast bootstrap support / SH-aLRT support, and numbers below nodes indicate the number of chromosome trees supporting the corresponding bipartition. The tree is arbitrarily rooted at the midpoint. B.) Maximum-likelihood phylogeny inferred from the concatenated genome-wide SNP dataset derived from all 12 chromosomes. Values at nodes indicate ultrafast bootstrap support / SH-aLRT support. The topology represents the best-supported tree under a concatenation framework, reflecting the overall phylogenetic signal across the genome. The tree is arbitrarily rooted at the midpoint.

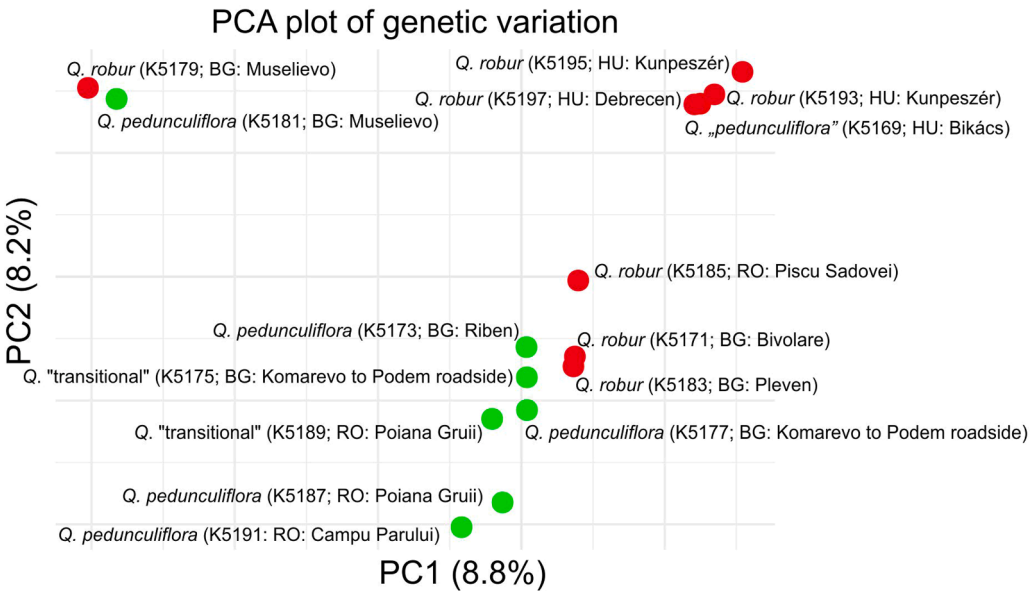


Fig. 4. Principal component analysis of genome-wide SNP variation in the *Quercus robur* complex. PC1 and PC2 explain 8.8% and 8.2% of the total variance, respectively. Colours indicate *a priori* taxonomic assignment based on morphometry (Hegedüs et al., 2025).

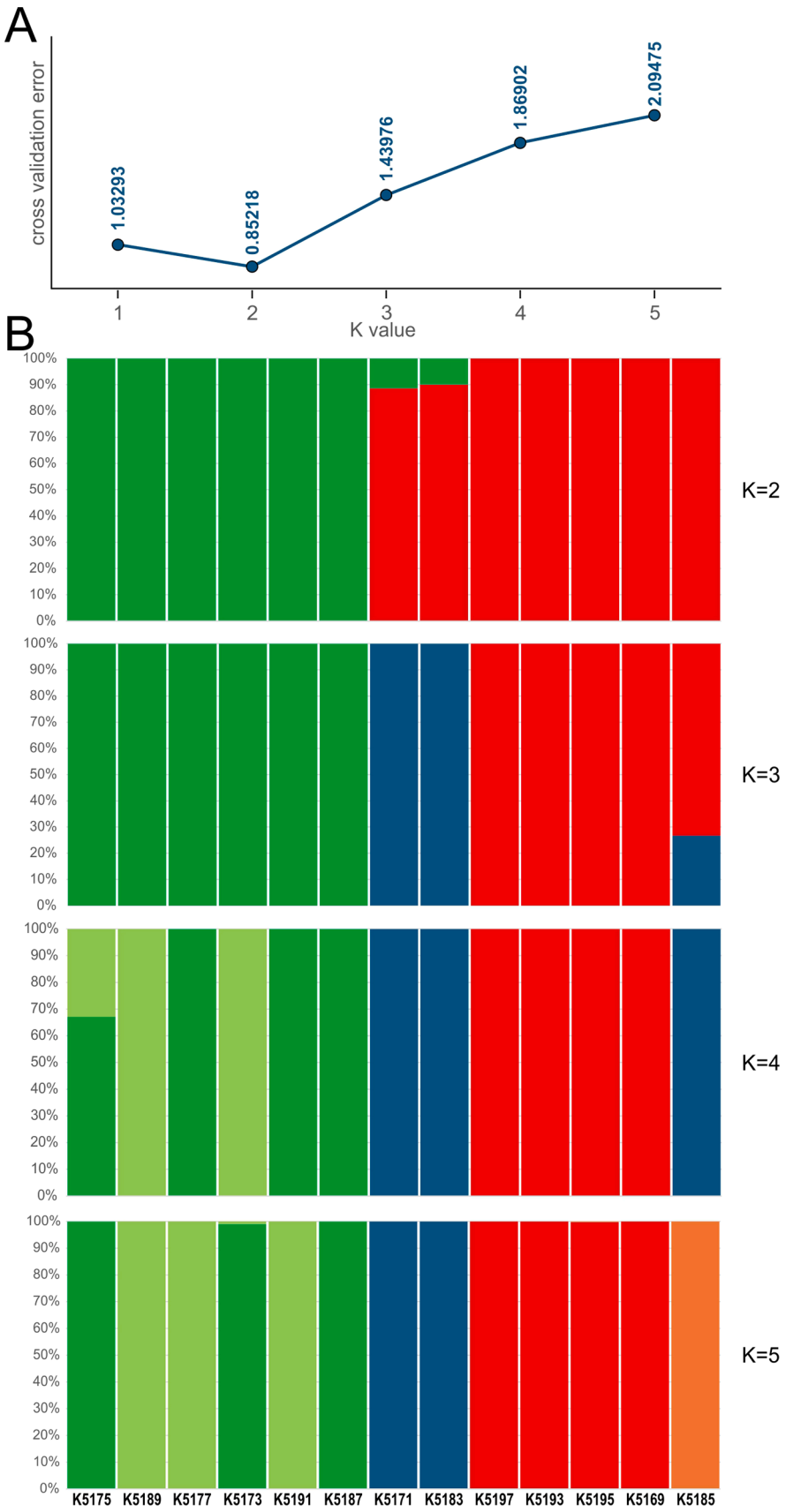


Fig. 5. Admixture analysis of the *Quercus robur* complex based on a reduced dataset of 50,000 highly discriminatory genome-wide nuclear SNPs. (A) Cross-validation error for ancestry models with K = 1–5. (B) Individual ancestry proportions for K = 2–5. Each vertical bar represents one individual, and colours indicate proportional assignment to the inferred genomic ancestry components. Individuals are ordered according to their broad taxonomic and geographic affinities.

that the genomic structure also contains a marked geographic component (Fig. 5B). The Romanian *Q. robur* sample from Piscu Sadovei showed mixed assignment between these *Q. robur*-associated components. Higher values of K resulted in further subdivision, particularly within the *Q. pedunculiflora*-associated group, but were associated with progressively higher cross-validation errors and therefore received less statistical support. The Bikács individual remained assigned to the same ancestry component as Hungarian *Q. robur* across K = 2–5, with no detectable contribution from the *Q. pedunculiflora*-associated component.

Overall, nuclear phylogenomic and population genomic analyses indicate that genomic structure reflects both geographic origin and the partial taxonomic differentiation observed within the *Quercus robur* complex, including additional structuring among *Q. robur*. Across genome-wide analyses, no Hungarian individual formed a distinct cluster corresponding to *Q. pedunculiflora*. Instead, all Hungarian samples were consistently associated with the *Q. robur* cluster in both phylogenomic and genetic structure analyses. In contrast, *Q. pedunculiflora* samples from the Balkans formed a distinct grouping, although not completely separated from *Q. robur*. The observed structure, therefore, reflects both geographic differentiation within *Q. robur* and partial genomic differentiation between the two taxa.

Genome-wide patterns of genetic differentiation between *Q. robur* and *Q. pedunculiflora* were highly heterogeneous (Fig. 6). Mean windowed F_{ST} values, calculated in 50-kb sliding windows with a 10-kb step, were generally low across most of the genome, typically ranging between 0.01 and 0.06. However, numerous localised peaks of elevated differentiation were observed across all chromosomes, with some windows exceeding F_{ST} values of 0.15–0.20 and rare outliers approaching ~0.23. These high-F_{ST} regions were scattered throughout the genome rather than concentrated in specific chromosomes, although certain chromosomes (e.g. chromosomes 4, 9, and 10–11) exhibited particularly pronounced peaks. Overall, the genomic landscape is characterised by a background of low differentiation punctuated by discrete regions of elevated divergence.

3.3. Candidate-gene dosage patterns

Analysis of normalised mapped dosage across 21 candidate genes previously implicated in drought response revealed substantial variation among loci. Some genes showed consistently higher normalised depth values across all samples, whereas others exhibited lower overall coverage levels (Table S1). Despite this among-gene variation, most candidate genes displayed broad overlap in normalised dosage values among individuals of *Quercus robur, Q. pedunculiflora*, and morphologically transitional samples. Gene-wise distributions of log-transformed normalised depth values indicated that variation among loci was generally greater than differences between biological groups (Fig. S2).

Per-gene comparisons using Wilcoxon rank-sum tests did not identify any candidate gene with a statistically significant difference between *Q. robur* and *Q. pedunculiflora* after multiple testing correction (all adjusted $P \geq 0.72$; Table 2). Although some loci showed weak tendencies toward higher normalised dosage in *Q. pedunculiflora* (Fig. S2), these patterns were inconsistent across genes and not statistically supported.

A global mixed-effects analysis yielded similar results. In the model using log-transformed normalised depth as the response, with gene identity and sample identity fitted as random intercepts, taxon had no significant overall effect on normalised mapped dosage (likelihood-ratio test: $\chi^2 = 1.24$, d.f. = 1, $P = 0.266$). The fitted coefficient for *Q. pedunculiflora* was positive ($\beta = 0.119 \pm 0.104$ SE), corresponding to an estimated 1.13-fold increase on the original scale, but the 95% confidence interval overlapped with no effect.

Because absolute dosage levels differed strongly among candidate genes, the analysis was repeated using within-gene standardised values. This approach also failed to detect a significant overall taxon effect (likelihood-ratio test: $\chi^2 = 1.05$, d.f. = 1, $P = 0.305$), although the fitted coefficient again indicated a weak positive tendency toward higher values in *Q. pedunculiflora* ($\beta = 0.410 \pm 0.399$ SE).

Principal component analysis based on the 21 candidate genes showed limited structuring among individuals (Fig. 7). The first two principal components explained 9.5% and 8.6% of the total variation, respectively. Samples from Hungary separated along the first principal component from the Balkan samples, while the second component separated a *Q. pedunculiflora* (K5191) from Câmpu Părului, Romania, as an outlier. Again, Balkan *Q. robur* occupied a somewhat transitional position between Balkan *Q. pedunculiflora* and Hungarian *Q. robur* (including the alleged Bikács individual).

Geographic comparisons yielded a similar pattern. Grouping samples into Hungary versus Balkans (Romania + Bulgaria) revealed no statistically supported differences in normalised mapped dosage. Per-gene inspection suggested slight tendencies toward higher values in Balkan samples, but overlap between groups remained extensive (Fig. 8; Table S1).

The global mixed-effects model with region as a fixed effect did not detect a significant overall geographic effect (likelihood-ratio test: $\chi^2 = 1.66$, d.f. = 1, $P = 0.197$). The fitted coefficient for the Balkan group was positive ($\beta = 0.147 \pm 0.110$ SE), corresponding to an estimated 1.16-fold increase, but this trend was not statistically supported.

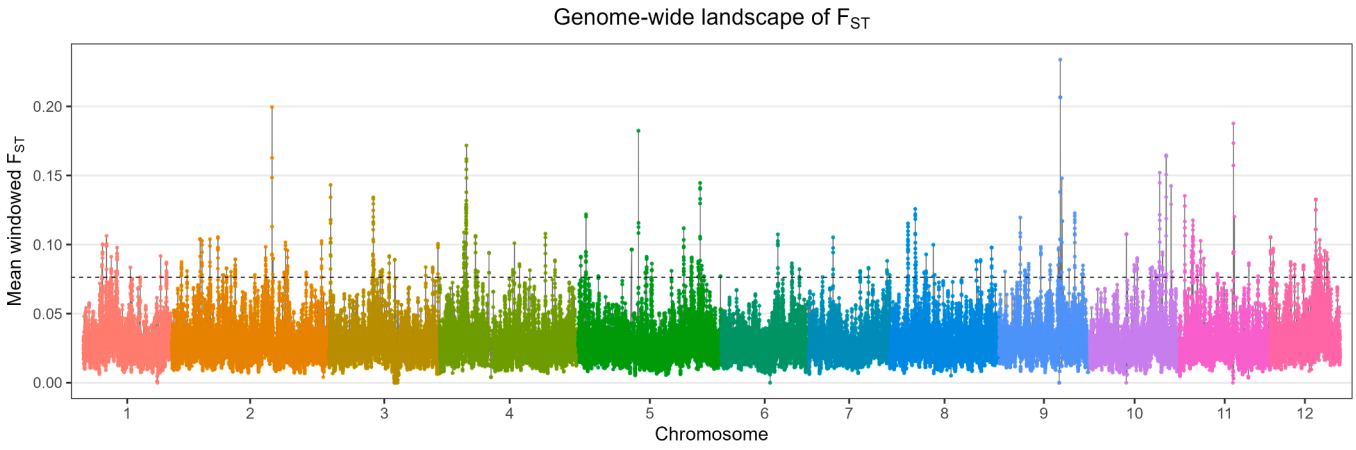


Fig. 6. Genome-wide landscape of genetic differentiation between *Quercus robur* and *Q. pedunculiflora*. Mean pairwise F_{ST} values were calculated in 50-kb sliding windows with a 10-kb step across the 12 chromosomes based on genome-wide SNP data. Points represent window-averaged F_{ST} values, with chromosomes distinguished by alternating colours. The dashed horizontal line indicates the genome-wide mean F_{ST}.

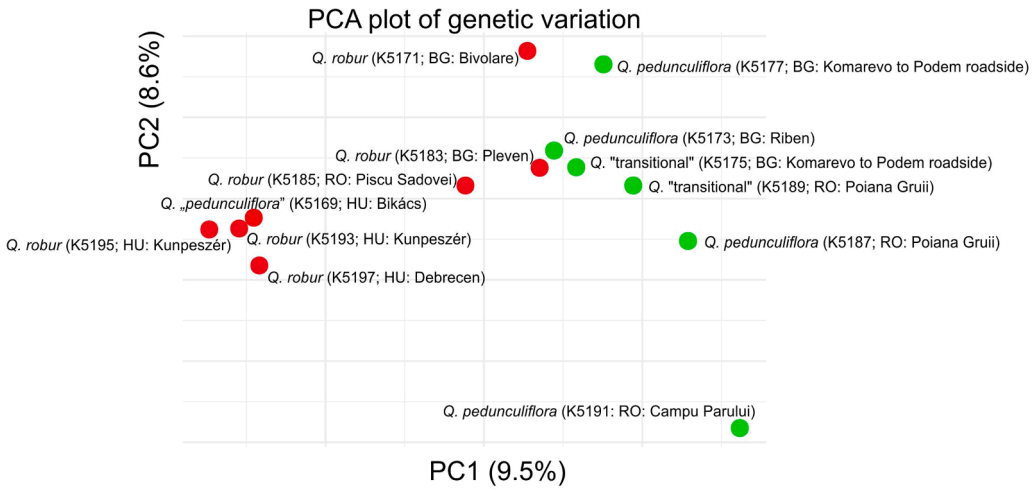


Fig. 7. Principal component analysis based on the 21 drought-tolerant candidate genes. PC1 and PC2 explain 9.5% and 8.6% of the total variation, respectively. Colours indicate *a posteriori* taxonomic assignment based on the above phylogenomic results.

Analyses based on within-gene standardised values led to the same conclusion (likelihood-ratio test: $\chi^2 = 2.07$, d.f. $= 1$, P $= 0.150$), with a similarly non-significant positive trend ($\beta = 0.571 \pm 0.381$ SE).

We also checked if any of our 21 candidates are sitting in high differentiation regions of the genome (Fig. 6). None of the 21 predefined drought-responsive candidate loci overlapped the top 1% of genomic windows showing the highest mean F_{ST} values.

Overall, the candidate loci previously associated with drought response did not show significant differences in normalised mapped dosage either between taxa or between the two broad geographic groups. The dominant pattern in the dataset was strong heterogeneity among candidate genes, whereas both taxonomic and geographic effects were weak and statistically unsupported, although with an observable tendency of having higher dosage values in Balkan and *Q. pedunculiflora* samples (Fig. 8; Table S1). These patterns are therefore interpreted as exploratory candidate-locus variation and as a basis for hypothesis generation rather than as evidence of adaptive differentiation or drought resistance.

4. Discussion

4.1. Genomic differences and taxonomic assignments

Our genome-wide analyses provide a consistent picture of structured genomic differentiation within the *Quercus robur* complex. In the plastome dataset, individuals assigned to the two taxa and morphologically transitional forms did not form reciprocally monophyletic groups, but instead showed taxonomically mixed clustering combined with substantial signal heterogeneity (Fig. 2). This pattern is consistent with previous findings in European white oaks (Leroy et al., 2020; Pakull et al., 2025) and specifically in the region (Bordács et al., 2002), where incomplete lineage sorting and extensive historical and ongoing introgression result in permeable species boundaries and widespread sharing of plastid haplotypes (Curtu et al., 2007; Hipp et al., 2020; Kremer and Hipp, 2020; Petit et al., 2002).

In contrast, the nuclear genome revealed substantially stronger structuring. Across chromosome-level phylogenomic analyses, PCA, and admixture analyses, *Q. pedunculiflora* samples formed a relatively coherent genomic group, whereas *Q. robur* samples were subdivided into Hungarian and Balkan genomic clusters (Figs. 3–5). Nonetheless, the concatenated phylogenomic tree, which echoes a 'consensus phylogenetic signal' despite genomic mosaicism (Kremer and Hipp, 2020), recovered the two taxa as reciprocally monophyletic (Fig. 3B). This nuclear genomic coherence is consistent with regarding *Q. pedunculiflora* as more than a simple ecological variant of *Q. robur*, although the plastid–nuclear discordance shows that its evolutionary history cannot be represented by a single bifurcating genealogy. Rather, the detected differentiation is consistent with a weakly differentiated evolutionary lineage (Kremer and Hipp, 2020; Kremer et al., 2024; Pakull et al., 2025). The critical question, however, is how such a lineage should be interpreted within the oak syngameon (Cannon and Petit, 2020; Kremer and Hipp, 2020; Hipp et al., 2020). In this regard, it is useful to recall the taxonomic relationship of *Q. robur* to most taxa of the European white-oak complex, and *Q. petraea* in particular, which are generally accepted at species rank, despite well-documented gene flow and incomplete reproductive isolation, because they remain ecologically and morphologically recognisable lineages separated by a circumscribable morphological and ecological gap (Kremer and Hipp, 2020; Kremer et al., 2024; Leroy et al., 2020).

In our case, this gap appears considerably narrower between *Q. robur* and *Q. pedunculiflora* (Apostol et al., 2017; Hegedüs et al., 2025). We therefore hypothesise that the two taxa may have diverged regionally and relatively recently, perhaps in more westerly versus more easterly refugial areas associated with the main European glacial refugia (Hewitt, 1999; Petit et al., 2003). Such a scenario is compatible with broader phylogeographic models for temperate European trees, in which eastern and south-eastern refugia, particularly around the Black Sea and adjacent regions, played an important role in the persistence of tree lineages through glacial cycles and in the retention of lineages adapted to warmer and drier climatic conditions (Svenning et al., 2008). A comparable analogy is delivered by the *Fagus sylvatica–F. orientalis* sister species (Renner et al., 2016; Denk et al., 2024), in which Oriental beech (sensu Denk et al., 2024) is repeatedly regarded as more drought-tolerant and climatically better suited to warmer and drier environments than European beech (Budde et al., 2023; Kurz et al., 2023; Mellert and Šeho, 2022). In our view, however, the ecological divergence between *Q. robur* and *Q. pedunculiflora* may have arisen more recently, and with too little accompanying morphological separation, to justify treatment of *Q. pedunculiflora* as a fully distinct species. We therefore prefer to interpret *Q. pedunculiflora* as *Q. robur* subsp. *pedunculiflora* rather than as a separate species.

The plastid–nuclear discordance therefore does not necessarily weaken the taxonomic interpretation of *Q. pedunculiflora*. In European white oaks, plastid genomes are frequently shared across species boundaries and often reflect regional phylogeographic history more strongly than species identity, owing to recurrent introgression, backcrossing and chloroplast capture (Bordács et al., 2002; Petit et al., 2002; Cannon and Petit, 2020). By contrast, nuclear genomes retain a stronger

signal of lineage cohesion because they integrate information across many independently inherited loci (Kramer and Hipp, 2020). Consequently, the absence of reciprocal plastid monophyly should not be taken as evidence against subspecific differentiation when a coherent nuclear genomic signal is present. Rather, in the context of the oak syngameon, the discordance indicates that organellar and nuclear genomes reflect different evolutionary histories. Our taxonomic interpretation is therefore based primarily on the multilocus nuclear signal, considered together with morphology and ecology, while the plastome is treated as evidence of historical gene flow and shared phylogeographic history rather than as a decisive marker of taxonomic boundaries.

The Muselievo specimens showed strongly outlying genomic positions in all nuclear genome analyses (Figs. 3 and 4). These two individuals were sampled along a historical roadside near the settlement of Muselievo and are locally recognised as remarkably old trees. Although they were appealing to use in our analyses as these two spatially neighbouring specimens displayed contrasting morphology (see Hegedüs et al., 2025), they had to be excluded from further analyses and discussion as suspected introduced specimens. Technical artefacts are unlikely to explain their divergent position, because sequencing-read characteristics of the two Muselievo individuals were highly comparable to those of the other specimens in our dataset. The apparently mixed ancestry proportions recovered by ADMIXTURE (Fig. S1) should likewise not be interpreted naively as evidence of recent admixture; in this context, they primarily indicate that these individuals are poorly represented by the two major ancestry components inferred from the remaining samples. Importantly, their inclusion did not alter the broad $K = 2$ ancestry pattern. Such sharp individual genomic anomalies are common when sampling old, culturally significant trees, as centuries of human-mediated long-distance seed transfer frequently mask natural evolutionary patterns at the local scale and result in geographic assignment errors (Degen et al., 2021; König et al., 2002; Tischenko et al., 2026). Excluding these highly localised anthropogenic signatures ensures that downstream analyses reflect the genuine regional genetic structure rather than artificial historical introductions.

The Bikács individual from Hungary provides a particularly important example in the context of the Hungarian occurrence of *Q. pedunculiflora*. Although previously suspected to be *Q. pedunculiflora* based on morphology (Pintér et al., 2025), this sample consistently clustered with Hungarian *Q. robur* in all nuclear analyses (Figs. 3–5.) and in the plastome phylogeny (Fig. 2). Within the scope of the present dataset, no Hungarian sample formed a genomic cluster corresponding to Balkan *Q. pedunculiflora*. The present analyses therefore do not support the presence of genomically distinct *Q. pedunculiflora* in the sampled Hungarian material.

The strongest admixture signal was detected in Balkan *Q. robur* individuals originating from the sympatric distribution area of the two taxa. In particular, the Pleven and Bivolare individuals showed partial assignment to the *Q. pedunculiflora* ancestry component in the admixture analysis (Fig. 5) and occupied somewhat transitional positions in PCA space (Fig. 4). Importantly, these same individuals were previously found to display intermediate quantitative micromorphological characters (Hegedüs et al., 2025). In the leaf indumentum analyses, although they still showed only the qualitative indumentum features typical of *Q. robur*, their simple-uniseriate trichome rays were longer than those of typical Hungarian *Q. robur*, yet clearly shorter than those observed in Balkan *Q. pedunculiflora*. Thus, the admixture signal revealed by the genomic analyses closely parallels the observed quantitative micromorphological intermediacy. This concordance strongly suggests that localised introgression contributes to the morphological variability observed in sympatric Balkan populations (Curtu et al., 2007). More broadly, similar correspondence between leaf morphology, pubescence-related traits, and genetic assignment has been reported repeatedly in European white oaks, indicating that quantitative leaf micro-traits can provide informative, although not infallible (see Kremer et al., 2002), proxies for underlying genomic introgression (Curtu et al., 2007; Enescu et al., 2013; Rellstab et al., 2016; Semerikova et al., 2025).

In contrast, morphologically transitional (i.e., showing intermediate indumentum characters in Hegedüs et al. (2025)) individuals did not occupy intermediate genomic positions, but were consistently associated with the *Q. pedunculiflora* cluster across phylogenomic and genetic structure analyses (Figs. 3–5). This indicates that trichome-based morphological intermediacy does not necessarily reflect genome-wide genetic intermediacy and suggests that these individuals may represent phenotypic variants within the *Q. pedunculiflora* lineage or individuals with incomplete expression of diagnostic traits. Similar mismatches between morphological intermediacy and genomic assignment have been reported in European white oaks, where hybrids can display a mosaic of phenotypes (Kremer et al., 2002; Truffaut et al., 2017; Reutimann et al., 2020).

It is noteworthy that the relationship between morphology and genomic background differed between the two types of indumentum characters examined here. The quantitative trait, namely the length of simple-uniseriate trichomes, showed intermediacy in admixed Balkan *Q. robur* individuals, whereas the more qualitative trait, i.e. the presence or absence of stellate and/or fasciculate trichomes, was not mirrored by genomic intermediacy in the individuals classified as “transitional” but genetically assigned to *Q. pedunculiflora*. This contrast suggests that different trichome traits may have distinct genetic architectures and different sensitivities to genetic background and environment. A trait such as trichome length may vary continuously and track admixture if it is influenced by additive effects across multiple loci, or by loci whose allelic effects scale with ancestry proportion (Wang et al., 2021). By contrast, the presence or absence of a particular trichome type may behave more like a threshold or developmental-switch trait, controlled by a more restricted regulatory module, dominance, epistasis, or canalised developmental decisions (Pattanaik et al., 2014); such traits may therefore remain parent-like even when genome-wide ancestry is admixed (Wang et al., 2021). A complementary explanation is that environmental effects may influence the development of these trichome characters differently (Hauser, 2014). Although this observation is based on a limited number of individuals, it may deserve further attention in future morphometric and developmental studies of oaks.

4.2. Candidate genes and drought-related genomic patterns

The analysis of candidate genes previously implicated in drought response was included as an exploratory component of the study and was not intended as a primary test of genomic differentiation or as a direct test of drought adaptation. Across the 21 loci examined, normalised mapped dosage values showed substantial overlap between *Quercus robur* and *Q. pedunculiflora*, with a weak tendency toward higher normalised dosage in the latter, and neither per-gene tests nor global mixed-effects models detected statistically supported differences. A similar result was obtained when we compared samples from the Pannonian Basin with those from the northern Balkans, irrespective of *a posteriori* taxonomic assignment (Fig. 8). Accordingly, these analyses should be regarded as exploratory screening of variation at predefined candidate loci and as hypothesis generation for future studies, rather than as evidence for differential drought adaptation.

In contrast to the genome-wide analyses, which revealed structured variation among individuals, the candidate-gene dataset did not capture a comparably structured pattern (Fig. 7). This contrast is not unexpected, because drought adaptation in long-lived forest trees is generally regarded as a polygenic and physiologically integrated trait (Kremer and Le Corre, 2012; Kremer and Hipp, 2020; Müller and Gailing, 2019; Tischenko et al., 2026), such that modest adaptive differentiation may be distributed broadly across the genome rather than concentrated in a small set of a priori candidate loci. Under conditions of low overall genomic differentiation (Fig. 6) and ongoing gene flow (Figs. 2–5.), variation at candidate loci may remain limited even across a broader geographic scale within the same species. Our observation is fully

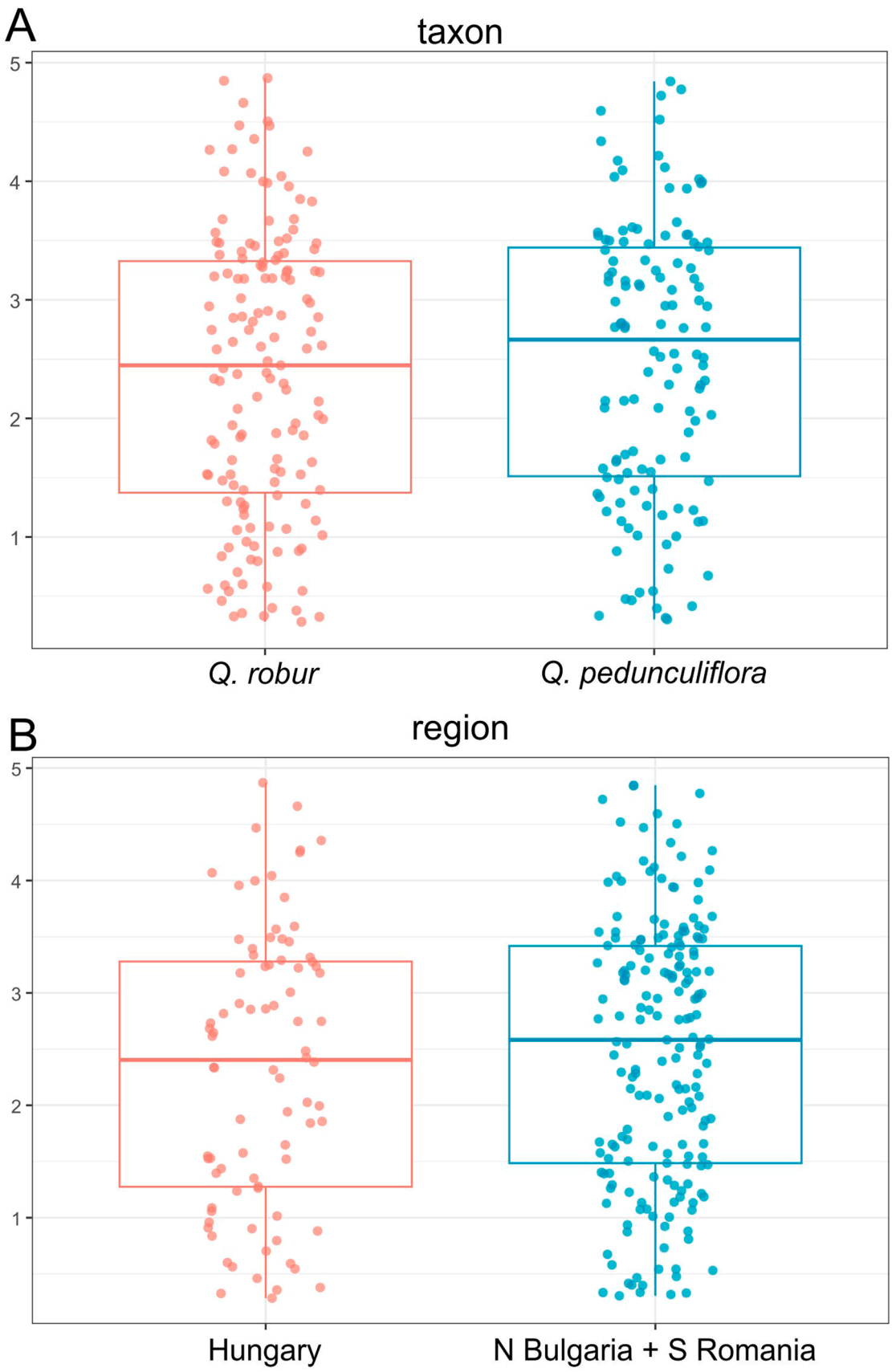


Fig. 8. Overall distribution of log-transformed normalised mapped dosage across the 21 drought-responsive candidate genes under two alternative grouping schemes. Each point represents one sample × gene observation, boxplots summarize medians and interquartile ranges, and whiskers indicate the spread of the data. (A) Comparison by taxon (*Q. robur* vs *Q. pedunculiflora*). (B) Comparison by geography (Hungary vs Balkan group, comprising northern Bulgaria and southern Romania).

consistent with the findings of Crăciunesc et al. (2015), who reported a lack of species-specific signal in allozyme variation of dehydrin genes in Romanian *Q. robur* and *Q. pedunculiflora*. Taken together, these results suggest that the candidate loci examined here do not provide a simple diagnostic signal separating the two taxonomic groups, but they do not allow conclusions about relative drought tolerance or adaptive performance.

At the same time, the limited sample size of the present study reduces the power to detect weak effects at the level of individual loci. Increasing sample size and genomic resolution will be necessary to determine whether these trends reflect biologically meaningful patterns or sampling-related variation. In addition, our opportunistic analysis focused on normalised mapped dosage, which is a useful exploratory proxy, but adaptive differences may also be reflected in allele-frequency shifts, regulatory variants, expression responses, phenology, and physiological plasticity (Lind et al., 2018; Feng et al., 2024). Direct inference of drought adaptation would additionally require independent environmental, phenotypic, physiological, or experimental evidence linking genomic variation to drought response (Popa et al., 2025; Hipp and Kremer, 2026). Finally, the white-oak literature supports the view that selection acts on a relatively small subset of loci, whereas much of the genome remains permeable to gene flow (Hardin, 1975; Hipp et al., 2019; Cannon and Petit, 2020; Kremer and Hipp, 2020; Ribicoff et al., 2025). This makes our result — namely, detectable genome-wide structure but no strong candidate-gene dosage separation — biologically plausible rather than contradictory. The candidate-gene results should therefore primarily be viewed as identifying patterns worthy of further testing in larger, environmentally characterised populations and in common-garden or physiological experiments.

4.3. The oak syngameon caught in the act

Oaks have long been regarded as one of the clearest examples of a syngameon (Cannon and Petit, 2020; Kremer and Hipp, 2020), that is, a set of otherwise distinct lineages connected by limited but recurrent gene exchange. Since the classic formulations of Lotsy, Hardin, Burger, and Van Valen (Hipp et al., 2019), the central paradox of oaks has been that closely related species can hybridise and introgress, yet remain morphologically, ecologically, and evolutionarily recognisable through time. Our genomic landscape recovered here is highly consistent with this view of oaks as a syngameon (Hardin, 1975). In such systems, lineage boundaries are not genome-wide impermeable, but semi-permeable: large parts of the genome may be exchanged relatively freely, whereas a more restricted subset of regions – perhaps those under divergent selection (Hipp et al., 2019) – resists introgression and contributes disproportionately to lineage integrity and ecological differentiation (Kremer and Hipp, 2020). As a consequence, oaks' nuclear genome evolves as a mosaic of discrete phylogenetic histories (Hipp et al., 2019, 2020; Plomion et al., 2018). At the genome-wide level, our analyses of the nuclear genome of *Q. robur* and *Q. pedunculiflora* closely accord with this interpretation: while the summary phylogenomic tree (Fig. 3A) displayed an elevated level of discordance between the underlying chromosomal information reflecting the mosaic nature of the oak genome, the concatenated phylogenomic tree (Fig. 3B), which can be regarded as a dominant average that maximises the global likelihood, recovered the two taxa as reciprocally monophyletic. This finding is in line with the observation of Hipp et al. (2019) that the oak genome retains an overarching phylogenetic signal despite its mosaic structure. The importance of this pattern lies precisely in the fact that introgression does not erase lineage identity: as in other oak complexes, the participating lineages remain morphologically and genomically coherent despite recurrent gene exchange (Cannon et al., 2024; Cannon and Petit, 2020). This framework also helps explain the contrast between the widespread sharing of plastid lineages (Fig. 2) and the stronger structure recovered from the nuclear genome (Figs. 3–5.): chloroplast capture may blur maternal history, whereas species cohesion is more strongly maintained at selected regions of the nuclear genome (Kremer and Hipp, 2020).

Similarly, the *k*-means clustering analysis (Fig. 5) based on 50k highly outlying SNPs can be regarded as a reconstruction of the discriminatory analysis of North-American white oaks (Hipp et al., 2019); just as there, these highly outlying SNPs were also found to be partly introgressed in a small proportion in some samples. The landscape of genomic islands of isolation is particularly well represented in our windowed F_{ST} landscape (Fig. 6), providing perhaps the clearest genomic expression of this pattern: most windows show low differentiation, consistent with broad genomic sharing, but this low background is punctuated by repeatable peaks of elevated divergence across multiple chromosomes. These peaks must be, at least partly, overlapping with the outliers recovered for the *k*-means clustering, and they perhaps represent loci responsible for trait divergence fuelling ecological divergence (Cavender-Bares and Pahlich, 2009). In this sense, the semi-permeable structure of oak genomes may be evolutionarily advantageous, because it allows lineage cohesion to be maintained while still permitting the movement of potentially adaptive variation across taxonomic boundaries (Cannon et al., 2024; Cannon and Petit, 2020; Kremer and Hipp, 2020). Notably, when we examined whether any of the 21 predefined drought-responsive candidate loci overlapped the top 1% of high-F_{ST} windows, we found no overlap. Thus, the strongest differentiation peaks in our dataset do not coincide with these predefined drought-response candidates.

In sum, we regard our findings as adding the *Q. robur–Q. pedunculiflora* system to the growing body of evidence that oak evolution proceeds through the interaction of lineage cohesion, ecological differentiation, and persistent introgression rather than through complete reproductive isolation. Having said that, we must emphasise that our comparison is restricted to only two, shallowly diverged taxa in the European white oak syngameon. More generally, the oak syngameon is best understood not simply as a set of isolated species pairs, but as a dynamic network of partially interfertile lineages, in which local introgression events may be embedded within a larger web of historical and ecological connections (Cannon and Petit, 2020; Cannon et al., 2024). The oak syngameon is not just a pattern of gene flow, but an evolutionary system that combines lineage cohesion, semi-permeable genomic barriers, and the potential for adaptive introgression (Cannon and Petit, 2020; Kremer and Hipp, 2020).

4.4. Implications for climate-adaptive forest management

Climate change is expected to substantially alter the environmental conditions of Central European forests in the coming decades. Projections indicate increasing mean temperatures, more frequent heat-stress events, and decreasing precipitation during the growing season (Bartholy et al., 2014; IPCC, 2021). These trends are likely to intensify drought stress and may threaten the long-term persistence, regeneration, and productivity of lowland oak forests (Krutovsky et al., 2025). In this context, climate-adaptive forestry and the use of climatically suitable forest reproductive material are receiving increasing attention in European forest policy and management (European Environment Agency, 2025; Stanturf et al., 2026). Southern and south-eastern provenances of widespread European tree species are therefore increasingly considered as potentially valuable genetic resources for forestry under warmer and drier future conditions (Bartha et al., 2018; Mátyás et al., 2018).

Against this background, our results offer a cautious but relevant perspective for management. Genome-wide analyses support the interpretation of *Q. pedunculiflora* as a weakly differentiated lineage within the *Quercus robur* complex rather than as a fully isolated species, and no evidence for the occurrence of *Q. pedunculiflora* in the sampled Hungarian material. The exploratory analysis of drought-responsive candidate genes did not reveal statistically supported differences between Hungarian and Balkan regions. Consequently, the present results do not

provide sufficiently strong genomic support for the immediate large-scale operational transfer of reproductive material from northern Balkan populations into Hungarian forests. However, the absence of strong genomic barriers, together with the overall genomic compatibility observed among trees, suggests that assisted gene flow initiatives, assisted by carefully designed provenance trials (Mátyás, 1994), may represent appropriate next steps for enhancing the adaptive potential of Pannonian *Q. robur* stands under future climatic conditions.

Such a stepwise approach is consistent with current thinking in climate-adaptive forestry, where the goal is not to replace local gene pools prematurely, but to test whether carefully selected non-local material can improve forest resilience without eroding locally adapted genomic backgrounds (St. Clair et al., 2020). Similar approaches are already being explored in other European forest tree systems. For example, populations of *Fagus orientalis* from south-eastern Europe and western Asia are being evaluated as potential sources of adaptive genetic variation for European beech forests, while experimental studies have also highlighted the need for careful long-term assessment of ecological and genetic consequences (Kurz et al., 2023).

Beyond their immediate practical value as potential donor populations, the south-eastern trees studied here may also be important for maintaining evolutionary processes within the European white-oak syngameon. The semi-permeable species boundaries characteristic of oaks may facilitate the redistribution of adaptive genetic variation through introgression, as has been demonstrated in other oak lineages (Leroy et al., 2020). In fragmented landscapes, such processes may help maintain adaptive potential and reduce the negative effects of isolation by enabling continued exchange of genetic variation among partially differentiated lineages (Pino et al., 2024). This may be especially relevant in south-eastern Europe, where many oak populations persist as relatively isolated forest fragments (Curtu et al., 2017).

Taken together, our results suggest that south-eastern populations of the *Q. robur* complex may represent valuable genetic resources for future climate-adaptive forestry in Central-Eastern Europe. The present data do not justify immediate operational transfer of reproductive material; they do support the conservation and further evaluation of these stands through expanded genomic sampling, provenance trials, and small-scale assisted gene-flow experiments aimed at enhancing forest resilience under future climatic conditions.

5. Conclusions

This study provides a genome-wide perspective on the taxonomic status and genomic patterns of the *Q. robur* complex in Central and South-Eastern Europe. In light of the oak syngameon, our results support the interpretation of *Q. pedunculiflora* as a weakly differentiated lineage consistent with subspecific rank as *Q. robur* subsp. *pedunculiflora*, rather than as a distinct species or a simple ecological variant of *Q. robur*. Clearly, a range-wide phylogeography within the *Q. robur* complex using highly discriminatory SNPs (see Hipp et al., 2019) can deliver a firm test to our taxonomic treatment. Although candidate gene analyses did not detect significant differences in drought-related loci, genome-wide data revealed structured variation among regions, suggesting that adaptive differentiation may be subtle and distributed across the genome. Given the ecological importance of *Q. robur* as a keystone species, the recognition of *Q. pedunculiflora* as *Q. robur* subsp. *pedunculiflora* highlights its potential relevance to climate-adaptive forest management, particularly for identifying tree taxa suitable for future climatic conditions in Hungary (Bartha et al., 2018). In particular, trees from northern Bulgaria and southern Romania may represent a valuable source of genetic variation to consider in forest regeneration or stand conversion efforts under changing environmental conditions. However, the limited number of samples analysed means the present results should be interpreted as a first genomic assessment rather than a definitive characterisation of the entire regional gene pool. Nevertheless, they provide the first robust genomic framework for future studies and offer a valuable basis for integrating evolutionary and applied perspectives in climate-adaptive forest management in Central-Eastern Europe.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT v5.4 to improve the English phrasing and flow of the manuscript. As none of the authors is a native speaker of an Indo-European language, we used the large language model for language editing and readability improvement, without changing the scientific meaning of our original text and without generating text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Ivett Hegedüs: Writing – review & editing, Writing – original draft, Visualization, Project administration, Formal analysis, Data curation, Conceptualization. **Dénes Bartha:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Nozim Shodiev:** Writing – review & editing, Resources, Methodology, Data curation. **Gábor Sramkó:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gábor Sramkó reports financial support was provided by National Research Development and Innovation Office. Ivett Hegedüs reports financial support was provided by University of Sopron Faculty of Forestry. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2026.101528.

Data availability

Raw Illumina sequencing reads were deposited in the National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA), under accession number PRJNA1496900 (to be released).

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