

Supplementary Materials

S1 — Genotyping procedure and cpDNA haplotypes

The DNA was extracted from silica-dried cambium samples using a commercial Qiagen DNeasy 96 Plant Kit (Qiagen, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of DNA was assessed by NanoDrop Spectrophotometer ND-1000 (Thermo Scientific) and gel electrophoresis with 1.00% (w/v) agarose. The samples were diluted to 3–10ng/μl concentration before PCR. For genotyping, ten plastid primer pairs selected from Deguilloux et al. (2003) and Weising & Gardner (1999) were analyzed to estimate the autochthony and eight nuSSRs primer pairs described by Steinkellner et al. (1997) and Kampfer et al. (1998) to describe patterns of genetic structure and diversity. These were amplified by PCR in multiplex reactions (Table S1) with the QIAGEN Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany) in a standard reaction mixture of 10 μl. The mixture contained standardly 5μl of 2x Type-it Multiplex PCR Master Mix (Qiagen), 3μl of RNase-free water (Qiagen), 1μl of the primer mix, and 1μl of 3–10ng template DNA.

Nuclear SSRs

The standard PCR program (for all multiplexes) consisted of the following steps: initial denaturation at 95°C for 15 min, min 30 cycles with denaturation at 94°C for 30 sec, followed by primer annealing at 56°C for 90 sec, and then extension at 72°C for 30 sec, ending with a final extension step at 60°C for 30 min. Primer sequences, fluorescent dyes used, and multiplex combinations are presented in Table S1.

The concentration of the amplified fragments after PCR was estimated by electrophoresis on a 1.5 % agarose gel. The PCR products of each reaction were diluted in HPLC water based on the observed concentration on the agarose gel. We subsequently carried out capillary electrophoresis using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc. CA) and the internal 400bp Size Standard (Analisis, Netherlands). The sizing of fragments was performed using the software GeneMapper v5.0 (Applied Biosystems).

cpSSRs

The cpSSRs were all combined into one multiplex. PCR was carried out in a GeneAmp® PCR-system 9700 thermocycler (Applied Biosystems) using following program consisting of following steps: initial denaturation at 96°C for 1 min and 35 cycles with denaturation at 96°C for 10 s, primer annealing at 50°C for 5 s and extension at 72°C for 1 min. Prior to capillary electrophoresis, the PCR reactions were purified applying gel filtration with use of Sephadex G-50. CpSSRs genotypes were resolved on an ABI PRISM™ 3100 DNA Genetic Analyzer (Applied Biosystems. Inc., Foster City, California, USA). The sizing of fragments was carried out with a GeneMarker v 1.8 (Applied Biosystems), utilizing the internal GENESCAN™-500 ROX™ Size Standard (Applied Biosystems).

Table S1. Summary of genotype loci including locus name, type, primer sequences, fluorescent dyes, multiplex combinations (including concentration in the multiplex of each primer), measured fragment sizes, and sources of primers.

Locus	Marker	Forward	Reverse	Fluorescent Dye	Multiplex	Reference
QpZAG15	nSSR	CGATTTGATAATGACACTATGG	CATCGACTCATTGTAAGCAC	Cyanine 5 (blue)	Mix1 (0.2 µM)	Steinkellner et al. (1997)
QpZAG104	nSSR	ATAGGGAGTGAGGACTGAATG	GATGGTACAGTAGCAACATTC	IRD700 (green)	Mix1 (0.4 µM)	Steinkellner et al. (1997)
QpZAG110	nSSR	GGAGGCTTCCTCAACCTACT	GATCTCTTGTTGCTGTATTT	Cyanine 5 (blue)	Mix2 (0.6 µM)	Steinkellner et al. (1997)
QrZAG7	nSSR	CAACTTGGTGTTCCGATCAA	GTGCATTTCTTTTATAGCATTCAC	DY-751 (yellow)	Mix2 (0.6 µM)	Kampfer et al. (1998)
QrZAG65	nSSR	CAGTGGTGTCAACTCCTCCCAG	GTCAGGTGACCATTCAAACCTAGAA	Cyanine 5 (blue)	Mix3 (0.6 µM)	Kampfer et al. (1998)
QrZAG87	nSSR	TCCCACCACTTTGGTCTCTCA	GTTGTCAGCAGTGGGATGGGTA	Cyanine 5 (blue)	Mix3 (0.1 µM)	Kampfer et al. (1998)
QrZAG5b	nSSR	TGAAGAGTAAGACCATTACATCA	GTATGTGAGTGTTTGTGGTTTGG	IRD700 (green)	Mix3 (0.8 µM)	Kampfer et al. (1998)
QrZAG20	nSSR	CCATTAAAAGAAGCAGTATTTTGT	GCAACACTCAGCCTATATCTAGAA	DY-751 (yellow)	Mix3 (0.6 µM)	Kampfer et al. (1998)
µdt1	cpSSR	ATCTTACACTAAGCTCGGA	TTCAATAACTTGTTGATCCC	6-Fam (blue)	Mix4 (0.3 µM)	Deguilloux et al. (2003)
ccmp6	cpSSR	CGATGCATATGTAGAAAAGCC	CATTACGTGCGACTATCTCC	6-Fam (blue)	Mix4 (0.8 µM)	Weising und Gardner (1999)
µkk4	cpSSR	TTGTTTACCTATAATTGGAGC	TAGCGGATCGGTTCAAACTT	6-Fam (blue)	Mix4 (0.8 µM)	Deguilloux et al. (2003)
µdt4	cpSSR	GATAATATAAAGAGTCAAAT	CCGAAAGGTCCTATACCTCG	6-Fam (blue)	Mix4 (4.0 µM)	Deguilloux et al. (2003)
µcd5	cpSSR	CCCCCGGATCTCTGTCAACTG	TAATAAACGAGAATCACATAA	HEX (green)	Mix4 (0.5 µM)	Deguilloux et al. (2003)
µkk3	cpSSR	TTAGATCGGGTAATCGTTCAA	AAGTGAATAAATGGATAGAGC	HEX (green)	Mix4 (0.4 µM)	Deguilloux et al. (2003)
ccmp2	cpSSR	GATCCCGGACGTAATCCTG	ATCGTACCGAGGGGTTTCAAT	HEX (green)	Mix4 (0.5 µM)	Weising und Gardner (1999)
µcd4	cpSSR	TTATTTGTTTTTGGTTTCACC	TTTCCCATAGAGAGTCTGTAT	Atto 550 (yellow)	Mix4 (0.8 µM)	Deguilloux et al. (2003)
ccmp10	cpSSR	TTTTTTTTTAGTGAACGTGTCA	TTCGTCGDCGTAGTAAATAG	Atto 550 (yellow)	Mix4 (0.8 µM)	Weising und Gardner (1999)
µdt3	cpSSR	TGTTAGTAATCCTTTCGTTT	AGGTATAAAGTCTAAGGTAA	Atto 550 (yellow)	Mix4 (0.8 µM)	Deguilloux et al. (2003)

All forward primers were labelled with a fluorescent dye.

S2 — Selection and characterization of three drought events

Drought events were determined based on the Standardized Precipitation Evapotranspiration Index (SPEI; Vicente-Serrano et al. 2010), using the R package SPEI (Beguería et al. 2014). As input data, homogenized weather station data from the Geosphere Austria meteorological station Retz (Lat: 48.754, Lon: 15.950) was used. (<https://www.zamg.ac.at/histalp>). This weather station, which is located approximately 10-20km from the studied oak populations, covered monthly data for precipitation and mean temperature from 1895 to 2020 (see Figure S1b). Potential evapotranspiration (PET) was calculated using mean temperature and latitude by applying the Thornthwaite-Function (R-package SPEI). SPEI was calculated using a 6-month time period.

The event's lowest SPEI value was seen in 2006 (-2.69). Due to an overlap with the event of 2000, it was decided to use the period 1992–1994 as the first drought event. 1992 marked the first year with a mean annual temperature higher than 10°C, followed by another record-breaking year, 1994 (10.5°C), while the whole period showed below-average precipitation levels. The event 1992–1994 had an average SPEI of -0.91 and an average yearly minimum SPEI of -1.95, minimum SPEI of -1.95, followed by a period 1995–1997 with an average SPEI of 0.22. 1987 serves as wet year control (619mm annual rainfall compared to an average of 483mm for 1980–1990) with average conditions before (1984–1986: 519mm) and afterward (1988–1991: 452mm). In addition to the drought event (1992–1994), two additional historical drought events (1917, 1947) were selected similarly. The year 1917 represents the year with the least precipitation sum of collected time series (1895–2020), showing an average SPEI of -1.24 and minimum SPEI of -2.11. 1947 was a year with low precipitation, occurring after a longer season (1935–1945) with good precipitation conditions during the growing season (May–September). The average SPEI for 1947 was -0.18 and minimum SPEI was -1.63.

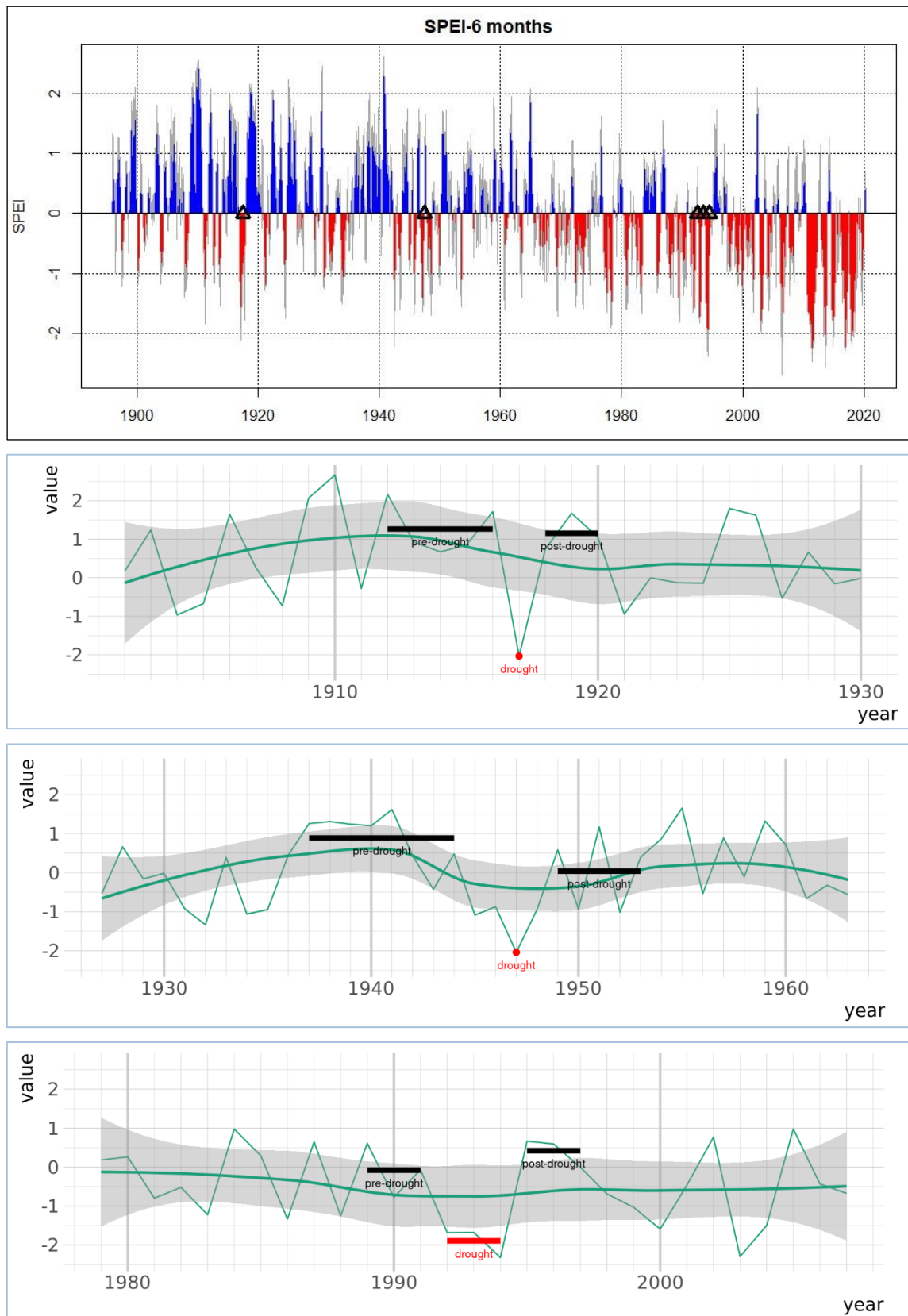


Figure S1a. Selected drought years and the SPEI time series. This figure displays the monthly SPEI (6-month aggregation length) for weather station Retz, highlighting the selected drought events (1917, 1947, 1992-1994) with triangular markers. In addition, the selected drought years with their pre-drought

and post-drought periods are shown. The X-axis denotes the years, and the Y-axis the annual vegetation period SPEI values (6th month ending in September).

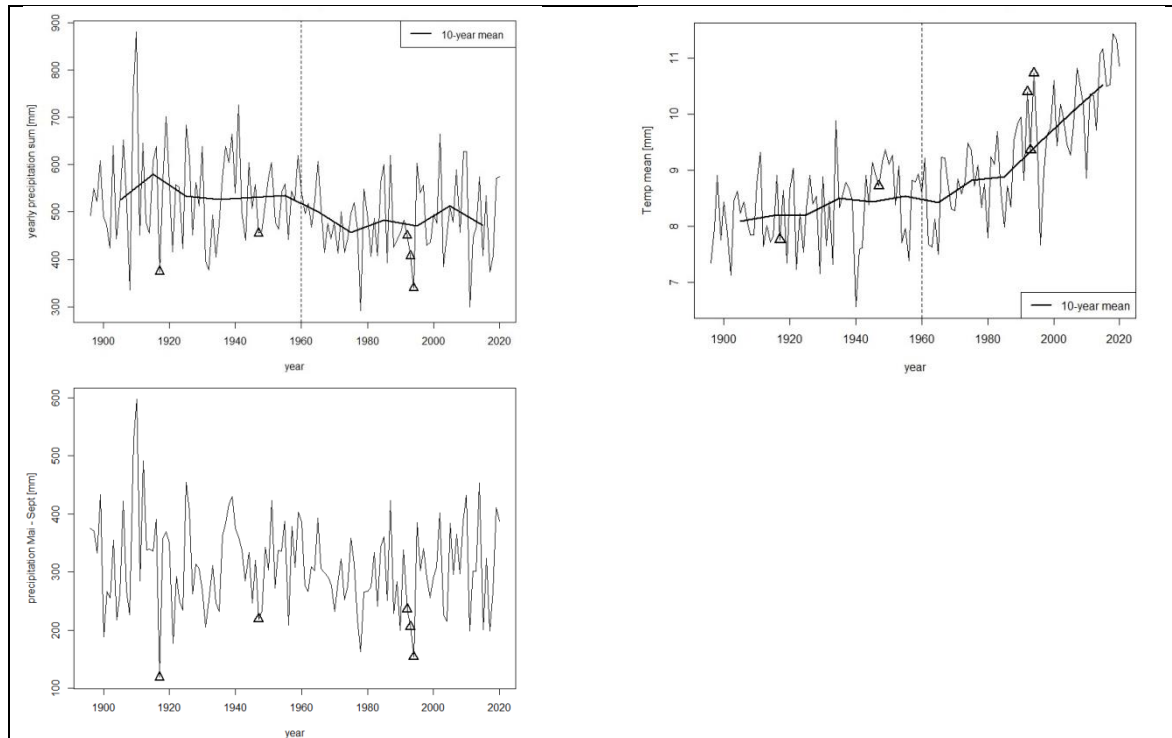


Figure S1b. Trends in mean annual precipitation, mean seasonal precipitation, and mean temperature estimated for the period 1900–2020 derived from the climatic data measured at station Retz. The triangles depict the years used for analysis of drought tolerance (1917, 1947, 1992, 1993, 1994).

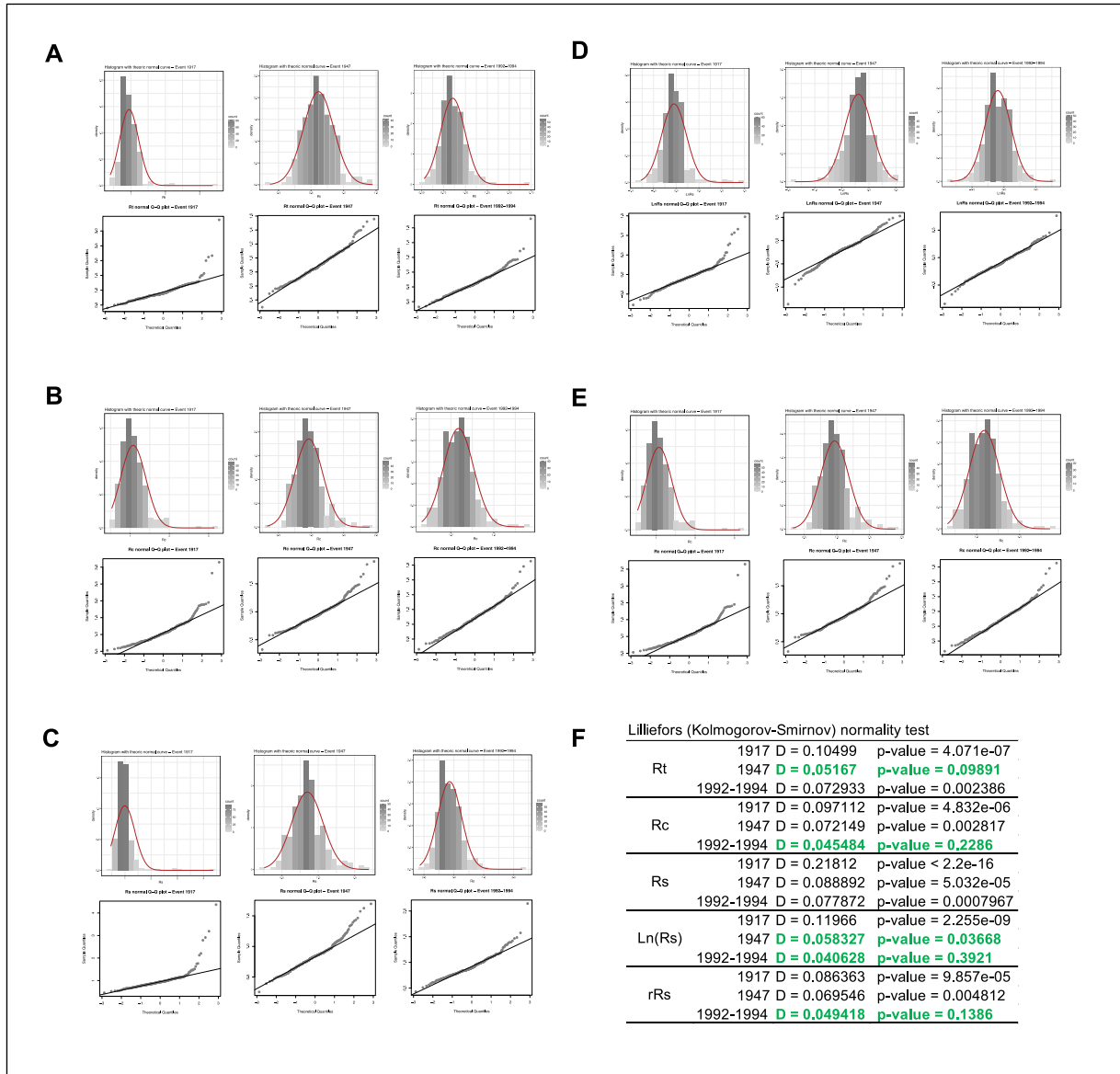


Figure S2. Histogram with normal distribution curve (red line) and Q-Q plot for all traits on all studied drought events A) Resistance. B) Recovery. C) Resilience. D) Natural logarithm of resilience. E) Relative resilience. F) Lilliefors test results for all traits and drought events, green highlighted those that significantly ($p\text{-value} \geq 0.01$) fit a normal distribution.

S3 — Population characterization specific measures of genetic variation

Table S2. Reference samples with different haplotypes revealed by PCR-RFLP analysis (Petit et al. 2002 and Tutková-van Loo & Burg 2004).

Sample	Locality	Species	Longitude	Latitude	Altitude	Country	Haplotype
A005	Klein Meiseldorf	<i>Q. robur</i>	15.828	48.676	330	Austria	17a
A007	Losenstein	<i>Q. robur</i>	14.464	47.935	400	Austria	7
A015	Hartberg	<i>Q. petraea</i>	16.090	47.673	490	Austria	5
A018	Altenburg	<i>Q. r x Q. p</i>	15.575	48.637	425	Austria	17a
A023	Altenburg	<i>Q. petraea</i>	15.585	48.624	360	Austria	17a
A026	Altenburg	<i>Q. petraea</i>	15.585	48.624	360	Austria	17a
A029	Schönbühel a.d. Donau	<i>Q. petraea</i>	15.362	48.273	315	Austria	2
A033	Karlstetten	<i>Q. petraea</i>	15.533	48.290	350	Austria	2
A038	Weyersdorf	<i>Q. petraea</i>	15.514	48.283	435	Austria	2
A043	Krems	<i>Q. petraea</i>	15.493	48.556	400	Austria	17a
A045	Krems	<i>Q. robur</i>	15.493	48.556	400	Austria	17a
A047	Krems	<i>Q. robur</i>	15.493	48.556	400	Austria	17a
A053	Etzmannsdorf	<i>Q. petraea</i>	15.642	48.615	395	Austria	2
A065	Waidhofen a.d. Ybbs	<i>Q. robur</i>	14.649	48.060	295	Austria	7
A071	Seitenstetten	<i>Q. robur</i>	14.795	47.970	340-360	Austria	7
A092	Karlsberg	<i>Q. robur</i>	14.328	46.716	650-690	Austria	7
A123	Trieben	<i>Q. petraea</i>	14.871	46.636	500-700	Austria	5
A144	Finkenstein, Ruine	<i>Q. petraea</i>	13.904	46.540	780-820	Austria	7
A166	Weissenstein	<i>Q. robur</i>	13.704	46.689	550-570	Austria	7
A188	Geschriebenstein	<i>Q. petraea</i>	16.425	47.381	700	Austria	2
A236	Breitenstein	<i>Q. petraea</i>	15.878	47.670	680	Austria	5
A241	Payerbach	<i>Q. petraea</i>	15.898	47.685	570	Austria	5
A246	Gleibsfeld	<i>Q. petraea</i>	16.148	47.680	380	Austria	5
A250	Gleibsfeld	<i>Q. petraea</i>	16.148	47.680	380	Austria	5
A251	Warth	<i>Q. petraea</i>	16.144	47.656	410	Austria	5
A256	Hafning	<i>Q. petraea</i>	16.108	47.688	500	Austria	5
A260	Hafning	<i>Q. r x Q. p</i>	16.108	47.688	500	Austria	5
A261	Schrattenbach	<i>Q. petraea</i>	16.003	47.786	560	Austria	5
A327	Altenmarkt	<i>Q. robur</i>	14.644	47.720	500	Austria	7
A330	Altenmarkt	<i>Q. robur</i>	14.644	47.720	500	Austria	7
A339	Galgenbühel	<i>Q. robur</i>	14.813	47.186	730	Austria	1
A343	Lamprechtshausen	<i>Q. robur</i>	12.939	47.959	425	Austria	1
A344	Lamprechtshausen	<i>Q. robur</i>	12.939	47.959	425	Austria	1
A371	Proleb, S-Hang	<i>Q. robur</i>	15.115	47.394	750	Austria	7
A374	Stoitzendorf	<i>Q. petraea</i>	15.861	48.656	310-320	Austria	5
A375	Stoitzendorf	<i>Q. petraea</i>	15.861	48.656	310-320	Austria	6
A378	Stoitzendorf	<i>Q. robur</i>	15.861	48.656	310-320	Austria	6

A379	Stoitzendorf	<i>Q. robur</i>	15.861	48.656	310-320	Austria	6
A380	Stoitzendorf	<i>Q. petraea</i>	15.861	48.656	310-320	Austria	6
A381	Stoitzendorf	<i>Q. petraea</i>	15.861	48.656	310-320	Austria	6
A382	Stoitzendorf	<i>Q. robur</i>	15.861	48.656	310-320	Austria	6
A385	Hochstraß	<i>Q. robur</i>	13.653	48.421	570	Austria	1
A386	Hochstraß	<i>Q. robur</i>	13.653	48.421	570	Austria	1
A387	Hochstraß	<i>Q. robur</i>	13.653	48.421	570	Austria	1
A390	Kritzling	<i>Q. robur</i>	13.518	48.546	430	Austria	7
A394	Dötzledt	<i>Q. robur</i>	13.728	48.285	440	Austria	1
A395	Wendling	<i>Q. robur</i>	13.636	48.196	410	Austria	1
A396	Poppreith	<i>Q. robur</i>	13.636	48.196	410	Austria	1
A398	Aistersheim	<i>Q. robur</i>	13.720	48.203	440	Austria	1
A399	Aistersheim	<i>Q. robur</i>	13.720	48.203	440	Austria	1
A405	Unterscharten	<i>Q. robur</i>	14.017	48.162	400	Austria	2
A428	Dunkelsteinerwald	<i>Q. robur</i>	15.376	48.226	400	Austria	2
A434	Wachberg	<i>Q. robur</i>	15.354	48.202	280	Austria	2
A435	Gmünd	<i>Q. robur</i>	15.002	48.754	520	Austria	17a
A437	Gmünd	<i>Q. robur</i>	15.002	48.754	490	Austria	17a
A459	Oberndorf	<i>Q. robur</i>	12.417	47.519	740	Austria	7
A520	Litschau	<i>Q. robur</i>	15.026	48.931	-	Austria	1

Table S3. Genotypes of haplotypes revealed by PCR-RFLP analysis Petit et al. (2002) in the present analysis with 10 cpSSR markers.

Haplotype	μdt1	μdt4	μkk4	ccmp6	μcd4	μdt3	ccmp10	ccmp2	μkk3	μcd5
1	79	145	111	102	101	127	117	165	97	74
2	79	145	111	102	99	127	116	165	97	74
5-6-7	79	144	110	102	100	128	117	164	97	74
17a	80	164	110	102	102	127	116	164	97	75

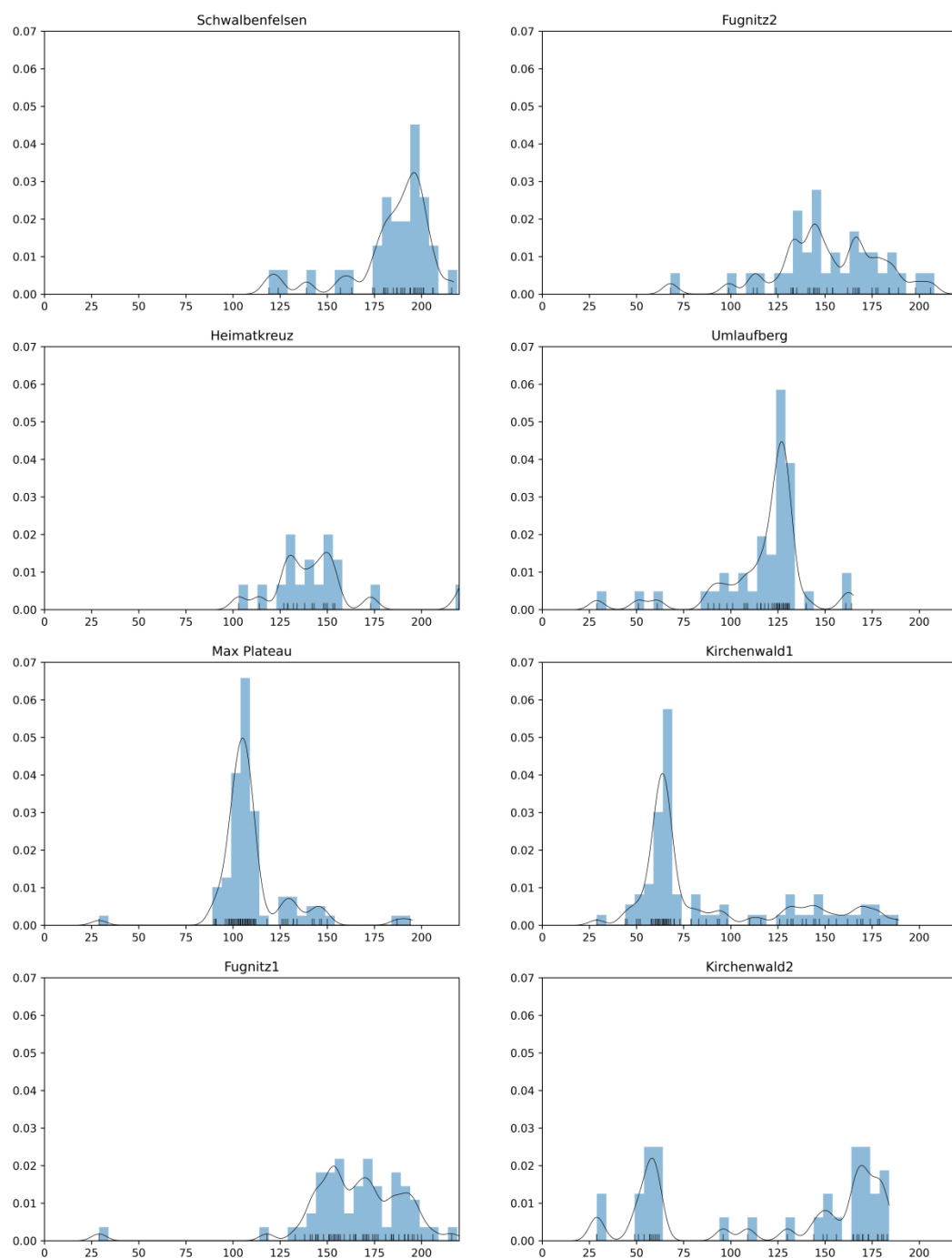


Figure S3. Histograms of tree ages (year 2023) for each location under study with age at the x-axis and frequency at the y-axis.

Table S4. Measures of genetic diversity within populations based on nuclear SSR. N = number of genotyped; Na = mean number of alleles per locus; Ho = observed heterozygosity; Hs = expected heterozygosity; pG = p-value for Gis; Ar = allelic richness (standardized number of alleles after rarefaction; rarefaction size = 30 individuals), centroid coordinates.

Population	Abb.	N	Na	Ho	Hs	Gis	pG	Ar	Latitude Longitude	Age (2023) Age M (2023)
Schwalbenfelsen	SF	32	16.5(± 1.2)	0.769 (±0.069)	0.89 (±0.019)	0.135 (±0.078)	0.001	12.43 (± 0.77)	48,8661 15,8400	119-216 183,9
Heimatkreuz	HK	38	17.8(± 1.7)	0.785 (±0.081)	0.89 (±0.022)	0.118 (±0.083)	0.001	12.73 (± 1.11)	48,8722 15,8404	103-245 182,7
Maxplateau	MP	83	20.4(± 2.0)	0.763 (±0.092)	0.868 (±0.034)	0.121 (±0.086)	0.001	12.10 (± 0.89)	48,8552 15,8505	90-194 111,2
Fugnitz1	FG1	60	19.3(± 2.5)	0.804 (±0.082)	0.884 (±0.023)	0.09 (±0.074)	0.001	12.24 (± 1.25)	48,8438 15,8432	110-229 168,6
Fugnitz2	FG2	37	13.6(± 1.5)	0.740(±0.0 80)	0.875 (±0.016)	0.154 (±0.092)	0.001	10.70 (± 0.83)	48,8490 15,8509	60-237 155,6
Umlaufberg	UB	44	16.3(± 1.7)	0.802 (±0.052)	0.874 (±0.026)	0.083 (±0.058)	0.001	11.66 (± 1.15)	48,8444 15,8933	51-164 118,9
Kirchenwald1	KW1	76	15.8(± 1.8)	0.762 (±0.090)	0.759 (±0.059)	-0.004 (±0.125)	0.498	9.011 (± 1.12)	48,8212 15,9428	44-188 90,3
Kirchenwald2	KW2	32	14.3(± 1.4)	0.761 (±0.092)	0.824 (±0.037)	0.077 (±0.098)	0.002	10.60 (± 0.99)	48,8213 15,9466	49-183 125,1

Table S5. Results of pairwise values of D_{JOST} (2008) calculated in R package *diveRcity* (Keenan et al. 2013) with associated confidence interval in [].

D_{JOST} diversity							
Population	Schwalbenfelsen	Heimatkreuz	Max Plateau	Fugnitz1	Fugnitz2	Umlaufberg	Kirchenwald1
Schwalbenfelsen	0,0041 [-0,0517; 0,0728]						
Heimatkreuz		0,0034 [-0,0058; 0,1269]					
Maxplateau			0,0018 [-0,0456; 0,0605]				
Fugnitz1				0,0351 [-0,0026; 0,0839]			
Fugnitz2					0,0772 [0,0412; 0,2256]		
Umlaufberg						0,0586 [-0,0036; 0,1343]	
Kirchenwald1							0,0685 [0,0415; 0,1621]
Kirchenwald2							
	0,1028 [0,0286; 0,1899]	0,1123 [0,0454; 0,1904]	0,1418 [0,0716; 0,2204]	0,1267 [0,0619; 0,2112]	0,1542 [0,0619; 0,2668]	0,092 [0,027; 0,1696]	0,0254 [-0,0122; 0,0816]

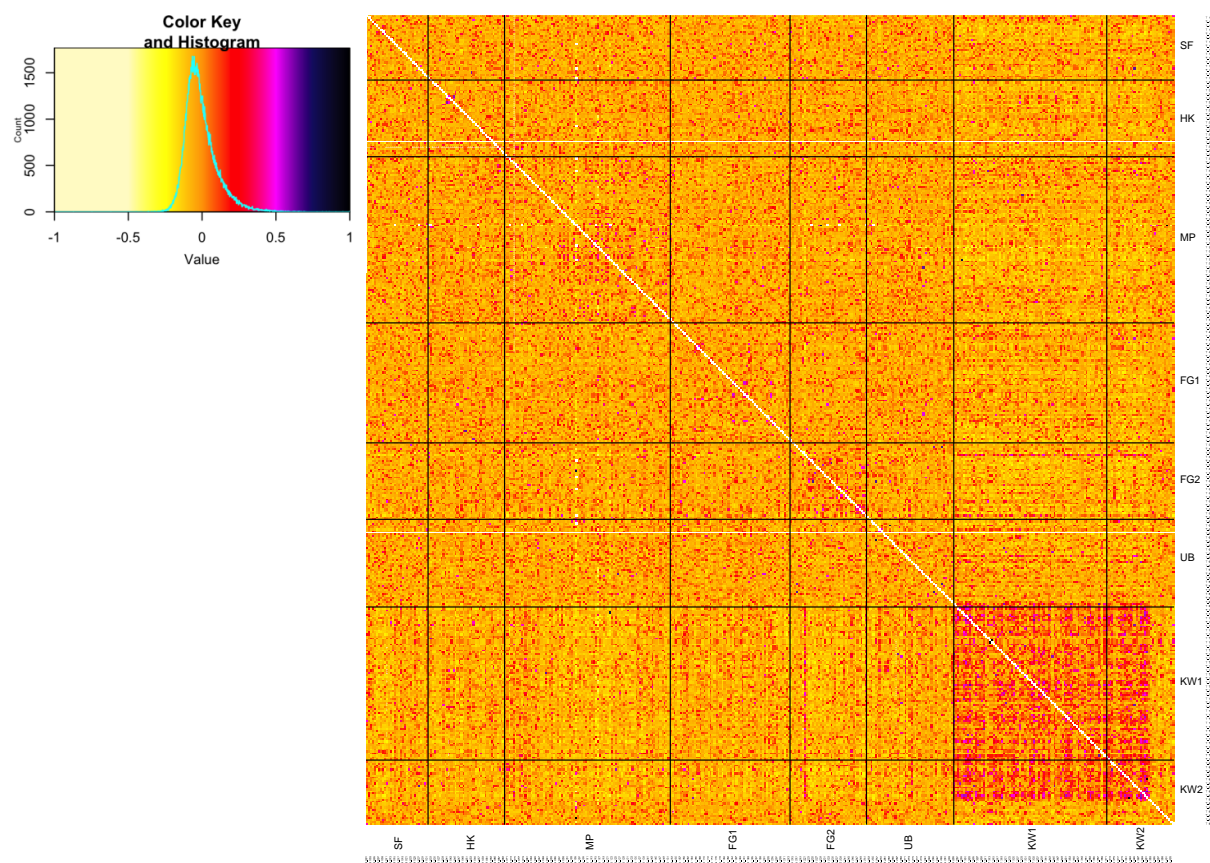


Figure S4. Heatmap plot of genetic relatedness matrix calculated in PolyRelatedness.

S4 — Analyses and selection of drought-resistant phenotypes

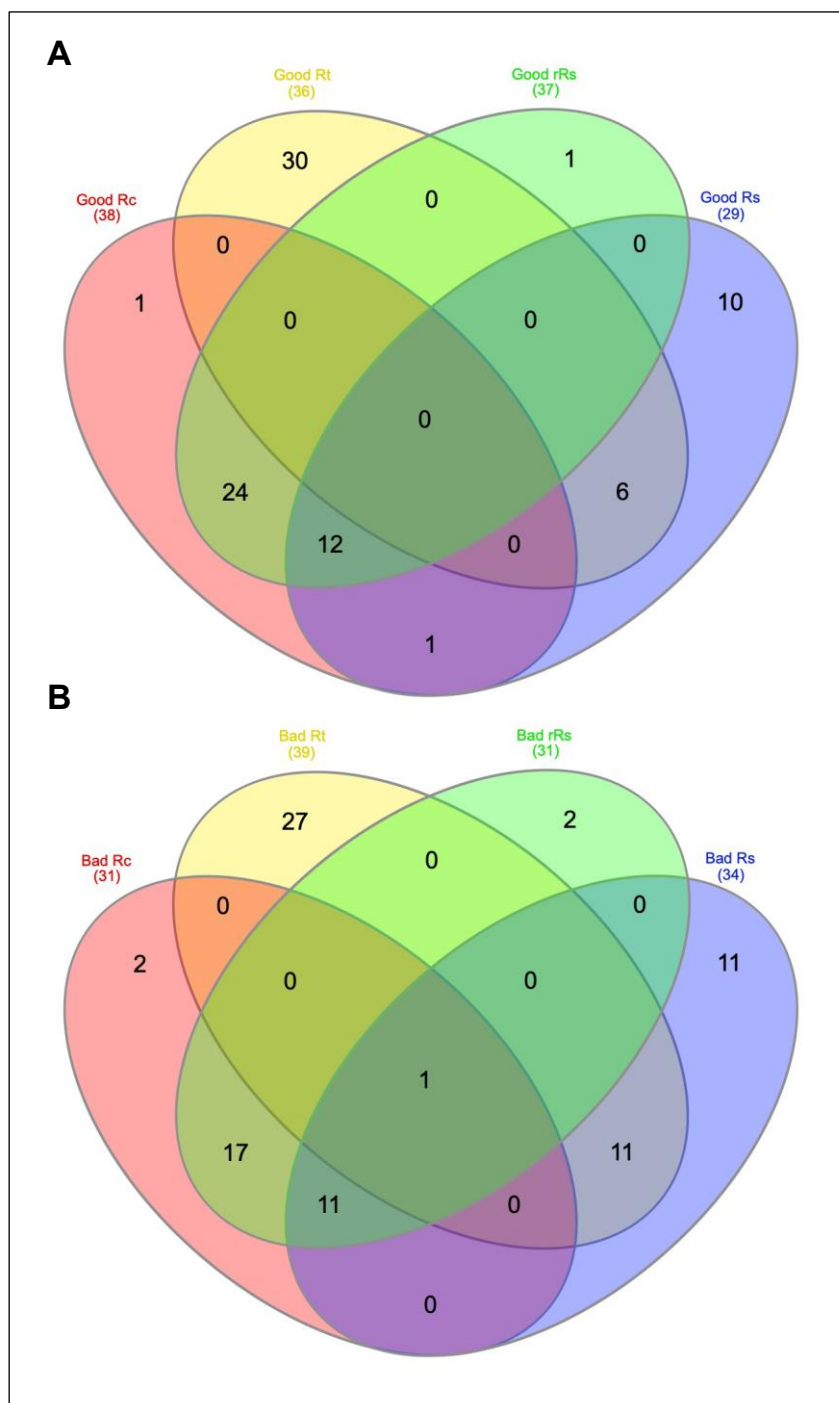


Figure S5. Venn diagrams for selected genotypes in both A) “good” and B) “bad” performance on the traits under study.

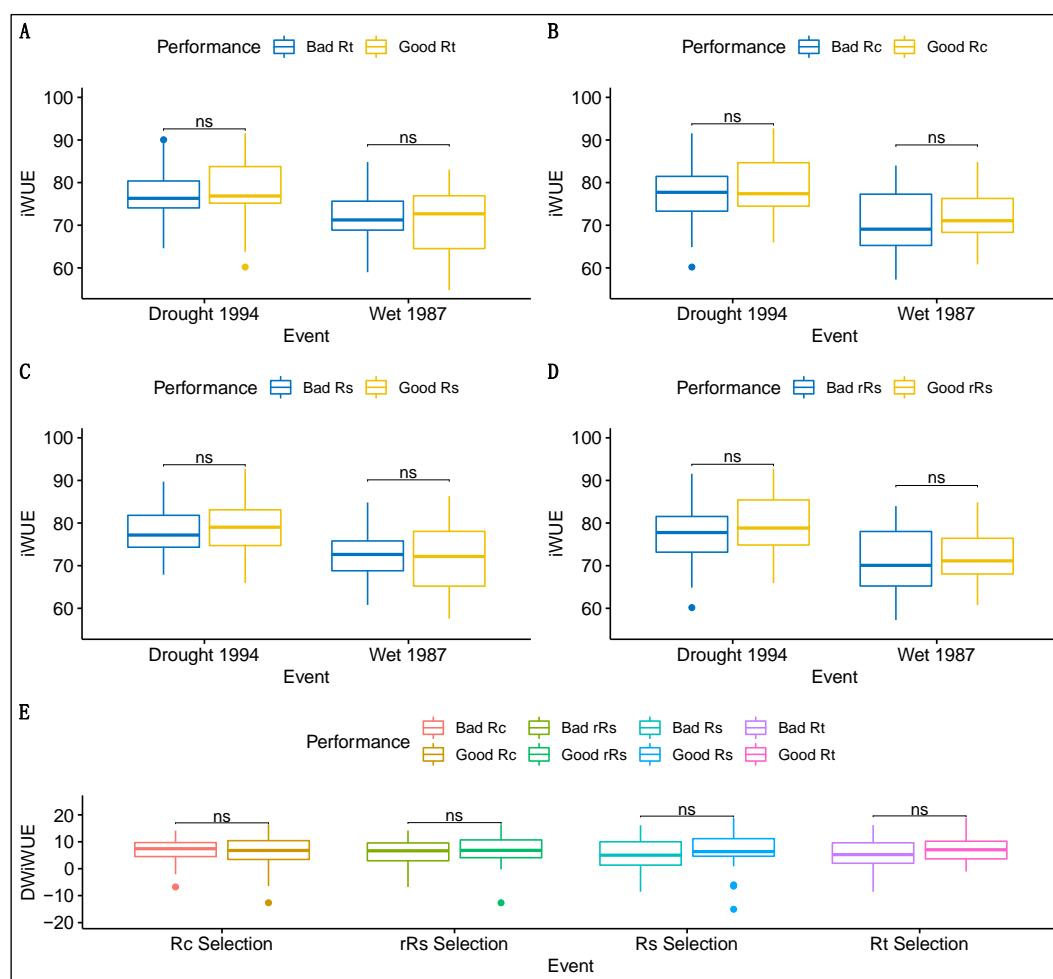


Figure S6. Box plots of intrinsic water use efficiency (iWUE) for selected groups (good and bad) for each trait, when isotopic analysis of $\delta^{13}\text{C}$ of latewood was performed: wet (1987) and dry (1994) years. A) Resistance. B) Recovery. C) Resilience. D) Relative resilience. E) Difference between dry and wet values (DWiWUE) box plots for all traits and groups. All pairwise t-test comparisons showed no significant differences ($p\text{-value} > 0.05$ - ns).

Table S6. Significant repeatability estimates ($p\text{-value} \leq 0.05$) for each studied trait including the standard error and 95% confidence interval.

Song characteristic	R estimate	standard error (SE)	95% Confidence interval	P value
Resistance (R_t)	0.082	0.038	0.003, 0.154	0.0145
Recovery (R_c)	0.088	0.035	0.019, 0.159	0.0059
Relative Resilience (rRs)	0.065	0.035	0.000, 0.133	0.0324

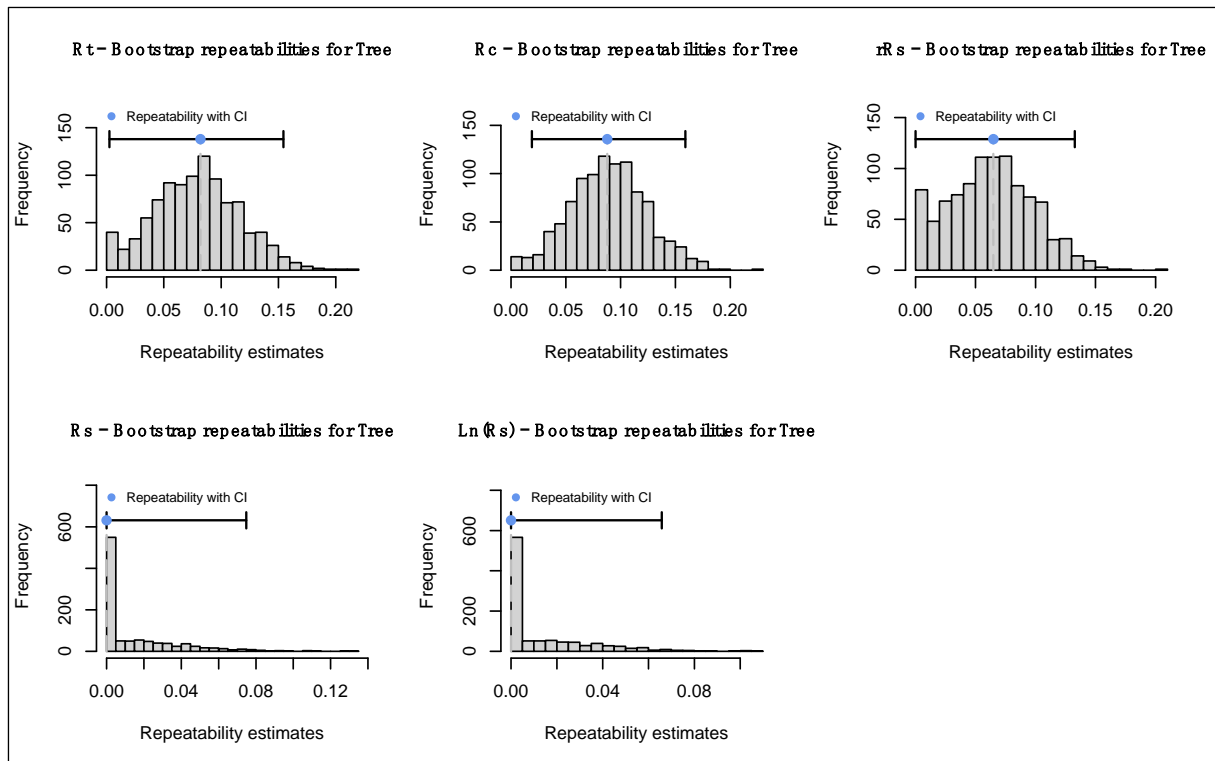


Figure S7. Repeatability estimates for each trait (R_t , R_c , Rs , $\ln(Rs)$, rRs). Distribution of the parametric bootstrap samples along with the point estimate and the limits of the confidence interval.

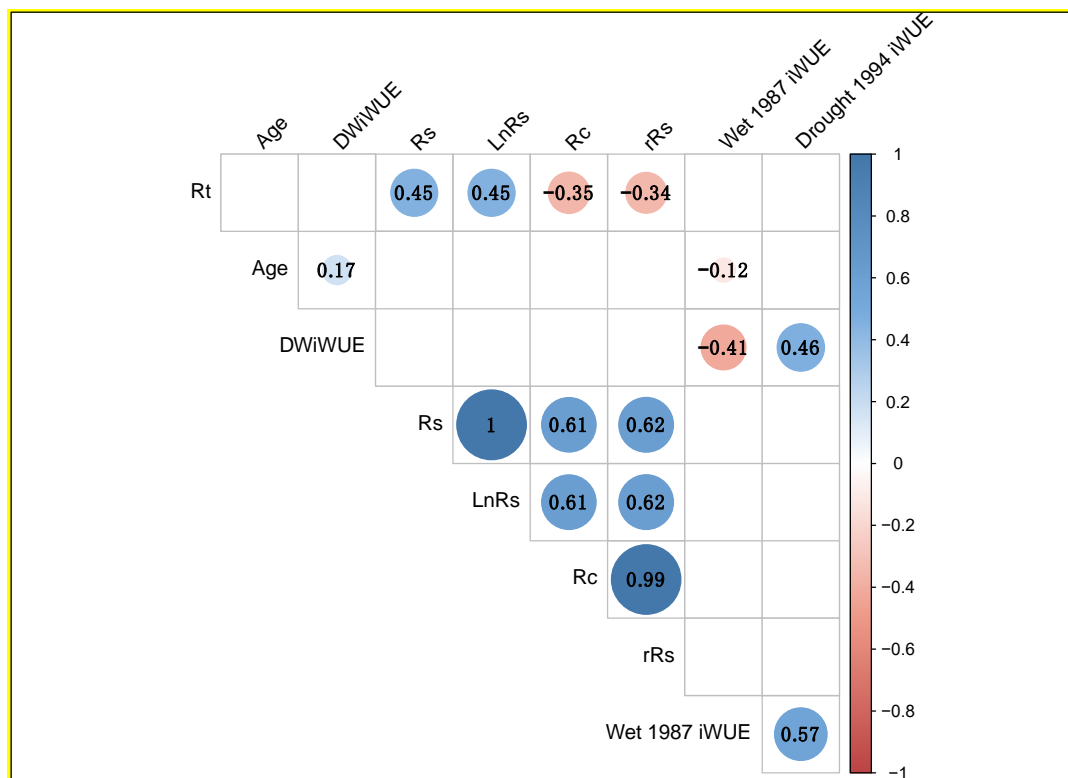


Figure S8. Correlogram based on the correlation matrix (Spearman) of all studied traits through all studied drought events (1917, 1947, 1992–1994) and corresponding age. The correlation matrix was ordered according to the degree of association between variables (hierarchical clustering) and the significance threshold was set at $p\text{-value} \leq 0.01$. Only significant correlation coefficients are plotted, sized and colored according to their value.

References

- Beguería, S.; Vicente-Serrano, S. M.; Reig, F. and Latorre, B. Standardized precipitation evapotranspiration index (SPEI) revisited: parameter fitting, evapotranspiration models, tools, datasets and drought monitoring. *Int. J. Climatol* **2014**, 34, 3001–23. <https://doi.org/10.1002/joc.3887>
- Deguilloux, M.-F.; Dumolin-Lapègue, S.; Gielly, L.; Grivet, D.; Petit, R.J. A Set of Primers for the Amplification of Chloroplast Microsatellites in *Quercus*: PRIMER NOTE. *Mol. Ecol. Notes* **2003**, 3, 24–27. <https://doi.org/10.1046/j.1471-8286.2003.00339.x>.
- Weising, K.; Gardner, R.C. A Set of Conserved PCR Primers for the Analysis of Simple Sequence Repeat Polymorphisms in Chloroplast Genomes of Dicotyledonous Angiosperms. *Genome* **1999**, 42, 9–19.
- Vicente-Serrano, S.M.; Beguería, S.; López-Moreno, J.I. A Multiscalar Drought Index Sensitive to Global Warming: The Standardized Precipitation Evapotranspiration Index. *J. Clim.* **2010**, 23, 1696–1718. <https://doi.org/10.1175/2009JCLI2909.1>.
- Steinkellner, H.; Fluch, S.; Turetschek, E.; Lexer, C.; Streiff, R.; Kremer, A.; Burg, K.; Glossl, J. Identification and Characterization of (GA/CT)_n Microsatellite Loci from *Quercus Petraea*. *Plant Mol. Biol.* **1997**, 33, 1093–1096.
- Kampfer, S.; Lexer, C.; Glössl, J.; Steinkellner, H. Characterization of (GA)_n Microsatellite Loci from *Quercus Robur*. *Hereditas* **1998**, 129, 183–186. <https://doi.org/10.1111/j.1601-5223.1998.00183.x>.