Supplemental Information

Raj et al. 10.1073/pnas.1103341108

SI Materials and Methods

Plant Material and Growth. Unrooted dormant stem cuttings of three hybrid *Populus* hybrid clones—DN34 [*Populus* × *canadensis* Moench var. eugenei (*P. deltoides* × *P. nigra*)], Walker [*P. deltoides* var. occidentalis × (*P. laurifolia* × *P. nigra*)], and Okanese [Walker × (*P. laurifolia* × *P. nigra*)] were each obtained from nurseries in two different Canadian provinces in Spring 2007 for a total of six hybrid clone populations of approximately 50 individuals each. Okanese and Walker were each sourced from AB (Alberta-Pacific Forest Industries) and SK (Agriculture and Agri-Food Canada, Agri-Environment Services Branch, Agroforestry Development Centre, Indian Head, SK; latitude 50° 32'N, longitude 104° 32'W). DN34 was obtained from MB (L.A. Quality Products) and SK.

Plants were grown and maintained in a climate-controlled growth chamber. Cuttings were imbibed in tap water in the dark for 48 h and planted in tall opaque pots (1 m length, 10 cm diameter) containing Sunshine Mix 1 (Sun Gro Horticulture) in a climate-controlled growth chamber with artificial canopy lights maintained between 200 and 400 μmol $m^{-2}s^{-1}$ on a 16-h photoperiod. The temperature of the chamber ranged from a day high of 22 °C and a night low of 17 °C, with humidity maintained at 55% to 65%. The cuttings were sprinkled with water daily until leaves emerged and later every 2 to 3 d after growth was established. Plants were fertilized (20:20:20 *N*-P-K; 1.5 g L^{-1}) at a rate of 600 mL per plant every 2 wk, and were last fertilized 1 wk before the water-withholding experiment. Pots were randomly rotated within and between canopies every 5 to 7 d.

Water-Withholding Experiment. Rooted cuttings were grown without water limitation for a minimum of 9 wk. Population height was used as a physical measure of developmental stage, and the water-withholding experiment was undertaken when each hybrid population was, on average, between 60 and 70 cm high. One half of each population was grown without further input of water, and the other half was well watered.

For each hybrid population, the onset of a physiological response to water deficit was monitored daily using an IR gas analyzer (LI-6400XT Portable Photosynthesis System; LI-COR Biosciences). Conductance measurements were made on the first fully expanded leaf 6 to 7 h (midday) after experimental dawn. A statistically significant difference in leaf stomatal conductance in water-deficient plants, for two consecutive days at midday, compared with well-watered plants, was used as an indicator of water stress (n = 5–6 per treatment, Welch unpaired t test, $P < 0.05$). On the third day, the first fully expanded leaf was harvested from three trees in each population—well watered and water-deficient—at two time points: predawn (1 h before lights were turned on) and midday (middle of the light period). The leaves were pooled and flash-frozen in liquid nitrogen. This was repeated three times, such that there were three biological replicate samples collected for each treatment at each time point, for each hybrid clone population. Leaves were harvested from the Okanese populations from AB and SK 13 d after the start of the water-limitation experiment. Leaf samples from the Walker populations from AB and SK were collected after 13 and 11 d, respectively. Leaf samples from the hybrid DN34 populations from MB and SK were collected after 11 and 13 d, respectively.

Transcript Abundance Analysis by Microarray. Frozen plant material was finely ground in liquid nitrogen, and total RNA was extracted as previously described (1). RNA quality was determined spectrometrically and electrophoretically. For each sample, 5 μg of total RNA was reverse-transcribed, labeled, and hybridized to the Poplar Genome Array according to manufacturer's protocols (Affymetrix) at the Centre for Applied Genome Evolution and Function at the University of Toronto. The Poplar Genome Array includes 61,251 probe sets representing more than 56,055 transcripts (Affymetrix). The GeneChip probe design is based on the Joint Genome Institute's *P. trichocarpa* genome project's predicted gene set v1.1 and all publicly available EST and mRNA sequences for all *Populus* species available through UniGene Build number 6 and GenBank. The array queries transcripts derived from 13 *Populus* species in addition to the fully sequenced *P. trichocarpa* genome.

The analysis of GeneChip data were conducted using the BioConductor suite of packages (2) in the R statistical programming language (3) by using the affy package (4). All 72 arrays were preprocessed together by using Microarray Suite version 5 (MAS5; Affymetrix) or GC-Robust Multiarray Analysis (gcrma) (5).

The transcript abundance analyses were conducted independently for each hybrid. The microarray study was analyzed as a 2 × 2 × 2 factorial complete randomized ANOVA design (two locations, two time points, two treatments) by using the LIMMA package (2) and a BH (6) false discovery rate-adjusted P value of 0.05. LIMMA fits a linear model to the data based on a user-defined experimental design matrix that corresponds to the RNA targets that have been hybridized on the arrays. A contrast matrix is then designed, which allows the coefficients defined by the design matrix to be combined into contrasts of interest. The linear model was parameterized by group means with a manually defined sum-to-zero contrast matrix to test for main effects and interaction effects by the process described by Wilkins et al. (7). This approach greatly simplifies the analysis of complex comparisons within the framework of a balanced factorial design.

Clustering Poplar Transcriptomes. PCC heat maps of whole-transcriptome profiles of were constructed for Okanese, Walker, and DN34 sourced from different locations (Fig. 2). All arrays were mas5-normalized and filtered. Heat maps included 19,720 probe sets that passed through the initial filter step (present calls in a minimum of 45 of 72 arrays; resultant expression measures were log2-transformed). All samples were clustered in the same order on both axes. The PCC was determined for each pair of samples within a hybrid. Samples were indicated by time of sample collection, location of origin, and water status.

Heat Maps of Treatment and Treatment:Location Interaction Effects Following Transcriptome Analyses. Heat maps were generated representing the relative abundance of drought-responsive transcripts in the three hybrid *Populus* clones obtained from two locations at predawn and midday time points (Fig. 3). Arrays were preprocessed using mas5 (present calls in a minimum of 45 of 72 arrays; resultant expression measures were log2-transformed). The number of probe sets indicated to the side of the heat map correspond to transcripts with significant treatment main effects only (BH-adjusted $P < 0.05$) and to transcripts with significant treatment:location interactions (BH-adjusted $P < 0.05$) at the specified time point. Each column represents a discrete biological sample, and all treatments are presented as biological triplicate replicates.

qRT-PCR. Six genes were selected for qRT-PCR validation. For each gene, the expression was analyzed in samples from the poplar hybrid genotypes Okanese, Walker, and DN34 from two different geographic locations and two different treatments each at predawn time point. The genes were chosen to represent a variety of different drought response patterns. Total RNA was prepared as described in the main text, and 3 μg of RNA were reverse-transcribed by using oligo(dT)18 primers and SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. qRT-PCR was performed by using iQ SYBR Green Supermix and the iCycler iQ real-time PCR detection system (Bio-Rad). For each condition, this was repeated for three biological replicates. The relative transcript abundance was determined based on the Pfaffl method (8, 9). Data were normalized to *ACTIN-7*. Where possible, primer sets were designed to span an intron to prevent amplification of genomic DNA. Melt-curve analysis was performed following amplification to confirm specificity of the amplification reaction. Sequences of primers used for qRT-PCR are given in Table S3.

Microsatellite Genotyping. The genetic identity of hybrid poplar clones from each location, as well as genetic identity of ramets of the same genotype obtained from different nurseries, was confirmed by genotyping the clones using previously characterized microsatellite markers [PMGC markers: International Populus Genome Consortium, SSR Resource (http://www.ornl.gov/sci/ipgc/ssr_resource.htm), ORPM markers (10), Table S4]. Markers were initially selected on the basis of coverage of each linkage group, and that they were known to map to multiple poplar species. Each of the nine markers were amplified by using DNA that was extracted from three to six plants per hybrid population from each location according to Doyle and Doyle (11). The reverse primer for each marker was labeled with 6-carboxy-fluorescein, and a 5′ GTTTCTT' sequence tail was added to the unlabeled primer to help with amplification and reduce stuttering. Amplified fragments were analyzed by using the ABI3730XL capillary sequencer (Applied Biosystems), and the results were supplied by the Centre for Applied Genomics at the Hospital for Sick Children (Toronto, ON, Canada).

Sequencing of Noncoding Regions. Promoter and noncoding regions for two unlinked nuclear loci, POPTR_0003s11430 (S8) and POPTR_0012s13180 (RD), were selected to study potentially highly variable regions and to assess the influence of location on sequence diversity. Three *Populus* hybrid genotypes (DN34, Walker, Okanese) from two different locations each were analyzed. Three individuals were used for each genotype and location. PCR primers were designed by using *P. trichocarpa* sequence information. PCR was performed on genomic DNA with the following primers: S8 forward, 5′ GTGCTTTTAGTTTTGGCTCAAG 3′; S8 reverse, 5′ GCTATACGAGAAACAACCAAATC 3′; RD forward, 5′ CCCAAAACCAAAAACGGAAGCAGC 3′; and RD reverse, 5′ GCATGATCTTTACAAGAACATTGC 3′. PCR fragments were cloned by using the pGEM-T Cloning kit (Promega), and at least six plasmids per location and genotype were sequenced individually. Sequence analysis yielded alignments of 615- and 1,018-bp length that were edited for rare PCR errors and assessed for allelic polymorphisms.

Global Methylation Analysis. Genomic DNA was extracted by using the DNeasy Plant Mini Kit (Qiagen), enzymatically hydrolyzed into nucleosides, and analyzed by isocratic cation-exchange HPLC as described previously (12) by using a Zorbax SCX column (300 × 4.6 mm, 5 μm; Agilent) with a 1200 series instrument (Agilent). Commercial standards (Sigma-Aldrich) were used for peak identification. 5-Methyl-2′-deoxycytidine (5mC) values were calculated based on a calibration curve of known molar ratios of 5mC and 2′-deoxycytidine. Total DNA methylation (as percentage) was calculated as (5mC / (2′-deoxycytidine + 5mC) × 100. Foliar tissue samples used for total DNA methylation analysis were identical to the foliar samples collected for RNA isolation and microarray hybridization. The effect of the independent factors—treatment and location—were then analyzed by using a 2 × 2 factorial ANOVA.

1. Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Mol Biol Rep* 11:113–116.
2. Gentleman RC, et al. (2004) Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* 5:R80.
3. R Development Core Team (2006) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna).
4. Gautier L, Cope L, Bolstad BM, Irizarry RA (2004) affy—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20:307–315.
5. Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F, Spencer F (2004) A Model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 99: 909–917.
6. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc, B* 57:289–300.
7. Wilkins O, Waldron L, Nahal H, Provart NJ, Campbell MM (2009) Genotype and time of day shape the Populus drought response. *Plant J* 60:703–715.
8. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.
9. Nordgård O, Kvaløy JT, Farmen RK, Heikkilä R (2006) Error propagation in relative real-time reverse transcription polymerase chain reaction quantification models: The balance between accuracy and precision. *Anal Biochem* 356:182–193.
10. Tuskan GA, et al. (2004) Characterization of microsatellites revealed by genomic sequencing of Populus trichocarpa. *Can J Forest Res* 34:85–93.
11. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15.
12. Rozhon W, Baubec T, Mayerhofer J, Mittelsten Scheid O, Jonak C (2008) Rapid quantification of global DNA methylation by isocratic cation exchange high-performance liquid chromatography. *Anal Biochem* 375:354–360.

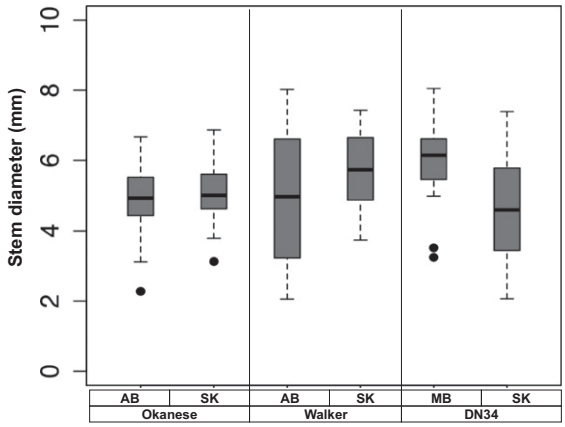


Fig. S1. Stem diameters: box plots of stem diameters of plants of *Populus* hybrid clones (*A*) Okanese, (*B*) Walker, and (*C*) DN34 sourced from two locations before the water-withholding experiment. Measurements were taken at the base of each plant. Contained within each box are the lower quartile, median, and upper quartile of the values ($n > 30$). Whiskers extend to the most extreme data point that is no more than 1.5 times the interquartile range from the box. Outliers are indicated as single points above or below each box.

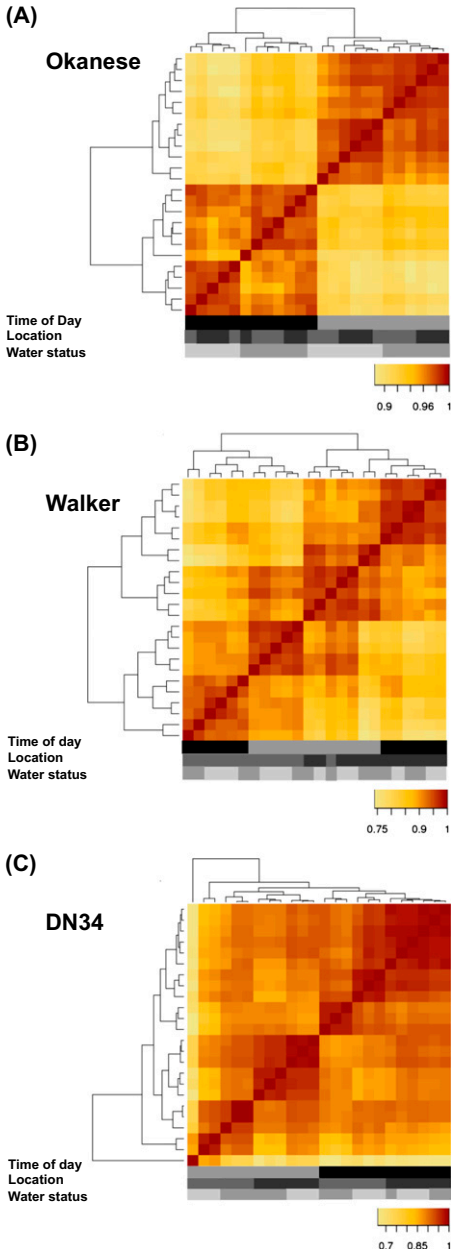


Fig. S2. Clustering poplar transcriptomes. PCC heat maps of whole-transcriptome profiles of (*A*) Okanese, (*B*) Walker, and (*C*) DN34 samples sourced from different locations. All arrays were gcrma-normalized and filtered. The heat maps include 18,694 probe sets that passed through the initial filter step (minimum expression $\log_2 100$ on at least three microarrays). All samples were clustered in the same order on both axes (left to right on the *x*-axis and bottom to top on the *y*-axis). The PCC was determined for each pair of samples within a hybrid. The color of each cell corresponds to the PCC for the compared samples, and the scale indicates the values. Samples are indicated by time of sample collection (predawn, black; midday, light gray), location of origin (AB, light gray; SK, dark gray), and water status (well watered, gray; water-deficient, light gray).

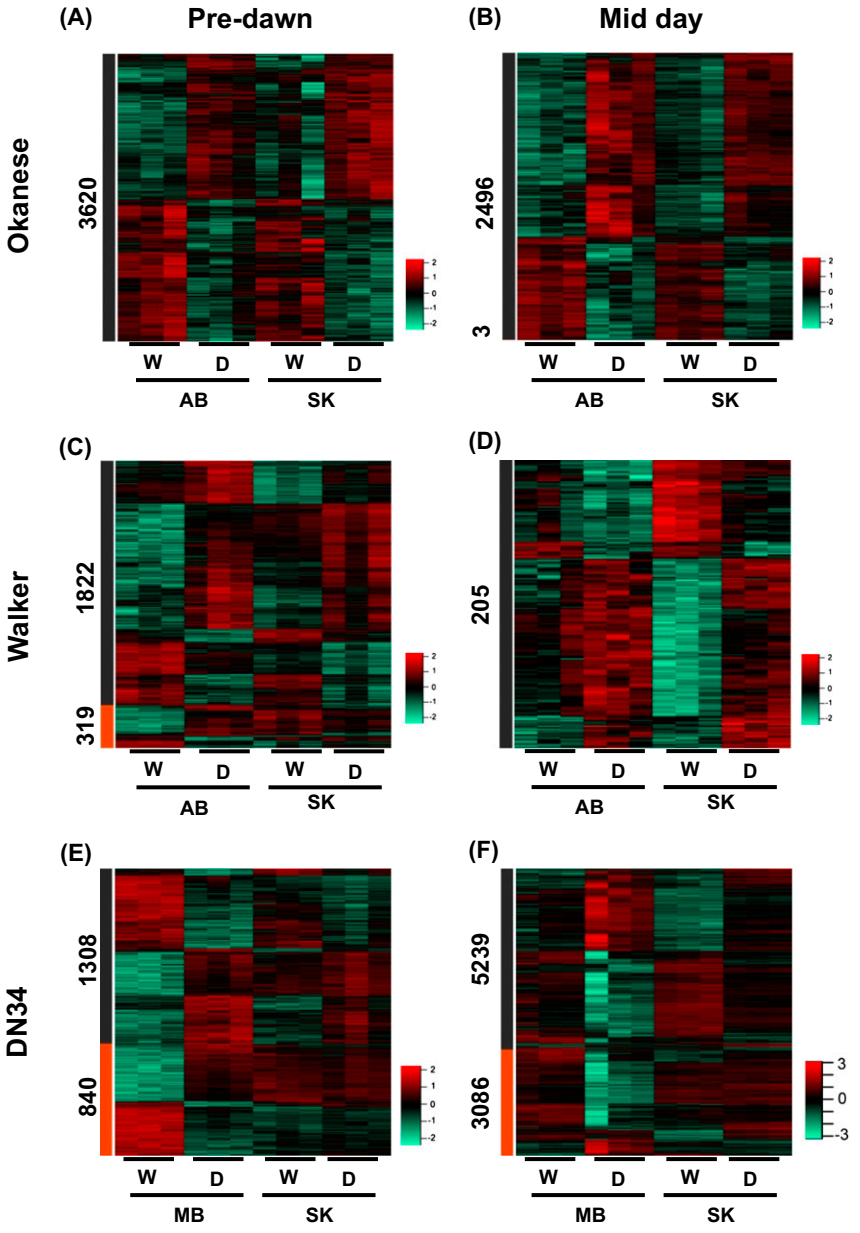


Fig. S3. Treatment and treatment:location interaction effects for each hybrid. Heat maps representing the relative abundance of drought responsive transcripts in the hybrid *Populus* clones (*A* and *B*) Okanese, (*C* and *D*) Walker, and (*E* and *F*) DN34 obtained from two locations at predawn (PD) and midday (MD) time points. All arrays were gcrma-normalized and filtered. The number of probe sets indicated to the side of the heat map correspond to transcripts with significant treatment main effects only (gray bar, BH-adjusted $P < 0.05$) and to transcripts with significant treatment:location interactions (orange bar, BH-adjusted $P < 0.05$) at the specified time point. Each column represents a discrete biological sample, and all treatments are presented as biological triplicate replicates. Red indicates higher, and green indicates lower, levels of transcript abundance. Expression levels are row-normalized. W, well watered samples; D, water-deficient samples.

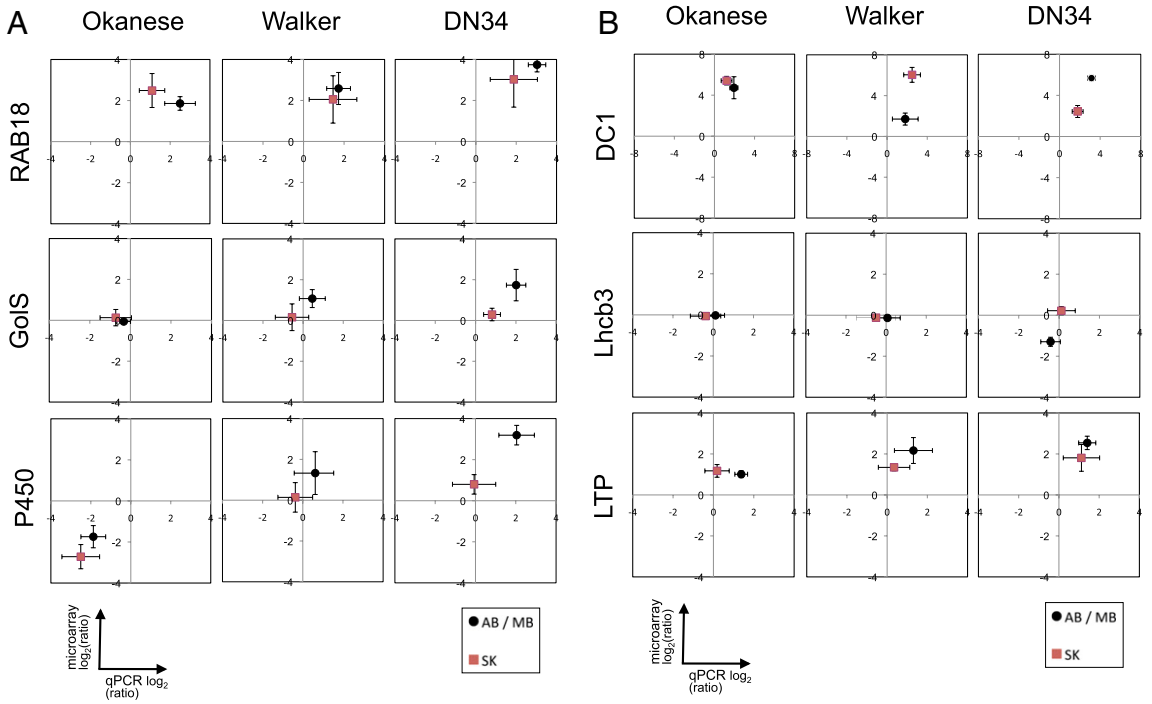


Fig. S4. qRT-PCR validation of transcript accumulation patterns at predawn. Log_2 ratio of the transcript accumulation under well watered conditions relative water-limited conditions as determined by microarray (*y* axis) and by qRT-PCR (*x* axis; $n = 3$; ±SE). Primer sequences are shown in Table S3.

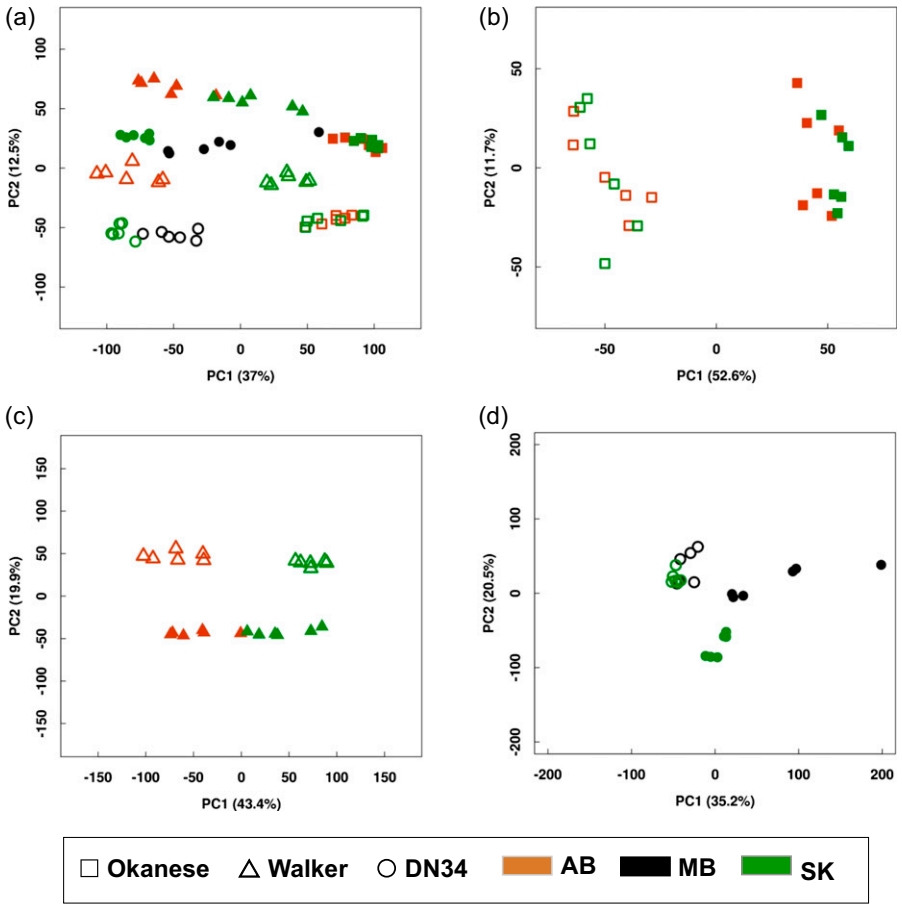


Fig. S5. PCA of whole-transcriptome profiles of hybrid poplar samples PC1 and 2. Variation in transcriptome abundance profiles is shown for transcriptome profiles corresponding to samples representing (*A*) all *Populus* hybrids, (*B*) Okanese (squares), (*C*) Walker (triangles), and (*D*) DN34 (circles) obtained from different locations (black, MB; orange, AB; green, SK) and harvested at different time points (predawn, open; midday, filled). All arrays were gcrma-normalized and filtered by intensity (minimum expression log2100 on ≥3 arrays) for a total of 18,694 probe sets.

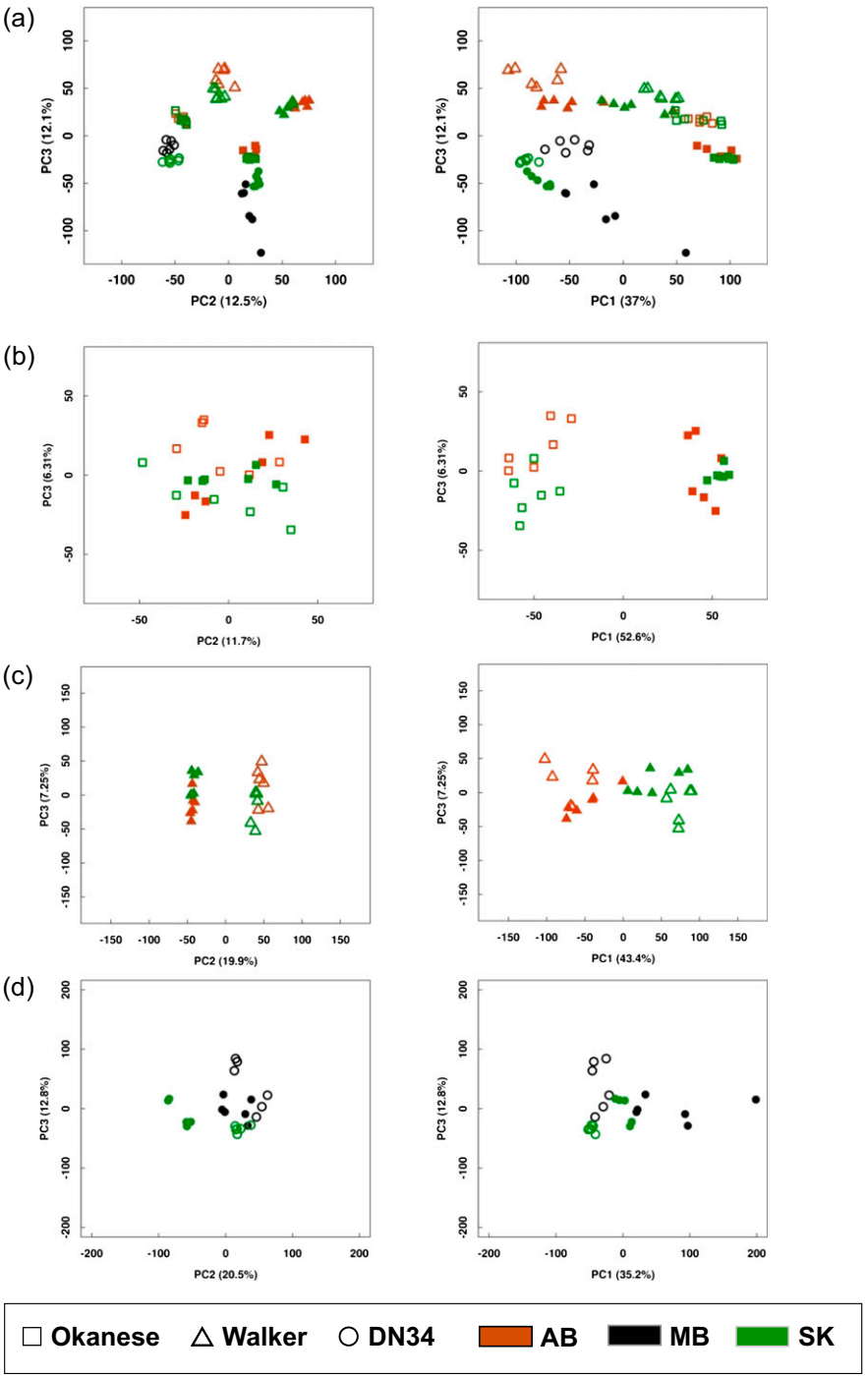
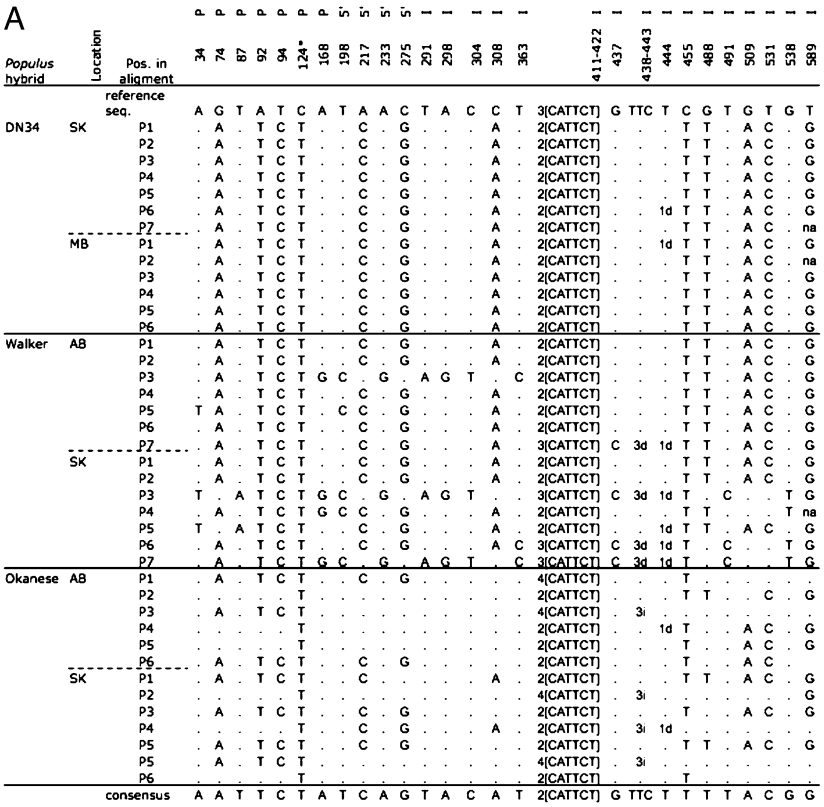


Fig. S6. PCA of whole transcriptome profiles of hybrid poplar samples PC1, 2, and 3. First, second, and third principal components of whole transcriptome profiles corresponding to samples representing (*A*) all *Populus* hybrids, (*B*) Okanese, (*C*) Walker, and (*D*) DN34 obtained from different locations (black, MB; orange, AB; green, SK) and harvested at different time points (predawn, open; midday, filled). All arrays were gcrma-normalized and filtered by intensity (minimum expression log2100 on ≥3 arrays) for a total of 18,694 probe sets.



A

Populus hybrid	Location	Pos. in aligment	P 34	P 74	P 87	P 92	P 94	P 124*	P 168	P 198	5' 217	5' 233	5' 275	I 291	I 298	I 304	I 308	I 363	I 411-422	I 437	I 438-443	I 444	I 455	I 488	I 491	I 509	I 531	I 538	I 589
		reference seq.	A	G	T	A	T	C	A	T	A	A	C	T	A	C	C	T	3[CATTCT]	G	TTC	T	C	G	T	G	T	G	T
DN34	SK	P1	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P2	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P3	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P4	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P5	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P6	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	1d	T	T	.	A	C	.	G
		P7	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	na
	MB	P1	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	1d	T	T	.	A	C	.	G
		P2	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	na
		P3	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P4	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P5	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P6	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
Walker	AB	P1	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P2	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P3	.	A	.	T	C	T	G	C	.	G	.	A	G	T	.	C	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P4	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P5	T	A	.	T	C	T	.	C	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P6	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P7	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	3[CATTCT]	C	3d	1d	T	T	.	A	C	.	G
	SK	P1	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P2	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P3	T	.	A	T	C	T	G	C	.	G	.	A	G	T	.	.	3[CATTCT]	C	3d	1d	T	.	C	.	.	T	G
		P4	.	A	.	T	C	T	G	C	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	.	.	T	na
		P5	T	.	A	T	C	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	.	1d	T	T	.	A	C	.	G
		P6	.	A	.	T	C	T	.	.	C	.	G	.	.	.	A	C	3[CATTCT]	C	3d	1d	T	.	C	.	.	T	G
		P7	.	A	.	T	C	T	G	C	.	G	.	A	G	T	.	C	3[CATTCT]	C	3d	1d	T	.	C	.	.	T	G
Okanese	AB	P1	.	A	.	T	C	T	.	.	C	.	G	.	.	.	.	.	4[CATTCT]	.	.	.	T	.	.	.	.	.	.
		P2	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	2[CATTCT]	.	.	.	T	T	.	.	C	.	G
		P3	.	A	.	T	C	T	.	.	.	.	.	.	.	.	.	.	4[CATTCT]	.	3d	.	.	.	.	.	.	.	.
		P4	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	2[CATTCT]	.	.	1d	T	.	.	A	C	.	G
		P5	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	2[CATTCT]	.	.	.	T	.	.	A	C	.	G
		P6	.	A	.	T	C	T	.	.	C	.	G	.	.	.	.	.	2[CATTCT]	.	.	.	T	.	.	A	C	.	
	SK	P1	.	A	.	T	C	T	.	.	C	.	.	.	.	.	A	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P2	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	4[CATTCT]	.	3d	.	.	.	.	.	.	.	G
		P3	.	A	.	T	C	T	.	.	C	.	G	.	.	.	.	.	2[CATTCT]	.	.	.	T	.	.	A	C	.	G
		P4	.	.	.	.	.	T	.	.	C	.	G	.	.	.	A	.	2[CATTCT]	.	3d	1d	.	.	.	.	.	.	.
		P5	.	A	.	T	C	T	.	.	C	.	G	.	.	.	.	.	2[CATTCT]	.	.	.	T	T	.	A	C	.	G
		P5	.	A	.	T	C	T	.	.	.	.	.	.	.	.	.	.	4[CATTCT]	.	3d	.	.	.	.	.	.	.	.
		P6	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	2[CATTCT]	.	.	.	T	.	.	.	.	.	.
		consensus	A	A	T	T	C	T	A	T	C	A	G	T	A	C	A	T	2[CATTCT]	G	TTC	T	T	T	T	A	C	G	G

Fig. S7. (Continued)

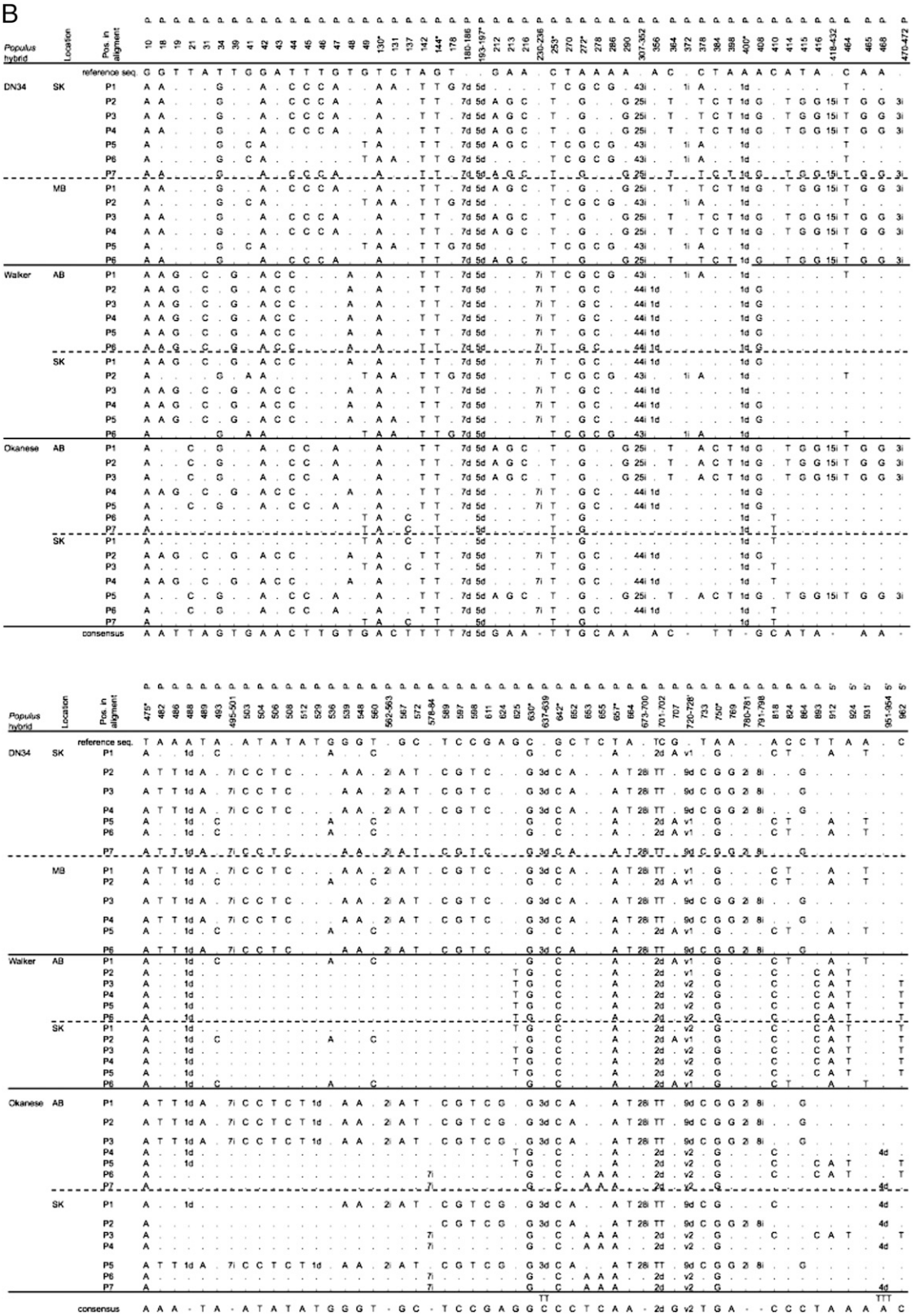


Fig. S7. (*A*) Polymorphic sites for promoter and noncoding regions of the locus POPTR_0003s11430 in the *Populus* hybrid genotypes. Sequences of the three hybrid genotypes DN34, Walker, and Okanese from two different geographic locations each are shown. The top line indicates the location of the substitution within the sequence (P, promoter, 5′, 5′ UTR; I, intron). Positions of polymorphic sites within the alignment are represented in the next line. Indels are shown as "i" (insertion relative to first sequence) or "d" (deletion relative to first sequence), with the number of base pairs given. Microsatellite polymorphisms are shown in brackets associated with the number of repeats. *Fixed polymorphism in the genotypes Okanese, Walker, and DN34 relative to the reference se-

Legend continued on following page

quence from the database (*P. trichocarpa* genome v2.2). (*B*) Polymorphic sites for promoter and noncoding regions of the locus POPTR_0012s13180 in the *Populus* hybrid genotypes. Sequences of the three hybrid genotypes DN34, Walker, and Okanese from two different geographic locations each are shown. The top line indicates the location of the substitution within the sequence (P, promoter; 5′, 5′ UTR). Positions of polymorphic sites within the alignment are indicated in the next line. Indels are shown as "i" (insertion relative to first sequence) or "d" (deletion relative to first sequence), with the number of base pairs given. A short stretch of 29 bp that was difficult to align was excluded from the alignment. Sequence variants at indel sites are represented by v1 (variant 1) and v2 (variant 2). *Fixed polymorphism in the genotypes Okanese, Walker, and DN34 relative to the reference sequence from the database (*P. trichocarpa* genome v2.2).

Table S1. Genotypes and clone age

Genotype	Propagation since
DN34	Early 1900s
Walker	1946
Okanese	1986

Table S2. Location of nursery sites used to obtain hybrid poplar cuttings and climate variables

Nursery location	Latitude (N)	Longitude (W)	Elevation (m)	Mean annual temperature, °C	Mean annual precipitation, mm	Days >5 °C
Boyle, AB	54° 53′	112° 51′	572	2.2	480	1,646
Indian Head, SK	50° 32′	104° 32′	585	3	398	1,725
Portage la Prairie, MB	49° 57′	98° 16′	259	3	525	1,883

Location details and historic climatic variables adjusted for specific location and elevation using the Climate PP model described previously (1).

1. Wang T, Hamann A, Spittlehouse D, Aitken SN (2006) Development of scale-free climate data for western Canada for use in resource management. *Int J Climatol* 26:383–397.

Table S3. Primer sequences used for qRT-PCR validation of microarray data

Name	Locus	Primer Sequence (5′→ 3′)
RAB18	POPTR_0003s13850	TGGAAGCACTAATGGCATGAC, TTCTTGCGCTCACCTCTCTTG
GOLS	POPTR_0008s02620	GTGGTGATTTGAATGCGAGTG, CTGCGAGTTCTCTTTGACGAT
P450	POPTR_0003s06460	CATTTTCACAGGACTTGATTGC, TCTGAAATTCCCCATTCCACG
DC1	POPTR_0002s01870	GAAATGGAAGAGAAGGAGAAGC, CTACCAACGAGGCAACAGGC
Lhcb3	POPTR_0001s41780, POPTR_0011s12680	CTCTTAGAGATGTTGTCTCCA, ATTCTCCAGTCAGGTATGAAG
LTP	POPTR_0016s10140	GAAGCAGCACAAGATGAGAAAGC, CACTGGACGGTTGGCAATGTTG

Table S4. List of primers for microsatellite markers

Primer name	Sequence (5′→ 3′)
PMGC_2501_LPL	CACAGGACGTTTTGGAGCAG
PMGC_2501_RPE	GTTTCTTAATTCGGACAGTCAGTCACC
ORPM_356_LPL	CCACGCTCGACAACATTTTA
ORPM_356_RPE	GTTTCTTAATCGTCCAATAAAAGCCACA
PMGC_2522_LPL	TCTGTTAATTTCTCAGCTGTTG
PMGC_2522_RPE	GTTTCTTTGCTTTACTAAACTTTTTACTGC
PMGC_2885_LPL	CATGATCAAATTGGATTTGAATG
PMGC_2885_RPE	GTTTCTTAAAGATGAACATGGCTAGCTC
PMGC_2658_LPL	GCCCTTGAATACCATGAGCG
PMGC_2658_RPE	GTTTCTTACCTTCAGTAGATCAGGTTAGTG
PMGC_2858_LPL	CTTACCATCTTTATCCTAATGC
PMGC_2858_RPE	GTTTCTTTTTCAAAAAATAAAAAGCAGCGC
PMGC_2818_LPL	AAGCTTCATCGTCCTGCTTG
PMGC_2818_RPE	GTTTCTTCGTATCAATTCACGACTCTCG
PMGC_93_LPL	ATCATGCGTTCGGCTACAGC
PMGC_93_RPE	GTTTCTTCTCAAACTCCAACTGTTATAAC
PMGC_2328_LPL	CAAAGGTGAAGTTACAGTCAC
PMGC_2328_RPE	GTTTCTTCCATTAGGCCATTATAGACAC
PMGC_2501_LPL	CACAGGACGTTTTGGAGCAG
PMGC_2501_RPE	GTTTCTTAATTCGGACAGTCAGTCACC

Table S5. The number of probe sets reporting transcripts with significant main effects or interactions in the *Populus* hybrid clones Okanese, Walker, and DN34, each sourced from different locations

Clone/detail	Probe sets
Okanese	
Location	91
Location at PD	259
Location at MD	0
Treatment	1,713
Treatment at PD	962
Treatment at MD	785
Treatment in AB	414
Treatment in SK	345
Time	9,698
Time in AB	6,320
Time in SK	7,671
Treatment:location interaction at PD	0
Treatment:location interaction at MD	1
Treatment.AB.PD	21
Treatment.AB.MD	544
Treatment.SK.PD	258
Treatment.SK.MD	20
Walker	
Location	7,939
Location at PD	7,277
Location at MD	4,754
Treatment	694
Treatment at PD	364
Treatment at PD only	355
Treatment at MD	35
Treatment in AB	94
Treatment in SK	350
Time	6,870
Time in AB	5,192
Time in SK	5,836
Treatment:location interaction at PD	13
Treatment:location interaction at MD	0
Treatment.AB.PD	628
Treatment.AB.MD	1
Treatment.SK.PD	13
Treatment.SK.MD	46
DN34	
Location	5,294
Location at PD	3,334
Location at MD	7,471
Treatment	4,849
Treatment at PD	1,525
Treatment at PD only	844
Treatment at MD	4,909
Treatment at MD only	4,669
Treatment in MB	5,288
Treatment in SK	288
Time	9,143
Time in MB	8,279
Time in SK	7,426
Treatment: location interaction at PD	1,228
Treatment: location interaction at MD	582
Treatment.MB.PD	3,916
Treatment.MB.MD	4,718
Treatment.SK.PD	0
Treatment.SK.MD	1,888

Arrays were preprocessed using MAS5 in R. Analyses were undertaken for each hybrid separately. Significant differences in transcript abundance were identified using a $2 \times 2 \times 2$ factorial complete randomized ANOVA (two locations, two time points, two treatments) in LIMMA with a BH false discovery rate-adjusted P value of 0.05. PD, predawn; MD, midday.

Table S6. Number of probe sets reporting transcripts with significant main effects or interactions in the *Populus* hybrid clones Okanese, Walker, and DN34, each sourced from different locations

Clone/detail	Probe sets
Okanese	
Location	871
Location at PD	1,359
Location at MD	33
Treatment	4,961
Treatment at PD	3,620
Treatment at MD	2,498
Treatment at MD only	2,496
Treatment in AB	2,023
Treatment in SK	1,962
Time	12,269
Time in AB	9,299
Time in SK	10,648
Treatment:location interaction at PD	0
Treatment:location interaction at MD	3
Treatment.AB.PD	1,279
Treatment.AB.MD	1,626
Treatment.SK.PD	1,489
Treatment.SK.MD	157
Walker	
Location	10,446
Location at PD	10,186
Location at MD	6,706
Treatment	2,452
Treatment at PD	1,964
Treatment at PD only	1,822
Treatment at MD	205
Treatment in AB	740
Treatment in SK	1,519
Time	9,093
Time in AB	7,461
Time in SK	7,462
Treatment:location interaction at PD	319
Treatment:location interaction at MD	0
Treatment.AB.PD	2,289
Treatment.AB.MD	2
Treatment.SK.PD	128
Treatment.SK.MD	250
DN34	
Location	7,656
Treatment	6,269
Treatment at PD	1,875
Treatment at PD only	1,308
Treatment at MD	7,014
Treatment at MD only	5,239
Treatment in MB	7,349
Treatment in SK	401
Time	10,862
Time in MB	10,491
Time in SK	8,327
Treatment:location interaction at PD	840
Treatment:location interaction at MD	3,086
Treatment.MB.PD	4,020
Treatment.MB.MD	8,060
Treatment.SK.PD	0
Treatment.SK.MD	1,820

Analyses were undertaken for each hybrid separately. Arrays were gcrma-normalized and filtered. Significant differences in transcript abundance were identified by using a $2 \times 2 \times 2$ factorial complete randomized ANOVA (two locations, two time points, two treatments) in LIMMA with a BH false discovery rate-adjusted P value of 0.05. PD, predawn; MD, midday.

Table S7. Microsatellite genotyping

Marker/size, bp	Okanese												Walker												DN34					
	AB						SK						AB						SK						MB			SK		
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	1	2	3
PMGC_2501																														
219	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
233	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
229	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
235	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ORPM_356																														
114	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
154	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
160	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PMGC_2522																														
139	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
155	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PMGC_2885																														
307	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
310	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
316	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
321	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
PMGC_2658																														
220	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
249	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
253	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
259	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PMGC_2858																														
76	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
95	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
PMGC_2818																														
129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
131	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
133	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
PMGC_93																														
344	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
347	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
353	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
356	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
362	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
367	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
PMGC_2328																														
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
91	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
108	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
128	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0

SSR markers used for fingerprinting the *Populus* hybrid clones Okanese, Walker, and DN34 cuttings sourced from two different locations each. Fragment size as well as an indication of presence (1) or absence (0) of each SSR amplicons is given.