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Phenotypic variation and quantitative trait locus identification for osmotic potential in an interspecific hybrid inbred F_2 poplar pedigree grown in contrasting environments

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Summary Elucidation of the mechanisms of dehydration tolerance in poplar (*Populus* sp.) trees will permit development of biochemical and molecular indicators to identify dehydration-tolerant genotypes during genetic selection. The objectives of this study were to characterize the degree of phenotypic variation in osmotic potential (a determinant of dehydration tolerance), determine the relationship between osmotic potential at full turgor and relative growth rate, and identify quantitative trait loci (QTL) for osmotic potential in an advanced-generation, interspecific poplar pedigree established in contrasting environments. A three-generation, sib-mated black cottonwood (*Populus trichocarpa* Torr. & Gray) and eastern cottonwood (*P. deltoides* Bartr.) segregating F_2 family (Family 331) was analyzed at a dry site east of the Cascade Mountain Range (Boardman, OR) and at a wet site west of the mountains (Clatskanie, OR). At the Boardman site, 2-year-old trees (59 clones) were either irrigated everyday (wet) or every other day (dry), whereas 3- and 4-year-old trees (58 clones) at the Clatskanie site were unirrigated.

At the Boardman site, the typically narrow range of osmotic potentials exhibited by grandparents and parents was greatly expanded in the F_2 population, spanning from –1.38 to –2.35 MPa under wet conditions, with a similar range under dry conditions (–1.40 to –2.15 MPa). Clones that had osmotic potentials ≤ –1.90 MPa generally displayed full maintenance of stem relative growth rates under dry conditions in contrast to clones with osmotic potentials that were ≥ –1.60 MPa, in which stem relative growth rates were reduced by an average of 38% in the dry treatment relative to the wet treatment. Although osmotic adjustments of 0.13 to 0.36 MPa were observed in nine out of 59 clones, adjustment typically occurred from relatively high baseline osmotic potentials. The range in osmotic potential at the wetter Clatskanie site at age three was higher (–1.27 to –1.84 MPa) and was further expanded the following year (–1.14 to –1.94 MPa), which had a wetter spring than the previous year, followed by a typically dry July. Seven QTL for osmotic potential were identified that each explained > 7.5% of the variation in osmotic potential. Given that four clones (7%) had osmotic potentials of –2.00 MPa or less and that QTL for osmotic potential have been identified, we suggest that there are opportunities to extend the limit of dehydration tolerance in *Populus*.

Keywords: dehydration tolerance, drought, osmotic adjustment, Populus deltoides, Populus trichocarpa, QTL, water potential.

Introduction

Plant growth rarely occurs at full potential for extended periods in field conditions and growth is often limited by drought in temperate regions. Use of woody crops for Quad-level (10^{15} BTU) energy production will require use of marginal agricultural lands (Tuskan 1998) where the occurrence of water stress will be recurrent, especially given the predictions of increased frequency and severity of droughts associated with the greenhouse effect (Neilson et al. 1989). For woody biomass to be a commercially viable energy source for biofuel production, a feedstock production goal of 20 Mg ha^{-1} $year^{-1}$ of dry aboveground biomass is required to reduce the cost of the energy source to \$2 GJ^{-1} (10^9 J) (Tuskan and Walsh 2001). Such aboveground productivities are currently obtainable in the Pacific Northwest, where cottonwood hybrids (*Populus trichocarpa* Torr. & Gray × *P. deltoides* Bartr.) are grown, and in subtropical regions, where *Eucalyptus* spp. are grown. Success in the Pacific Northwest is chiefly the result of a comprehensive genetic improvement program and nearly ideal water and nutrient conditions. The ability of *Populus* clones to tolerate drought is critical to the wood products industry, which must use combinations of silvicultural treatments to obtain high dry mass yields over a typical 6–7 year rotation length (up to a maximum of 12 years), which captures the maximal productivity of the trees in their juvenile growth phase. A long-term goal of the commercial poplar industry is to increase the limits of dehydration tolerance of poplar to maintain productivity on drought-prone sites, thereby expanding the land base suitable

for short-rotation woody crops with and without irrigation.

Some plant species, and some individuals within a species, can tolerate increasing water deficit stress by maintaining low osmotic potential at full turgor (π_o) or by accumulating solutes in tissues, thereby lowering π_o (osmotic adjustment; Morgan 1984), or both. Tyree and Jarvis (1982) suggested that low π_o should enhance the ability of plants to take up water from dry soils and may be as important as root growth in facilitating water uptake. Whether such mechanisms promote growth during drought stress or simply minimize the impact of drought on productivity and facilitate recovery from drought is unclear. There are few assessments of the fundamental relationship between π_o or osmotic adjustment and growth of trees under field conditions. With values of leaf π_o typically stabilizing from the third growing season on, assessments of these variables at 3–4 years of age would provide a good representation of values at rotation age (6–7 years). Many poplar clones currently being established as short-rotation crops are not drought tolerant; however, there is evidence of genetic variability (Tyree et al. 1978, 1979, Tschaplinski and Tuskan 1994, Tschaplinski et al. 1994) implying that drought-tolerant clones can be identified and established as crops. The possibility of establishing drought-tolerant clones in drier areas of the United States, such as the east of the Cascade Mountain Range in Washington and throughout the Midwest, will increase the amount of land with the potential to support short-rotation biomass plantations.

Low π_o is indicative of dehydration tolerance and also occurs as part of a generalized stress response, suggesting that a common genetic or biochemical regulatory network may be involved. Elucidation of the mechanisms involved in regulating π_o will permit development of biochemical and molecular indicators that can be used during genetic selection for rapid stress tolerance screening in traditional breeding programs. The rapid development of genomic tools (Tuskan et al. 2004*b*), coupled with the establishment of multiple generation poplar pedigrees and a marker-saturated genetic linkage map for poplar (Yin et al. 2004), permits the identification of quantitative trait loci (QTL) and eventually, the genes controlling traits such as dehydration tolerance. Establishment of the pedigrees at different sites allows an assessment of QTL detection in contrasting environments. The main objectives of this study were to: (1) characterize the phenotypic variability and range of π_o in an interspecific, multiple-generation poplar pedigree grown under contrasting field conditions; (2) determine the correlation between π_o and productivity in that pedigree; and (3) identify QTL for π_o.

Materials and methods

Germplasm and site description

Boise Cascade Corp. (BCC) established a large drought stress facility at Boardman, OR (45.8° N, 119.5° W), in which poplar trees could be subjected to various degrees of water stress to determine the effects on productivity. The BCC established cuttings of 59 clones from the University of Washington's (UW) black cottonwood (*Populus trichocarpa*) and eastern cottonwood (*P. deltoides*) F_2 Family 331, including the two grandparents (*P. trichocarpa* '93–968' (female), *P. deltoides* 'ILL-129' (male)), two F_1 hybrid parents (53–246 (female), 53–242 (male)) and 55 full-sib F_2 progeny. Two ramets per clone per block were established from cuttings planted on May 18, 1993 at a spacing of 1.5 m (within row) by 3.0 m (between rows). Operational irrigation/fertilization regimes, pest control measures and site tending were applied. The characteristics of the site have been described previously (Gebre et al. 1998). Briefly, the soil classification is Kimberly-Quincy-Xeric Torriorthens, which is well- to excessively-drained (USDA 1983). Annual maximum and minimum air temperatures at the site from May through October were 26.9 and 10.5 °C, respectively. The site typically receives ~20 cm of precipitation annually with most of that occurring as snowfall. Site fertilization requirements were determined weekly by complete foliar nutrient analyses.

The same clones of Family 331 were additionally established and maintained by James River Corp. at a hydric site west of the Cascade Mountains at Clatskanie, OR (46.1° N, 123.3° W) on April 23, 1993, with cuttings also planted at a 1.5 m × 3.0 m spacing. Being in the Columbia River delta, this high fertility site required no additional inputs of water or nutrients to achieve high aboveground growth rates. Mean annual precipitation is 161 cm. Mean daily maximum and minimum temperatures for the area during the growing season (May–October) from 1993 through 1996 were 23.4 and 11.5 °C, respectively. The January through May period was wetter (+28 cm) in 1996 than in 1995 and the temperature in May was on average 5 °C less in 1996 than in 1995. At the time of leaf sampling for osmotic potential in late July, the site had received only 2 cm of precipitation that month.

Water treatments

Soil water content at the drier Boardman site was determined by neutron probe analysis. Trees in three of the four replicate blocks at the Boardman site received the daily operational irrigation at a rate of ~91 cm during the growing season. Trees in the fourth replicate block received one-half of this rate (i.e., water was turned off every other day) to mimic drought conditions. A soil water-release curve was generated to determine the soil matric potentials achieved by the different irrigation treatments to 35 cm depth (Figure 1). The soil matric potential of the drought treatment was below –0.30 MPa on four sampling dates and reached a minimum of –0.56 MPa on Day 262 (August 10), compared with –0.11 MPa and generally stable values for the well-irrigated, wet treatment. Midday temperatures exceeded 40 °C during the first week and were only slightly lower (~5 °C) in the second week.

Growth measurements

Tree height (H) and stem diameter at breast height (D) were determined annually before leaf flush and at the end of the growing season in October of each year for the Boardman site (two ramets per clone). Additionally, D was determined for a

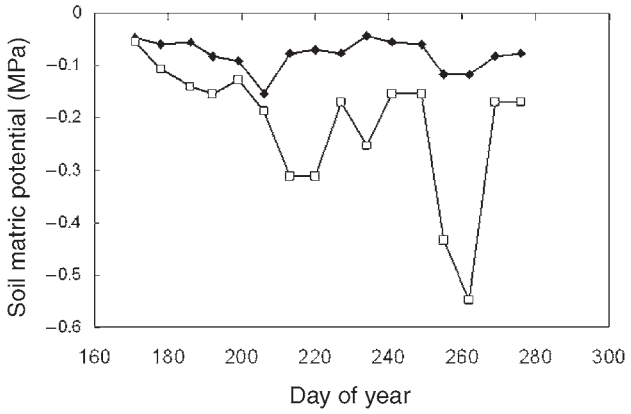

Figure 1. Soil matric potential of the wet (◆; operational irrigation regime of 91 cm) and dry (□; 50% of the irrigation of the operational regime) treatments at Boardman, OR during the 1994 growing season. Trees were sampled in their second growing season.

2-week period (June 13–30) preceding the leaf sample collections for the determination of π_o. The *D* of clones at the Clatskanie site was determined on June 4 and 56 days later on July 30, 1996 (six ramets per clone). Periodic and annual diameter and height measurements were used to calculate D^2H and stem diameter relative growth rate (RGR) estimates for clones under each treatment. We calculated RGR as the difference in natural log of D^2H at two points in time divided by the interval between the measurements.

Determination of osmotic potential

We measured π_o to assess the degree of dehydration tolerance among clones in the different irrigation treatments. Trees were sampled in their second growing season at Boardman and in their third and fourth growing seasons at Clatskanie. Leaves (leaf plastochron index (LPI) 9–11) on fully exposed lateral shoots of two ramets per clone from one block were collected (1000–1600 h local time) to determine π_o with a Wescor vapor pressure osmometer (Wescor Inc., Logan, UT). All clones of the F_2 Family 331 were sampled within a 2-week period (July 28–August 11, 1994) at Boardman. The shoots were rehydrated overnight in distilled water, after which one leaf per shoot was removed, frozen in dry ice, thawed and π_o of the extracted sap measured with an osmometer.

The 3- and 4-year-old trees of the same 58 clones from Family 331 were studied at Clatskanie in 1995 and 1996, respectively. Six ramets per clone were sampled on July 15 and 16, 1995, and six additional ramets per clone were sampled the following growing season on July 30 and 31, 1996, a year with a cooler and wetter spring than the preceding year, followed by a typically dry summer. Comparison of the 2-year-old "wet" and "dry" data from the Boardman site with the 3- and 4-year-old data from the Clatskanie site provided an initial assessment of the environmental effect on π_o, with Boardman having more sunny, low humidity, hot and dry days during the growing season than the cooler, wetter and cloudy coastal Clatskanie site.

Genetic data and map construction

In a concurrent study (Sewell et al. unpublished data), genotypic segregation data from simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers were used to construct a sex-average framework map for the F_2 Family 331. Evenly spaced markers were selected from a previous mapping study of Family 331 (Tuskan et al. 2004*a*). Fully informative markers (i.e., a marker that is heterozygous for different alleles in each of the F_1 parents) were preferentially chosen when available. The framework map was constructed at LOD 5 using MapMaker (Lander et al. 1987) and JoinMap (Stam 1993). Briefly, the map has 637 SSRs and 533 AFLPs, an average marker interval of 3.8 cM and linkage disequilibrium occurring over relatively small regions. Methods pertaining to SSR and AFLP analyses for *Populus* followed Tuskan et al. (2004*a*) and Yin et al. (2004), respectively. Map construction followed Sewell et al. (1999).

Data analysis

Osmotic potentials presented are clonal means of two ramets per clone per treatment at Boardman and six ramets per clone per year at Clatskanie. Significant ($P < 0.05$) osmotic adjustment to drought stress treatment for each clone, was determined by Student's *t* tests. The relationships between osmotic potential parameters and growth parameters at each site and between sites were determined by correlation analyses.

A web-based interval mapping program (http://qtl.cap.ed.ac.uk/; Seaton et al. 2002) was used to detect associations between the segregation of genetic markers and phenotypic variability for π_o. This method uses a least-squares approach (Haley and Knott 1992) to simultaneously analyze multiple markers of an outbred pedigree (Knott et al. 1997). Each linkage group was scanned at 1-cM intervals for locations explaining a high proportion of the phenotypic variance (i.e., evidence for a QTL) with a conventional interval analysis. The QTL were reported at two thresholds, a significant level ($P = 0.01$) and a suggestive level ($0.05 = P > 0.01$), for linkage group-wise tests. Separate thresholds were used in an attempt to avoid Type I and II errors associated with point-wise versus genome-wide analyses (Lander and Kruglyak 1995). Initially, the data sets from each of the four water treatments (i.e., wet and dry conditions from 1994 at Boardman and untreated conditions from 1995 and 1996 at Clatskanie) were analyzed individually (results not shown). These treatments were then organized into a data set for each site (i.e., two treatments per site) and a single data set including all four treatments, and re-analyzed with "treatment" as a fixed effect. Additive and dominance effects were calculated for each data set with an F_2 model (Seaton et al. 2002).

Results

Osmotic potential of F_2 Family 331 at Boardman (1994)

The survey of osmotic potential at full turgor (π_o) of F_2 Family 331 at Boardman demonstrated tremendous variability

among the full-sib progeny, with π_o ranging from a high of –1.38 MPa (least tolerant) for Clone 1120 to a low of –2.35 MPa (most tolerant) for Clone 1087 under well-watered conditions (Table 1). Of the grandparents and parents, *P. deltoides* 'ILL-129' (male) had the lowest π_o (–1.90 MPa), *P. trichocarpa* '93–968' (female) had the highest π_o (–1.55 MPa) and the F_1 hybrid parental clones 53–246 (female), 53–242 (male) were intermediate (–1.84 and –1.71 MPa, respectively). A paired *t* test revealed a significant difference in π_o between trees in the wet and dry treatments, but the mean π_o of

Table 1. Mean osmotic potential at full turgor (π_o) for clones of Family 331 under wet and dry conditions at Boardman, OR during the 1994 growing season. Abbreviations: OA = osmotic adjustment (i.e., the difference between π_o-Dry and π_o-Wet); and D^2H = ratio of stem diameter at breast height squared times height. Relative growth rate (RGR) of stem diameter at breast height (June 13 to June 30) is shown, including the proportional maintenance of RGR Dry/Wet as an indication of drought tolerance.

Clone	π_o-Wet (MPa)	π_o-Dry (MPa)	OA (MPa)	RGR-Wet $day^{-1} \times 100$	RGR-Dry $day^{-1} \times 100$	RGR (Dry/Wet)	D^2H-Wet (cm^3)	D^2H-Dry (cm^3)
ILL-129	–1.90	–1.88	–0.02	0.576	0.564	0.980	10980	16876
53-242	–1.71	–1.63	–0.08	0.558	0.443	0.794	22176	20696
53-246	–1.84	–1.75	–0.09	0.633	0.440	0.695	18865	19861
93-968	–1.55	–1.49	–0.06	0.764	0.351	0.459	27514	8171
1059	–1.59	–1.73	0.14	0.836	0.544	0.650	12030	8528
1060	–1.56	–1.68	0.12	0.721	0.623	0.865	9839	4915
1061	–1.62	–1.62	0.00	0.719	0.537	0.747	9035	8765
1062	–2.04	–1.90	–0.14	0.737	0.566	0.767	20506	12417
1064	–1.61	–1.76	0.15	1.103	0.781	0.708	10878	6286
1065	–1.82	–1.65	–0.17	0.785	0.746	0.950	12964	12638
1067	–1.68	–1.82	0.14	0.927	0.812	0.877	8622	8523
1068	–1.90	–1.74	–0.16	0.596	0.733	1.229	9138	11883
1069	–1.60	–1.60	0.00	0.986	0.564	0.572	9564	5613
1071	–1.52	–1.60	0.08	0.739	0.644	0.871	2905	2664
1072	–1.65	–1.76	0.11	0.847	0.639	0.755	8619	8633
1073	–1.59	–1.48	–0.11	0.709	0.504	0.711	9388	7967
1075	–1.85	–2.15	0.30	0.794	0.425	0.535	12754	11917
1076	–1.56	–1.87	0.31	0.656	0.498	0.759	7012	10248
1077	–1.66	–1.80	0.14	0.787	0.404	0.513	5695	4249
1078	–1.69	–1.62	–0.07	0.761	0.611	0.803	7616	11650
1079	–1.76	–1.74	–0.02	0.829	0.688	0.830	8947	9364
1084	–1.84	–1.85	0.01	0.995	0.558	0.561	9533	5875
1086	–1.87	–1.84	–0.03	0.447	0.649	1.452	5154	4212
1087	–2.35	–2.08	–0.27	0.313	0.696	2.223	7169	10263
1090	–1.47	–1.63	0.16	0.818	0.574	0.702	10632	7245
1093	–1.58	–1.87	0.29	0.750	0.665	0.886	11950	8857
1095	–1.67	–1.67	0.00	0.728	0.677	0.930	10995	8789
1101	–1.52	–1.66	0.14	1.079	0.369	0.342	5451	4335
1102	–1.56	–1.82	0.26	0.962	0.589	0.612	3110	3648
1103	–1.87	–1.92	0.05	0.281	0.494	1.758	5047	8141
1104	–1.76	–1.66	–0.10	0.508	0.504	0.992	6912	7407
1106	–1.63	–1.85	0.22	0.813	0.468	0.575	3873	6626
1112	–1.70	–1.50	–0.20	0.770	0.473	0.614	9416	11007
1114	–1.88	–1.98	0.10	0.699	0.698	0.998	16018	12965
1118	–1.70	–1.93	0.23	0.787	0.728	0.925	10407	6514
1120	–1.38	–1.40	0.02	0.874	0.620	0.710	5290	2205
1121	–1.50	–1.70	0.20	0.944	0.521	0.553	10741	9249
1122	–1.70	–1.69	–0.01	0.876	0.625	0.713	13200	21188
1126	–1.83	–1.87	0.04	0.767	0.422	0.550	8707	3056
1127	–1.62	–1.71	0.09	0.941	0.922	0.980	3078	10257
1128	–1.69	–2.04	0.35	0.813	0.600	0.738	13334	16248
1130	–1.96	–1.96	0.00	0.535	0.791	1.479	3354	5323
1131	–1.60	–1.74	0.14	1.536	0.448	0.292	14035	4219
1133	–1.41	–1.53	0.12	0.845	0.495	0.585	6880	2251
1136	–1.69	–1.60	–0.09	0.803	0.692	0.861	7348	7934
1140	–1.72	–1.83	0.11	0.716	0.462	0.645	13334	12773
1149	–2.17	–1.99	–0.18	0.616	0.513	0.832	22261	17164

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Table 1 Cont'd. Mean osmotic potential at full turgor (π_o) for clones of Family 331 under wet and dry conditions at Boardman, OR during the 1994 growing season. Abbreviations: OA = osmotic adjustment (i.e., the difference between π_o-Dry and π_o-Wet); and D^2H = ratio of stem diameter at breast height squared times height. Relative growth rate (RGR) of stem diameter at breast height (June 13 to June 30) is shown, including the proportional maintenance of RGR Dry/Wet as an indication of drought tolerance.

Clone	π_o-Wet (MPa)	π_o-Dry (MPa)	OA (MPa)	RGR-Wet $day^{-1} \times 100$	RGR-Dry $day^{-1} \times 100$	RGR (Dry/Wet)	D^2H-Wet (cm^3)	D^2H-Dry (cm^3)
1151	–2.06	–1.94	–0.12	1.009	0.804	0.796	11335	10761
1158	–1.77	–1.80	0.03	0.855	0.834	0.975	6733	2554
1162	–1.48	–1.61	0.13	0.906	0.514	0.567	5003	3049
1163	–1.72	–1.80	0.08	0.665	0.743	1.117	13903	11665
1169	–1.60	–1.77	0.17	0.579	0.562	0.971	14943	11944
1173	–1.78	–1.78	0.00	0.942	0.841	0.893	24017	13698
1174	–1.74	–1.65	–0.09	0.702	0.726	1.035	15149	13353
1182	–1.72	–1.67	–0.05	0.687	0.721	1.050	2031	1613
1186	–1.69	–1.82	0.13	0.344	0.700	2.036	2096	5906
1580	–1.92	–2.01	0.09	0.438	0.577	1.316	18377	19006
1582	–1.52	–1.64	0.12	0.861	0.647	0.751	11864	8265
1587	–1.58	–1.77	0.19	0.695	0.155	0.223	7803	4032

all clones was similar under both well-watered and dry conditions (–1.71 versus –1.76 MPa, respectively), and π_o under dry conditions was positively correlated with that under wet conditions ($r^2 = 0.45$; $P = 0.01$; d.f. = 57).

Under dry conditions, π_o ranged from a high of –1.40 MPa for Clone 1120 to a low of –2.15 MPa for Clone 1075, as a result of a 0.30 MPa adjustment relative in the wet treatment in the case of the latter clone. Of the nine F_2 progeny of Family 331 that displayed statistically significant osmotic adjustments of 0.13–0.36 MPa (Clones 1128 (0.36 MPa), 1075 (0.30 MPa), 1102 (0.27 MPa), 1118 (0.23 MPa), 1106 (0.22 MPa), 1121 (0.20 MPa), 1587 (0.18 MPa), 1090 (0.17 MPa) and 1101 (0.13 MPa)), eight were in the top 45% (26/59) of the fastest growing clones under well-irrigated conditions. However, these eight clones were also in the top 41% (24/59) of clones with the greatest proportional reductions in stem diameter RGR in the dry versus wet treatments (Table 1). They were also generally in the bottom half of RGR rankings under drought conditions, averaging a 42% reduction in RGR. With the exception of Clone 1075, all clones that displayed osmotic adjustment to water stress did so from a high π_o under well-watered conditions (greater than –1.70 MPa). As such, the degree of osmotic adjustment under dry conditions was positively correlated with π_o under wet conditions ($r^2 = 0.31$; $P = 0.01$; d.f. = 57).

In contrast with the poor growth of clones displaying osmotic adjustment during drought, nine of the 12 clones with the lowest π_o (less than –1.88 MPa) under dry conditions were in the top half of clones with RGR that was least affected by drought, with RGR maintained at > 80% of that in the wet treatment (Table 1). Overall, only 13% of the variation in proportional reduction in RGR was explained by the regression with π_o ($r^2 = 0.13$; $P = 0.01$; d.f. = 57). However, RGR of clones with π_o greater than –1.75 MPa was reduced by 25%, and was significantly ($P = 0.04$) different from the 5% reduction in RGR in clones with π_o less than –1.75 MPa. Of the six clones that were least affected by drought, four maintained low π_o, and all were –1.82 MPa or less. Based on the observed distribution, a low π_o under dry conditions was advantageous at the Boardman site, i.e., mean RGR of clones that had π_o of –1.90 MPa or lower was 12% greater in the dry treatment than in the wet treatment, whereas mean RGR of clones that had π_o of –1.60 MPa or greater was reduced by 38% in the dry treatment relative to that in the wet treatment.

There was also considerable clonal variation in morphology. For example, Clone 1120 was a slow-growing clone at Boardman with the highest π_o, bronzing leaf margins of the upper leaves, heavy interveinal chlorosis of the lower leaves and the underside of leaves exuding water. Clone 1087 was an average productivity clone that had thick xeromorphic leaves, the lowest π_o and displayed only minor leaf loss of the lowest leaves. Clone 1075 was a fast-growing clone, displaying only minor leaf loss under stress. The hot (about 40 °C), dry weather in July resulted in considerable leaf loss in many clones, whereas it caused only minimal loss of lower leaves in other clones (e.g., Clone 1087).

Osmotic potential of clones of F_2 Family 331 at Clatskanie (1995–1996)

The mean π_o of all clones was 0.19 MPa higher at Clatskanie than at Boardman (–1.52 versus –1.71 MPa, Figure 2). Although the trees at Clatskanie were 3 years old, they were about the same size as the 2-year-old trees sampled at Boardman. Despite the contrasting environments between sites, clonally replicated π_o data collected at the cooler and wetter Clatskanie site was positively correlated with π_o at the drier and sunnier Boardman site ($r^2 = 0.36$; $P = 0.01$; d.f. = 56). However, year-to-year variation was high at the Clatskanie site: the coefficient of determination was lower between years 3 and 4 ($r^2 = 0.20$; $P = 0.01$; d.f. = 56) than between sites. Although the individuals with above average π_o had a higher π_o in 1996 (–1.40 to –1.49 MPa) than in 1995 (–1.50 to –1.59 MPa), the distribution of π_o values was flatter in 1996 with more clones having lower π_o, whereas the mean family π_o

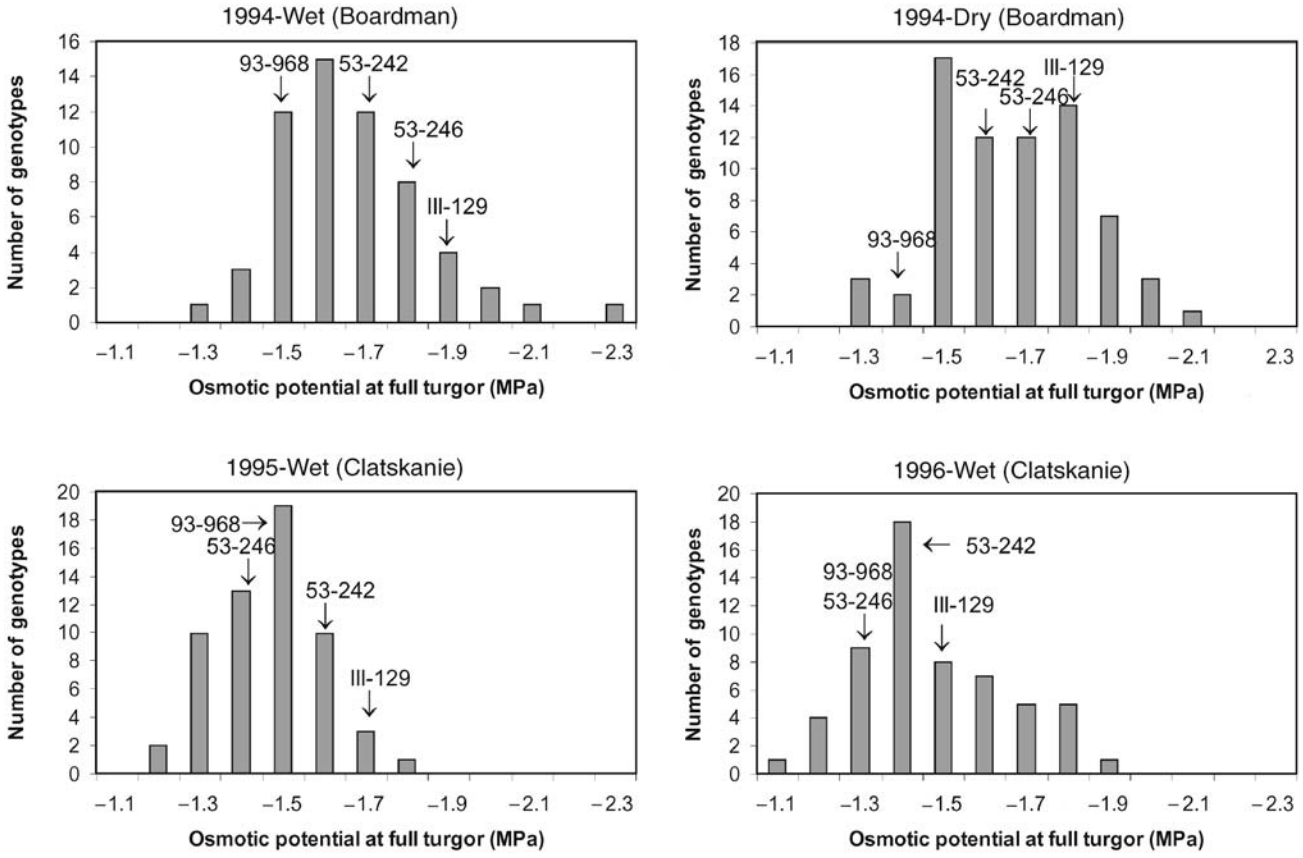

Figure 2. Distribution of osmotic potential at full turgor (π_o) of 2-year-old clones of Family 331 in the wet and dry treatments at the dry Boardman, OR site in 1994, and 3- and 4-year-old clones sampled at the wetter Clatskanie, OR site in 1995 and 1996, respectively. Clones were grouped into 0.1 MPa bins. Grandparents and parents are located in each figure.

was similar (–1.52 MPa) in both years (Figure 2).

Correlation of stem D and RGR with osmotic potential

At Boardman, D^2H at the end of the first growing season (i.e., before bud flush of the second growing season) was negatively correlated with π_o ($r^2 = 0.22$; $P = 0.01$; d.f. = 57) for the trees in the wet treatment (Table 2). Stem D^2H for the same trees measured during the second growing season was not significantly correlated with π_o, but RGR was negatively correlated with π_o ($r^2 = 0.20$, $P = 0.01$, d.f. = 57). Stem D^2H of the clones in the dry treatment was also correlated with π_o in year 2, but the relationship was weak ($r^2 = 0.077$; $P = 0.01$; d.f. = 57), and RGR was not correlated with π_o. At Clatskanie, D^2H and RGR during the fourth growing season were correlated with π_o, but π_o explained only 14 and 15% of the variation in these growth variables, respectively (Table 2). Overall, the relative clonal

Table 2. Correlation coefficients (r) and coefficients of determination (r^2) for the regressions of osmotic potential at full turgor (π_o) with the growth variables, stem diameter at breast height (D), relative growth rate (RGR) of stem diameter at breast height, and stem volume (D^2H; stem diameter at breast height squared times height). Growth data for Family 331 were collected at Boardman, OR (BR) and Clatskanie, OR (CL) field sites with the age of the trees indicated in parenthesis. The probability (P) that the slopes of the regressions are significantly different from zero at the given degrees of freedom (d.f.) are shown.

Growth variable	Site	Treatment	r	r^2	P, d.f.
D^2H (end Year 1)	BR	Wet	–0.472	0.223	0.01, 57
D^2H (Year 2)	BR	Wet	–0.240	0.058	> 0.05, 57
D^2H (Year 2)	BR	Dry	–0.277	0.077	0.05, 57
RGR (Year 2)	BR	Wet	–0.443	0.196	0.01, 57
RGR (Year 2)	BR	Dry	–0.115	0.001	> 0.05, 57
D^2H (June Year 4)	CL	Wet	–0.369	0.136	0.01, 56
RGR (Year 4)	CL	Wet	–0.387	0.149	0.01, 56

growth performance was similar between sites. Stem diameter in June of the third growing season at Boardman was correlated with that in June of the fourth growing season at Clatskanie ($r^2 = 0.45$; $P = 0.01$; d.f. = 56).

QTL identification

Twelve QTL (seven significant and five suggestive) were identified for π_o, where individual QTL accounted for 5.5 to 19.1% of the variation in π_o (Table 3). Eight of these QTL (***QI.1***, *I.2*, ***II***, ***III***, ***VI***, ***VIII***, ***XII*** and *XV*) were detected using the pooled data across all treatments (QTL detected at the significant threshold are in bold type). The remaining four QTL were detected only at a single site. Of these, one QTL (*QXIII.1*) was detected using the 1994 wet and dry treatment data from Boardman and three (*Q XI*, ***XIII.2*** and *XVIII*) using the 1995 and 1996 untreated data from Clatskanie. Each QTL, whether detected from data pooled across all treatments or pooled across each site, was also detected using the data analyzed from each individual treatment within that site.

An interspecific cross between two highly divergent species allows for the assumption that the alleles at the QTL may be fixed in alternate forms within each parental species (Bradshaw and Stettler 1995). Under this assumption, an F_2 model was used to evaluate gene action from the additive and dominance effects (Table 3). Seven of the twelve QTL exhibited a dominance effect significantly different from zero for at least one of the treatments, which suggests a deviation from additivity. Five of these QTL (***QII***, ***VI***, *XI*, ***XII*** and *XV*) exhibited a positive effect (indicating some degree of dominance or overdominance) and two (*QXIII.1* and ***XIII.2***) a negative effect (indicating some degree of underdominance).

The direction of effect (i.e., the parental species that contributed the superior allelic effect) for each QTL was determined from those estimated additive effects that were significantly different from zero for at least one of the treatments. The superior allelic effect arose from the parent with the lowest value of π_o (*P. deltoides* 'ILL-129' (male) for six QTL (***QI.1***, *I.2*, ***II***, ***III***, ***XIII.2*** and *XVIII*) and the parent with the highest value of π_o (*P. trichocarpa* '93–968' (female)) for three QTL (*QVIII*, ***XII*** and *XIII.1*).

Discussion

Although stem diameter growth of clones was correlated between the Boardman and Clatskanie sites, most clones did not grow as well at Clatskanie and clonal rankings shifted between sites. These subtle differences suggest that care must be exercised in extrapolating growth classifications from one environmental condition to another, such as found in the hot, dry and sunny continental climate at the Boardman site and the cooler, humid and cloudy coastal climate at the Clatskanie site. Results of several poplar clonal trials at multiple field sites have also provided evidence of genotype × environment interactions in growth responses (Mohn and Randall 1973, Riemenschneider et al. 2001).

Maintenance of RGR during drought varied between clones and can be considered a general measure of drought tolerance. However, the capacity for osmotic adjustment did not facilitate maintenance of RGR under drought stress in our study. Although a positive relationship between osmotic adjustment and the stability of grain yield during drought has been demonstrated in wheat, chickpea, and sorghum (Morgan 1983, Wright et al. 1983, Ludlow et al. 1990, Morgan et al. 1991, Blum et al. 1999), there have been fewer examples where RGR of aboveground biomass (e.g., stem or leaves) was positively correlated with osmotic adjustment (Munns 1988, Passioura 1988). Exceptions include the studies of Wright et al. (1996) and Tangpremsri et al. (1995) on sorghum and of Morgan (1995) and Blum et al. (1999) on wheat that suggest a positive relationship between osmotic adjustment and shoot growth.

The poor growth response of clones displaying osmotic adjustment to reduced irrigation may be associated with their high RGR when irrigated, resulting in low concentrations of tissue organic solutes and high π_o, making them the most vulnerable to subsequent drought. Among the F_2 clones that demonstrated osmotic adjustment, almost all declined from a high π_o and osmotic adjustment was generally accompanied by a marked decline in RGR. Such reductions in RGR would make more organic solutes available for osmotic adjustment that would have otherwise have been consumed in growth. Munns (1988) suggested that osmotic adjustment competes with growth processes for organic solutes. Osmotic adjustment and low π_o are typically considered together as drought tolerance traits; however, our results indicate that these traits can be uncoupled. Although osmotic adjustment did not sustain RGR during drought, the maintenance of low π_o was characteristic of clones that maintained RGR during drought.

The relatively low degree of phenotypic variation in D^2H and RGR explained by the correlation with π_o was predicted, because full sib-mating produces many allelic combinations that result in growth depression (i.e., inbreeding depression). Given the observed relationship between low π_o and the maintenance of growth during drought, and that turgor maintenance is required to survive dehydration, it is evident that low π_o is a key determinant of dehydration tolerance. Therefore, the variability in π_o observed in this interspecific hybrid pedigree can be exploited, especially given the identification of several QTL for π_o. Seven percent of clones exhibited a value of π_o that is considered extremely low (less than –2.0 MPa), especially for a fast-growing hardwood species. Among the woody species that we have studied, such low values are typically exhibited by dehydration-tolerant oak species, such as chestnut oak (*Quercus prinus* L.) (Tschaplinski et al. 1998). Further research will involve conducting candidate gene studies in the marker intervals of interest, with the search narrowed by subjecting the grandparent clones to drought and using NimbleGen's *Populus* whole-genome microarray to determine which transcripts are up-regulated in the marker interval. Subsequent up- and down-regulation of the target genes, followed by phenotypic validation of transgenic transformants, will provide information on the functional role of the candidate genes that underlie the identified QTL.

Eight QTL were identified from the data set pooled across all treatments and sites (Table 3), indicating that these QTL

Table 3. The quantitative trait loci (QTL) identified for osmotic potential at full turgor (π_o) from data analyzed across all treatments and sites (π_o-all), or across treatments within sites at Boardman and Clatskanie (π_o-BR and π_o-CL, respectively), with "treatment" as a fixed effect.

LG[1]	Analysis	QTL (cM)[1]	Adjacent marker	*P*	Var%[2]	Site	Treatment[3]	Gene action[4]	
								Add estimate	Dom estimate
Ia	π_o-all	*QI.1* (22)	p_2789	0.0004	11.6	BR	1994 dry	–0.080*[5]	0.050
							1994 wet	–0.102***	0.060
						CL	1995 untr.	–0.057	0.070
							1996 untr.	–0.144***	0.042
Ib	π_o-all	*QI.2* (48)	p_2786a	0.0268	5.8	BR	1994 dry	–0.092*	0.054
							1994 wet	–0.076*	0.074
						CL	1995 untr.	–0.045	0.043
							1996 untr.	–0.041	–0.023
II	π_o-all	*QII* (52)	p_684/p_2797	0.0000	19.1	BR	1994 dry	–0.123***	0.154*
							1994 wet	–0.072	0.105
						CL	1995 untr.	–0.104*	0.020
							1996 untr.	–0.203***	–0.005
III	π_o-all	*QIII* (0)	p_2696	0.0003	11.9	BR	1994 dry	–0.157***	–0.057
							1994 wet	–0.048	0.041
						CL	1995 untr.	–0.064	0.026
							1996 untr.	–0.164***	–0.049
VI	π_o-all	*QVI* (61)	w_12	0.0002	12.8	BR	1994 dry	0.026	0.208***
							1994 wet	0.031	0.154**
						CL	1995 untr.	0.014	0.060
							1996 untr.	0.031	0.011
VIII	π_o-all	*QVIII* (25)	p_2610	0.0035	8.9	BR	1994 dry	0.069*	0.045
							1994 wet	0.031	–0.012
						CL	1995 untr.	0.052	–0.010
							1996 untr.	0.047	–0.019
XI	π_o-CL	*QXI* (16)	p_029	0.0463	7.1	CL	1995 untr.	0.017	0.033
							1996 untr.	0.024	0.141***
XII	π_o-all	*QXII* (14)	p_495	0.0091	7.5	BR	1994 dry	0.006	0.074
							1994 wet	–0.009	0.066
						CL	1995 untr.	0.036	0.047
							1996 untr.	0.081***	0.117**
XIII	π_o-BR	*QXIII.1* (25)	p_649	0.0156	9.8	BR	1994 dry	0.104*	–0.127*
							1994 wet	0.098*	–0.039
	π_o-CL	*QXIII.2* (56)	o_016	0.0088	11.4	CL	1995 untr.	–0.013	–0.085
							1996 untr.	–0.074*	–0.129**
XV	π_o-all	*QXV* (0)	p_2585	0.0317	5.5	BR	1994 dry	–0.004	0.130*
							1994 wet	0.032	0.078
						CL	1995 untr.	0.013	0.058
							1996 untr.	0.038	0.144**
XVIII	π_o-CL	*QXVIII* (42)	p_2880	0.0184	9.6	CL	1995 untr.	–0.047	0.005
							1996 untr.	–0.109***	–0.053

[1] Linkage group number (LG) and QTL location in centiMorgans (cM) refer to map developed by Sewell et al. (unpublished).
[2] Percentage of variation explained by QTL.
[3] See Materials and methods for description of treatments.
[4] Additive estimate = (TT – DD)/2; Dominance estimate = TD – (TT + DD)/2, where T is the allele from *P. trichocarpa* and D is the allele from *P. deltoides*.
[5] Asterisks indicate level of statistical significance: *, 0.05 = *P* > 0.01; **, 0.01 = *P* > 0.005; and ***, *P* = 0.005.

were consistently expressed regardless of treatment or site. However, the finding that four additional QTL were only detected at a single site was unsurprising, because the ability to detect the QTL may depend on an environment allowing those QTL full expression. Although π_o was correlated between sites, differences between environmental conditions between sites and even between years at the same site introduced considerable variability that reduced the magnitude of these relationships. That is, a generally wet site that experiences a wetter than normal period followed by a dry period will affect π_o of some clones differently than a period of typically high water availability followed by a similarly dry period. Preconditioning, whether in response to drought, excessive water or protracted shade will affect the observed clonal π_o.

This is not the first study that has detected QTL for dehydration tolerance in plants, but it is the first study involving a long-lived woody perennial species. The QTL for dehydration tolerance in rice (*Oryza sativa* L.) have been detected by Lilley et al. (1996), who reported five QTL for lethal osmotic potential, and Zhang et al. (2001) who identified five QTL for osmotic adjustment. The biochemical networks that result in high concentrations of solutes contributing to low π_o likely function as generalized stress responses. Maintenance of low π_o is a key characteristic that contributes to drought tolerance, but other characteristics such as foliar morphological characteristics that minimize water loss, reduced stomatal conductance and root growth during drought also contribute to the overall drought tolerance of a clone. The F_2 progeny segregated for an extensive array of characteristics that can play a role in drought tolerance, and some of these traits have been mapped in woody species. For example, Brendel et al. (2002) identified and mapped four significant and four suggestive QTL in maritime pine (*Pinus pinaster*) for wood cellulose carbon isotope composition, a time-integrated measure of water-use efficiency. Similarly, Casasoli et al. (2004) reported detecting 17 QTL for carbon isotope discrimination of leaves in *Castanea sativa*, explaining 4.1 to 13.2% of the phenotypic variation.

Seven QTL for π_o were identified that each explained > 7.5% of the variation in osmotic potential in our study. The ability to consistently detect QTL across sites and treatments and the observation that both parents contribute superior allelic effects at different QTL, suggest that osmotic potential is heritable in *Populus*. With further work, these markers may prove useful in traditional genetic selection or genetic manipulation of targeted genetic loci, or both. Furthermore, the newly available genomic sequence for *Populus* (http://genome.jgi-psf.org/) will facilitate the identification of genes associated with these QTL. These genes can then be exploited through transgenesis to enhance dehydration tolerance in elite clones that are highly productive when irrigated, but are currently relatively drought intolerant. The large individual effect of a QTL indicates that a gene may be influencing several subcomponents of osmotic potential. Such responses may be regulated by a single transcription factor or other regulatory steps within specific biochemical pathways. As a cautionary note, the magnitude of the effect of a QTL may have been overestimated in this study because of the small population size.

References

Blum, A., J. Zhang and H.T. Nguyen. 1999. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crop Res. 64:287–291.

Bradshaw, H.D., Jr. and R.F. Stettler. 1995. Molecular genetics of growth and development in *Populus*. IV. Mapping QTL with large effects on growth, form, and phenology traits in a forest tree. Genetics 139:963–973.

Brendel, O., D. Pot, C. Plomion, P. Rozenberg and J.-M. Guehl. 2002. Genetic parameters and QTL analysis of $\delta^{13}C$ and ring width in maritime pine. Plant Cell Environ. 25:945–953.

Casasoli, M., D. Pot, C. Plomion, M.C. Monteverdi, T. Barreneche, M. Lauteri and F. Villani. 2004. Identification of QTLs affecting adaptive traits in *Castanea sativa* Mill. Plant Cell Environ. 27: 1088–1101.

Gebre, G.M., T.J. Tschaplinski, G.A. Tuskan and D.E. Todd. 1998. Clonal and seasonal differences in leaf osmotic potential and organic solutes of two hybrid poplar clones grown under field conditions. Tree Physiol. 18:653–658.

Haley, C.S. and S.A. Knott. 1992. A simple regression model for interval mapping in line crosses. Heredity 69:315–324.

Knott, S.A., D.B. Neale, M.M. Sewell and C.S. Haley. 1997. Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor. Appl. Genet. 94:810–820.

Lander, E.S. and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241–247.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newberg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.

Lilley, J.M., M.M. Ludlow, S.R. McCouch and J.C. O'Toole. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427–1436.

Ludlow, M.M., F.J. Santamaria and S. Fukai. 1990. Contribution of osmotic adjustment to grain yield of *Sorghum bicolor* (L.) Moench. under water-limited conditions. II. Water stress after anthesis. Aust. J. Agric. Res. 41:67–78.

Mohn, C.A., and W.K. Randall. 1973. Interaction of cottonwood clones with site and planting year. Can. J. For. Res. 3:329–332.

Morgan, J.M. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Aust. J. Plant Physiol. 34:607–614.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant. Physiol. 35:299–319.

Morgan, J.M. 1995. Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. Field Crop Res. 40:143–152.

Morgan, J.M., B. Rodriguez-Maribona and E.J. Knights. 1991. Adaptation to water-deficit in chickpea breeding lines by osmoregulation—relationship to grain yields in the field. Field Crops Res. 27:61–70.

Munns, R. 1988. Why measure osmotic adjustment? Aust. J. Plant Physiol. 15:717–726.

Neilson, R.P., G.A. King, R.L. DeVelice, J. Lenihan, D. Marks, J. Dolph, B. Campbell and G. Glick. 1989. Sensitivity of ecological landscapes and regions to global climate change. U.S. Environmental Protection Agency, Environmental Research Laboratory, EPA-600-3-89-073, NTIS-PB-90-120-072-AS, Washington, D.C., 103 p.

Passioura, J.B. 1988. Root signals control leaf expansion in wheat seedlings growing in drying soils. Aust. J. Plant Physiol. 15: 687–693.

Riemenschneider, D.E., W.E. Berguson, D.I. Dickmann, R.B. Hall, J.G. Isebrands, C.A. Mohn, G.R. Stanosz and G.A. Tuskan. 2001. Poplar breeding and testing strategies in the nort-central U.S.: Demonstration of potential yield and consideration of future research needs. For. Chron. 77:245–253.

Seaton, G., C.S. Haley, S.A. Knott, M. Kearsey and P.M. Visscher. 2002. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics 18:339–340.

Sewell, M.M., B.K. Sherman and D.B. Neale. 1999. A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151:321–330.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J. 3:739–744.

Tangpremsri, T., S. Fukai and K.S. Fischer. 1995. Growth and yield of sorghum lines extracted from a population for differences in osmotic adjustment. Aust. J. Agric. Res. 46:61–74.

Tschaplinski, T.J. and G.A. Tuskan. 1994. Water-stress tolerance of black cottonwood and eastern cottonwood clones and four of their hybrid progeny. II. Metabolites and inorganic ions that constitute osmotic adjustment. Can. J. For. Res. 24:681–687.

Tschaplinski, T.J., G.A. Tuskan and C.A. Gunderson. 1994. Water-stress tolerance of black cottonwood and eastern cottonwood clones and four of their hybrid progeny. I. Growth, water relations and gas exchange. Can. J. For. Res. 24:346–371.

Tschaplinski, T.J., G.M. Gebre and T.L. Shirshac. 1998. Osmotic potential of several hardwood species as affected by throughfall manipulation of an upland oak forest during a dry year. Tree Physiol. 18:291–298.

Tuskan, G.A. 1998. Short-rotation forestry: What we know and what we need to know. Biomass Bioenergy 14:307–315.

Tuskan, G.A. and M. Walsh. 2001. Short-rotation woody crop systems, atmospheric carbon dioxide and carbon management. For. Chron. 77:259–264.

Tuskan, G.A., L.E. Gunter, Z.K. Yang, T.M. Yin, M.M. Sewell and S.P. DiFazio. 2004*a*. Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. Can. J. For. Res. 34:85–93.

Tuskan, G.A., S.P. DiFazio and T. Teichmann. 2004*b.* Poplar genomics is getting popular: The impact of the poplar genome project on tree research. Plant Biol. 6:2–4.

Tyree, M.T. and P.G. Jarvis. 1982. Water in tissues and cells. *In* Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler. Springer-Verlag, Berlin, pp 35–77.

Tyree, M.T., Y.N.S. Cheung, M.E. MacGregor and A.J.B. Talbort. 1978. The characteristics of seasonal and ontogenetic changes in the tissue-water relations of *Acer*, *Populus*, *Tsuga*, and *Picea*. Can. J. Bot. 56:635–647.

Tyree, M.T., M.E. MacGregor and S.I. Cameron. 1979. Ontogenetic factors affecting the osmotic pressure and bulk modulus of elasticity of poplar leaves. Rep. 14. *In* Poplar Research, Management and Utilization in Canada. Eds. D.C.F. Fayle, L. Zsuffa and H.W. Anderson. Ont. Min. Nat. Resour. For. Res. Inf. Pap. No. 102, 11 p.

USDA. 1983. Soil survey of Morrow County, Oregon. Soil Conservation Service and Oregon Agricultural Experimental Station, pp 35–96.

Wright, G.C., R.C.G. Smith and J.R. McWilliam. 1983. Differences between two grain sorghum genotypes in adaptation to drought stress. I. Crop growth and yield responses. Aust. J. Agric. Res. 34:615–626.

Wright, P.R., J.M. Morgan and R.S. Jessop. 1996. Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: plant water relations and growth. Field Crops Res. 49:51–64.

Yin, T.-M., S.P. DiFazio, L.E. Gunter, D. Riemenschneider and G.A. Tuskan. 2004. Large-scale heterospecific segregation distortion in *Populus* revealed by a dense genetic map. Theor. Appl. Genet. 109:451–463.

Zhang, J., H.G. Zheng, A. Aarti et al. 2001. Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. Theor. Appl. Genet. 103: 19–29.