

Early effects of water deficit on two parental clones of *Populus nigra* grown under different environmental conditions

Claudia Coccozza^{A,B,D}, Paolo Cherubini^B, Nicole Regier^B, Matthias Saurer^C, Beat Frey^B and Roberto Tognetti^A

^AEcoGeoFor Lab, Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio (STAT), Università degli Studi del Molise, Contrada Fonte Lappone, I-86090 Pesche, Italy.

^BWSL Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland.

^CPSI Paul Scherrer Institute, CH-5232 Villigen, Switzerland.

^DCorresponding author. Email: claudia.coccozza@unimol.it

Abstract. Global climate change is expected to induce a dramatic increase in the frequency and intensity of drought events in the Mediterranean region. Their effects might be particularly severe in short rotation forestry systems, such as poplar plantations, with high water demands. The aim of this study was to examine the clone-specific reaction of plant-water relations and growth to a dry-down cycle in two parental clones of *Populus nigra* L.: Poli, which is adapted to the dry/hot climatic conditions of southern Italy, and 58–861, which prefers the cooler and moister conditions typical in northern Italy. Plants were grown in controlled conditions in an airconditioned greenhouse, under three different irrigation regimes for 44 days. Drought stress resulted in a general decrease in plant size and predawn water potential in both clones. Although the control trees grew somewhat taller and retained leaves longer than those in other treatments, the two clones responded differently to water stress. Under severe stress conditions, Poli showed proline accumulation in old leaves to preserve plants from drought damage, without reduced stomatal activity, as shown by low values of $\delta^{13}\text{C}$. In 58–861, the accumulation of ABA in roots during drought probably stimulated stomatal control, increasing drought avoidance in this drought-sensitive clone. Although in 58–861 the expression of aquaporin genes PIP1–2 and TIP1–3 was enhanced, in Poli gene expression was downregulated. We analysed only part of the aquaporins genes, but we assume that these clones exhibited contrasting water transport strategies during drought. Clone 58–861 seems to increase the permeability of the vascular tissue by overexpressing aquaporin genes, probably in order to facilitate water transport, and Poli appears to increase water conservation in the root cells by downregulating aquaporins.

Additional keywords: ABA, aquaporins, carbon isotope composition, proline, water stress.

Introduction

The greenhouse effect is expected to increase global mean temperatures by 1.4–5.8°C by the middle of this century. Although fresh water resources may not be seriously limited under such future global warming (Betts *et al.* 2007), there is still an increased risk of drought in some regions because of reduced soil moisture caused by enhanced evapotranspiration from soil under a warmer environment. One tree that may be particularly affected by increased drought is the poplar.

Poplars are fast growing trees that are highly susceptible to water deficit. However, significant variation in poplar's drought response has been recorded (Ceulemans *et al.* 1978; Gebre and Kuhns 1991; Marron *et al.* 2002). This variation between genotypes affects several physiological and morphological traits (Harvey and van den Driessche 1997; Marron *et al.* 2002), in particular: plant growth and leaf area (Souch and Stephens 1998; Ren *et al.* 2007); potential for osmotic adjustment (Gebre *et al.* 1998); sensitivity of leaf expansion,

extent of leaf abscission and adjustment of the root : shoot ratio (Liu and Dickmann 1992; Chen *et al.* 1997; Tschaplinski *et al.* 1998) and vulnerability of xylem to cavitation (Cochard *et al.* 2007). Poplars under water-stress conditions have been found to vary biochemically in terms of accumulation of carbohydrates (Bogeat-Triboulot *et al.* 2007) as well as proline and ABA (Ren *et al.* 2007).

Proline is an important amino acid that plays a role in plants' response and adaptation to drought stress (Bates *et al.* 1973). Its concentration has been found to increase in the leaves and roots of Mediterranean shrubs under water-stress conditions (Ain-Lhout *et al.* 2001; Shvaleva *et al.* 2006), as well as in poplars under osmotic stress (Watanabe *et al.* 2000). Proline is an osmoprotectant, capable of mitigating the impacts of drought (Stewart and Larher 1980) and salt (Dix and Pearce 1981) in higher plants. Indeed, the proline accumulation process may preserve the structure and activity of enzymes while protecting membranes from damage from the reactive oxygen species that

are produced in response to drought (Hare and Cress 1997; Hong *et al.* 2000). However, until now the variability of proline content in poplar clones from the Mediterranean region subjected to drought conditions has never been studied.

Different poplar genotypes appear to differ in their responsiveness to water stress mediated by ABA (Cochard *et al.* 1996), and clonal differences in $\delta^{13}\text{C}$ have been found for gas-exchange and stem-growth traits in poplar (Voltas *et al.* 2006). Plant $\delta^{13}\text{C}$ values reflect isotope fractionation processes at the leaf level, which are correlated with intrinsic water-use efficiency (WUE), i.e. the amount of water transpired per unit carbon gain (Farquhar *et al.* 1982; Farquhar *et al.* 1989). Stable carbon isotopes are increasingly being used as a powerful tool for investigating the balance between photosynthesis and stomatal conductance in C_3 plants. The positive relationship between WUE and $\delta^{13}\text{C}$ arises through their independent linkages to the ratio of internal to ambient CO_2 concentrations (C_i/C_a) (Farquhar *et al.* 1989; Guehl *et al.* 1995). Stomatal closure in water-stressed poplars may be elicited by ABA, which has been proposed as the principal factor in the signalling cascade that operates between roots and shoots triggered by drought (Davies *et al.* 1994).

Aquaporins are essential for water transport within plants and are differentially expressed during water stress. Plant aquaporins are classified into four subgroups: plasma-membrane intrinsic proteins (PIPs), tonoplast-intrinsic proteins (TIPs), Nodulin26-like intrinsic proteins (NIPs) and small basic intrinsic proteins (SIPs) (for a review, see Kaldenhoff *et al.* 2007; Katsuhara *et al.* 2008). In poplar, more than 60 genes encoding aquaporins have been annotated (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html). In the roots of poplar, the functions of aquaporins have, however, hardly been investigated and, to our knowledge, the regulation of aquaporins during drought has never been studied. Thus, in this study, we analysed the expression of six aquaporins genes in poplar roots. The PIP and TIP genes are the closest homologues to the aquaporin genes of other plants, which have been shown to be important during drought and other osmotic stress (Pih *et al.* 1999; Aharon *et al.* 2003).

We hypothesised that reduced water availability would increase the proline and ABA contents as well as WUE in poplar, and that the magnitude of responses induced by drought stress would differ in clones with distinct geographic origins. In addition, the expression pattern of aquaporin-encoding genes was assumed to be downregulated in roots under water stress, with differences between genotypes: the lower gene expression in stressed plants being associated with higher hydraulic resistance (Secchi *et al.* 2007).

For a successful breeding program for poplars with drought tolerance in areas with recurrent drought spells, the first requirement should be to identify poplar genotypes that show functional adaptation (including gene expression) with an acceptable yield under water deficit. The objectives of this study were, therefore: (1) to analyse the impact of recurrent water deficits on plant ecophysiological performance and aquaporin gene expression patterns in the roots of two parental clones of *Populus nigra* L. with different geographic origins; (2) to highlight the relationships between growth traits and water relations, and (3) to evaluate the relationships between

drought tolerance and proline, ABA and carbon isotopic composition.

Materials and methods

Plant material and experimental design

The two *Populus nigra* L. genotypes originated from Italian populations. The female parent 58–861 comes from northern Italy (45°09'N, 7°01'E), near the Dora Riparia River close to The Alps at 597 m above sea level. It is adapted to the cool and moist conditions of the area, opening its buds later and setting the apical bud earlier than the Poli clone, and may be considered 'drought-sensitive'. The male parent Poli comes from Southern Italy (40°09'N, 16°41'E), near the Sinni River in the plain beside the Ionio Sea at 7 m above sea level. It is adapted to the dry/hot climatic conditions of the area and may be considered 'drought-tolerant' (Gaudet *et al.* 2008).

In early March 2007, 18 2-year-old woody cuttings of two clones were planted separately into 10 L plastic pots filled with a mixture of 42% crust humus, 42% wood fibre, 4% clay, 1.25 g Floranid permanent and 1.25 g NP 20 : 20 with a pH of 5.5–6.2 and grown in an air-conditioned greenhouse under well watered (control plants) conditions. The plants were exposed to extra light (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density) for 15 h day^{-1} , and temperature and humidity were kept constant (20°C at night and 25°C during daytime, with 40–60% RH). After 6 weeks of growth, plants of similar height were selected for the experiment. The experimental layout was a randomised block design with two factors (clone and watering regime) and three replicates per clone-treatment combination. At the beginning of the experiment on 18 April 2007, all pots were watered to field capacity. The pots were weighed weekly to determine the amount of water loss, which was completely replenished for the control plant pots. From pots assigned to water stress, water was withheld for 3 weeks followed by 2 weeks watering with 25% of the amount of water added to the control plants.

Soil water tension

Soil water tension (Ψ_{soil}) was monitored daily throughout the experiment with two tensiometers for each clone-treatment combination (TS1 Self Refilling Tensiometer, UMS, München, Germany), placed at the same depth and in substrates with identical physical structure and chemical composition.

Pre-dawn leaf water potential

Pre-dawn water potential (Ψ_{pd}) was measured once a week with a pressure chamber (PMS Instruments Co., Corvallis, OR, USA) during the whole experiment to monitor plant water status. For all the clones and treatments, Ψ_{pd} measurements were performed on fully expanded leaves ($n=3$), located halfway from the crown along the stem. The evening before Ψ_{pd} was measured the plants were enclosed in black polyethylene bags to stop transpiration. Measurements were made the following day in the early morning with the leaves still enclosed in the plastic bag and considered predawn.

Stem and leaf morphology

The number of leaves and the stem height were measured ($n=3$) once a week to evaluate the growth responses of trees with

different watering regimes. Prints were taken weekly of the first fully expanded leaf from the apex (leaf plastochron index, LPI=8). LPI is based on the leaf rank from the first fully open, but not yet completely expanded, apical leaf (Dickmann 1971). Prints were measured with an area meter (Li-3100, Li-Cor, Lincoln, NE, USA).

Proline determination

At the end of the experiment, the plants were harvested and leaves and roots separated. The plant material was immediately put into liquid nitrogen, and stored at -80°C until proline analysis. Proline concentrations were determined by the acid-ninhydrin procedure of Bates *et al.* (1973). Leaf and root samples (0.5 g) were ground and then homogenised with 3% sulfosalicylic acid (10 mL). The homogenised sample was filtered through filter paper (595 1/2) (Whatman, Kent, UK). The supernatant (2 mL) was mixed equal volumes of acid-ninhydrin and acetic acid. The mixture was incubated in a boiling water bath (100°C) for 1 h, and the reaction then stopped in an ice bath. The reaction mixture was extracted with toluene (4 mL) and absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis. Three replicates were measured for each sample.

ABA measurement

Leaves and roots were harvested, immediately put into liquid nitrogen then stored at -80°C until extraction. Twenty milligrams of leaf (without midribs) and root tissues were extracted overnight in 1.5 mL distilled water in the dark at 4°C on a shaker (Barta and Loreto 2006). The extracts were centrifuged at 10 000g for 25 min. The ABA content of the supernatants was then quantified in an enzyme-linked immunosorbent assay (ELISA) using the Phytodetek-ABA kit (AGDIA, Elkhart, IN, USA) according to the indications of the manufacturer. All assays were made in triplicate. The hormone concentrations were calculated using a standard curve of ABA and the relative optical density, according to the ELISA technique.

Carbon isotope composition

An aliquot of the freeze-dried material was weighed into tin capsules for carbon isotope analyses of the leaves and roots. The samples were combusted in an elemental analyser and the evolving CO_2 measured on an isotope-ratio mass spectrometer (delta-S, Finnigan MAT, Bremen, Germany). The isotope ratio

$^{13}\text{C}/^{12}\text{C}$ is expressed as a relative deviation from the international standard VPDB as a $\delta^{13}\text{C}$ -value in per mil.

RNA extraction and first strand cDNA synthesis

Frozen fine roots were ground to a powder in liquid nitrogen. RNA was extracted using the Agilent plant RNA isolation mini kit according to the manufacturer's instructions (Agilent Technologies AG, Basel, Switzerland). RNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and RNA quality was assessed using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). First strand cDNA synthesis was performed with the QuantiTect Reverse Transcription Kit (Qiagen AG, Hombrechtikon, Switzerland), using 200 ng total RNA. The quality of the first strand cDNA was tested via PCR using primers for actin and elongation factor 1, β subunit (Table 1). Absence of genomic DNA in the samples was verified by choosing an intron-spanning amplicon for elongation factor 1, β subunit.

Primer design and real-time RT-PCR

Primers for aquaporin genes (*NIP1-2* and *5-1*, *TIP1-1* and *1-3*, *PIP1-2* and *SIP 1-2*) (Table 1) were designed using Primer 3 software (Rozen and Skaletzki 2000) for amplification of gene fragments around 100 bp in length and annealing temperature of 60°C . Sequences for primer design were downloaded from the *Populus trichocarpa* Torr. & Gray v1.1 database at the Joint Genome Institute (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; Tuskan *et al.* 2006). Primer sequences are shown in Table 1. Primer specificity was tested against the genome sequence via insilico PCR, available at <http://www.popgenie.db.umu.se> (Sjödin *et al.* 2009).

Real-time RT-PCR was performed using the FastStart Universal SYBR Green Master Mix (Roche Diagnostics, Mannheim, Germany) on an ABI 7500 fast real-time PCR system (Applied Biosystems Inc., Foster, CA, USA). Reactions of 15 μL total volume contained 5 μM of forward and reverse primers and 1 μL of 1:10 or 1:100 diluted cDNA. PCR efficiencies were determined using the program LinRegPCR (Ramakers *et al.* 2003). Relative gene expression was calculated with qBase software (Hellems *et al.* 2007), using transcripts of actin2, ubiquitin and elongation factor1 and β subunit as references. This software allows multiple reference genes to be included to calculate the relative gene expression. Three technical replicates were performed for each of the three biological replicates.

Table 1. Sequences of the primers used in real-time RT-PCR
Primers were designed on Joint Genome Institute gene models as denoted

Gene	Abbreviated forward primer	Reverse primer	JGI gene model ID
Nodulin-intrinsic protein	NIP1-2 ATT TCA ACC CTG CTG TCA CC	GTT GCT CCG ATG ACT TGA CA	gw1.IV.2596.1
Nodulin-intrinsic protein	NIP5-1 CCT TGA TTT TCC TGC TCC TG	TAT TGG TCC TGC TGT TGC TG	estExt_Genewise1_v1.C_LG_I5715
Plasmamembrane-intrinsic protein	PIP1-2 CAA GCC CAG TTT GTT CCA TT	GGC CCT TGA AGA AAT ACA CG	grail3.0049030302
Small intrinsic protein	SIP1-2 TAG GCA CAC CAC TTG GGA TT	ATT GGT GGT GCC TTG AAA AG	eugene3.00141013
Tonoplast-intrinsic protein	TIP1-1 CTC CAC TGT CGC TTG CTT G	TTC CAT ACA CCA ACC CCA GT	grail3.0025002201
Tonoplast-intrinsic protein	TIP1-3 TTT GGT CCT GCT GTT GTG AG	ATA GAC AAG GGC AGC AAT GG	estExt_fgenes4_pg.C_LG_X1886

Table 2. ABA, proline and carbon isotope measures at the end of the experiment (day 44 of the experiment, on 31 May 2007)
For each measure, significant differences between clones, treatments and leaf ages, and interactions among variables (ANOVA test) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant

Plant part	Measure	Clone (C)	Treatment (T)	Leaf age (L)	C × T	T × L	C × L
Leaves	ABA	***	*	***	***	*	NS
	Proline	NS	NS	*	NS	NS	NS
	Carbon isotope	***	NS	***	**	NS	*
Roots	ABA	***	**	NA	*	NA	NA
	Proline	NS	NS	NA	NS	NA	NA
	Carbon isotope	***	NS	NA	NS	NA	NA

Statistical analysis

The experiment was set up in a completely randomised block design with three replicates for each clone-treatment combination. Data were averaged on a plant basis and the individual means were used for the analysis. The effects of watering treatments on the water potential and growth parameters were tested with the statistical package Statistica (StatSoft Inc., Tulsa, OK, USA), using repeated-measures analyses of variance. Proline, ABA, carbon isotope values and enzyme activities refer to the last sampling only. Data were subjected to ANOVA, without considering the effects of time of exposure. Statistical comparisons were considered significant at $P < 0.05$.

Results

Dry-down cycle

Control pots had the highest water content, whereas the severely stressed ones had the lowest. Interaction 'clone' × 'watering

treatment' was not significant, whereas 'day of treatment' × 'watering treatment' ($P < 0.001$) was significant.

A considerable degree of agreement between Ψ_{soil} and the dry-down course induced by differential water supply was observed, with a consistent reduction in Ψ_{soil} after irrigation events (Fig. 1). There were significant differences in Ψ_{soil} between clones ($P < 0.001$) and between days of experiment ($P < 0.001$) and watering treatments ($P < 0.001$). There were significant interactions 'clone' × 'watering treatment' ($P < 0.001$) and 'day of treatment' × 'watering treatment' ($P < 0.001$). There was no significant 'clone' × 'watering treatment' interaction in the daily analyses. However, significant differences between watering treatments were observed.

Leaf water relations

In both clones, the Ψ_{pd} of control plants ranged around -0.4 MPa throughout the experiment, whereas the Ψ_{pd} of moderately

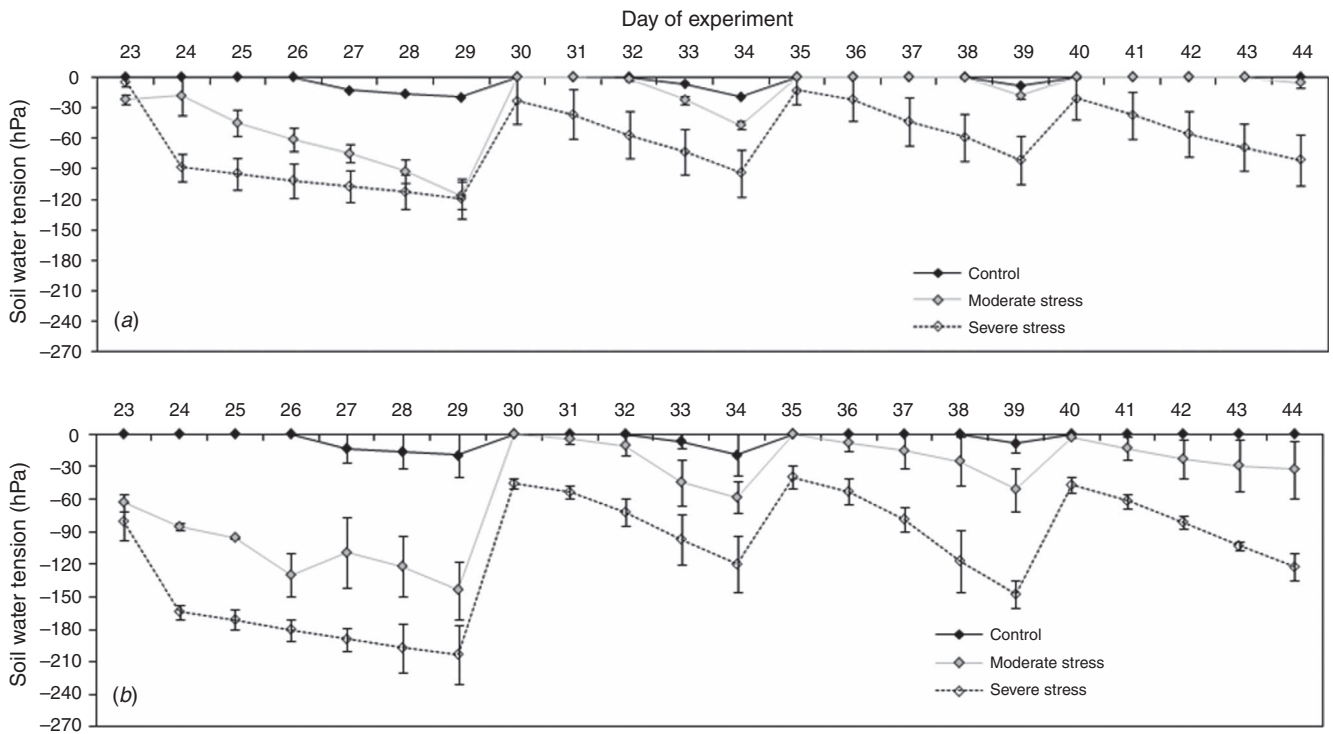


Fig. 1. Tensiometer readings, as Ψ_{soil} , for *Populus nigra* clones (a) Poli and (b) 58–861 during the water-stress experiment. The graphs show, for each measure, the values for the mean and s.e. (three replicates).

stressed plants declined to about -0.7 and that of severely stressed ones to about -0.9 MPa (Fig. 2). Differences in water availability between clones were related to the course of Ψ_{pd} during the experiment. In Poli, water-stressed plants responded promptly to the beginning of water stress and maintained a relatively low Ψ_{pd}

thereafter. In 58–861, water-stressed plants experienced a major reduction in Ψ_{pd} only towards the end of experiment. Significant differences in Ψ_{pd} were observed between clones, and between days of experiment and irrigation treatments (Fig. 2). ‘Clone’ \times ‘day of treatment’ ($P < 0.01$) and ‘day of

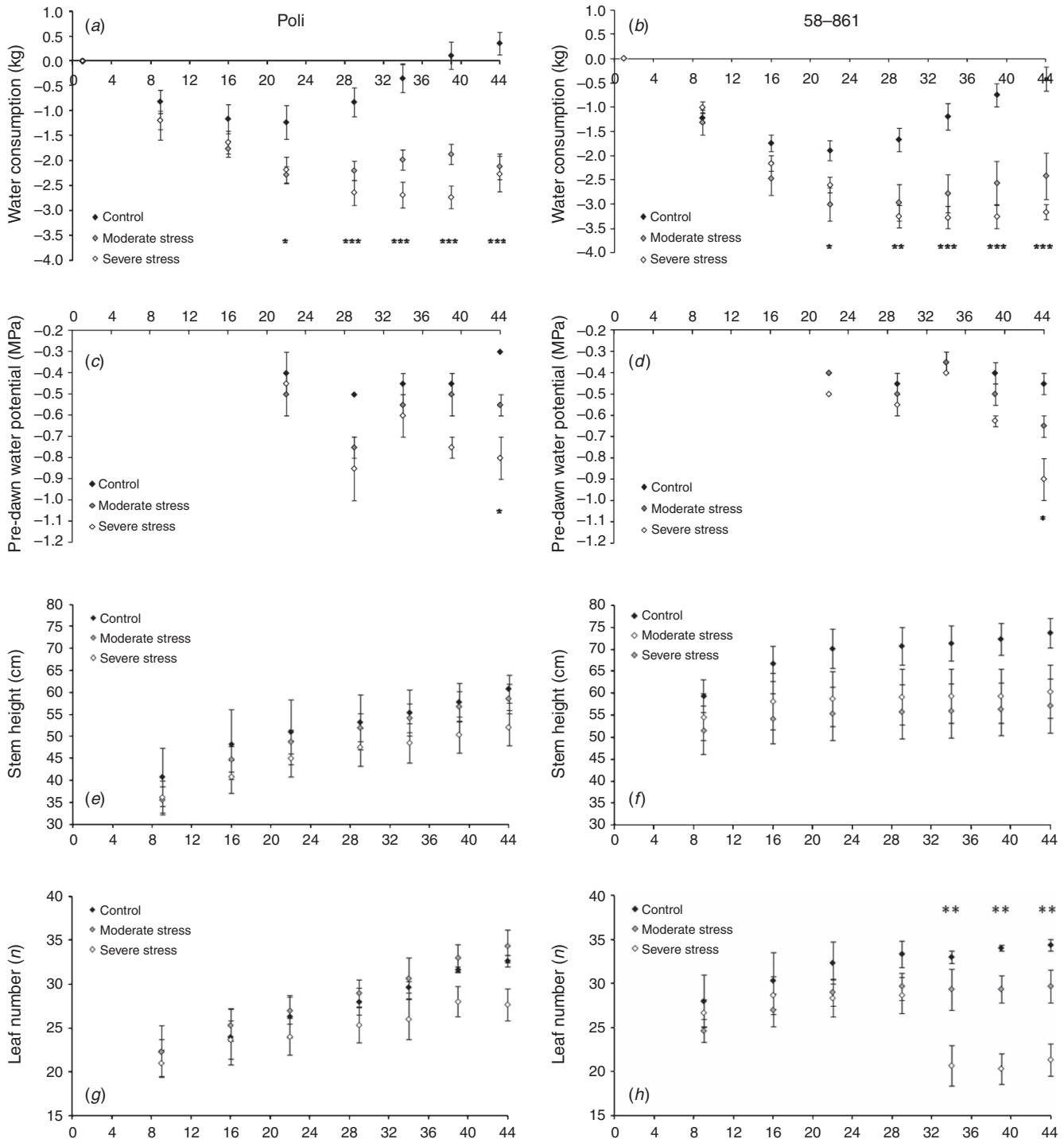


Fig. 2. Water consumption, Ψ_{pd} , stem height and leaf number for *Populus nigra* clones Poli and 58–861 during the water-stress experiment. The graphs show, for each measure, the values for the mean and standard errors, and the statistical analysis of the three watering treatments. Significant differences between treatments for each variable (ANOVA test) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

treatment' \times 'watering treatment' ($P < 0.01$) interactions were also significant.

Plant structure

The leaf area of the two clones differed significantly. Poli had smaller leaves than 58–861 at all measured stem heights. Poli showed only minor differences between treatments, and its leaf area was between 2.8 and 17.7 cm², whereas 58–861 had leaf areas between 7.1 and 73.1 cm². The leaf area of Poli did not vary markedly throughout the stem length at any measurement time. Both severe and mild exposure to drought stress resulted in no significant reductions in the surface area of the first fully developed leaf from the apex in either clone. The decrease in surface area of the eighth leaf from the top of severely stressed 58–861 was not significantly different from that in other treatments. In contrast, the leaves of the same node in Poli showed a consistent decline in surface area for the duration of experiment: 52% in well watered plants, 61% in moderately stressed plants and 43% in severely stressed plants.

By the end of experiment, the stem height of the two clones showed no significant differences between treatments (Fig. 2). The control plants grew somewhat taller and retained leaves for longer than those in other treatments. Significant differences in stem height ($P < 0.0001$) were observed between clones, and between day of experiment and watering treatments in leaf number of 58–861 (Fig. 2). 'Clone' \times 'day of treatment' ($P < 0.001$), 'clone' \times 'watering treatment' ($P < 0.001$) and 'day of treatment' \times 'watering treatment' ($P < 0.01$) interactions were significant for the number of leaves, while only the interaction 'clone' \times 'watering treatment' ($P < 0.05$) was significant for stem height. Intra-diurnal analyses revealed significant differences between clones for the whole experimental period and highly significant differences between watering treatments towards the end of the experiment for leaf number (Fig. 2). Significant differences between clones were also found for stem length in the inter-diurnal analysis (Fig. 2).

Plant biomass was affected marginally by treatments in Poli. In 58–861, the effect of increasing water stress was significant on root biomass, with a marked reduction from control to severe-stress treatments. Significant differences in leaf ($P < 0.001$), stem ($P < 0.001$) and root ($P < 0.001$) biomass were found between clones (data not shown).

Proline content

Proline content did not vary significantly among the different treatments in young leaves of Poli (Table 2; Fig. 3), but in old leaves (the first formed) proline content increased consistently from that of the control to that in severely stressed plants. In 58–861 (Fig. 3), the proline content in young leaves was higher in control plants than in other treatments, although in old leaves there were no significant differences due to the watering regime. No significant differences were found between clones, watering treatments, or leaf ages (Table 2); but there were significant 'clone' \times 'watering treatment', 'clone' \times 'leaf-age' and 'watering treatment' \times 'leaf-age' interactions. No significant differences in the proline content in roots between watering treatments were found in either clone (Table 2).

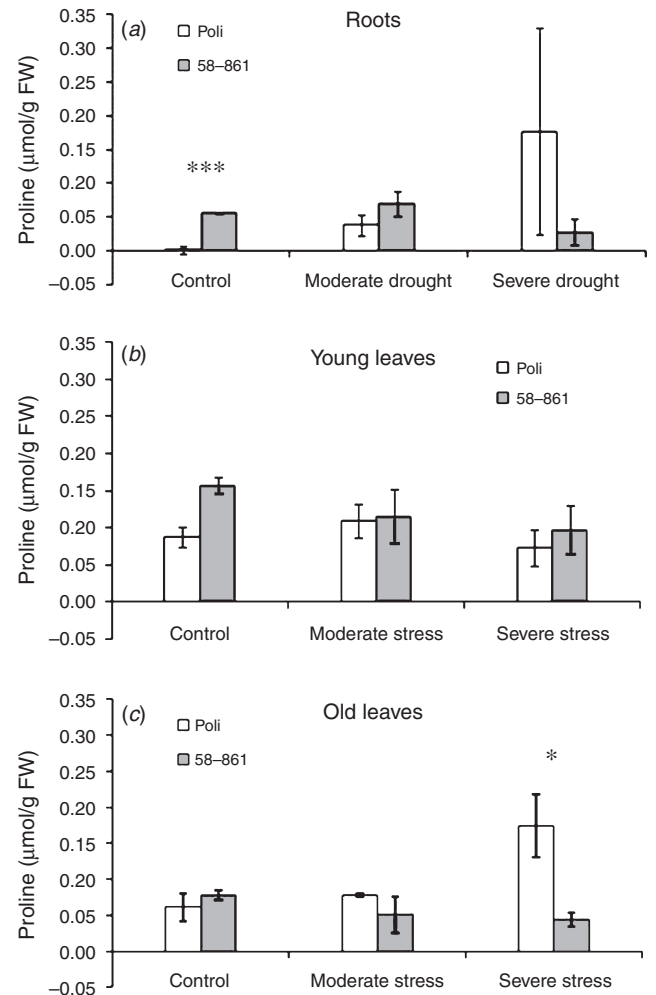


Fig. 3. Changes in proline content in the leaves and roots of *Populus nigra* clones submitted to three water treatments. Data are means \pm s.e. ($n = 3$). Significant differences between treatments for each variable (ANOVA test) are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ABA concentration

ABA concentrations in roots, and young and old leaves were significantly higher in 58–861 than in Poli (Fig. 4). Moreover, significant differences in leaves were found according to leaf age and treatments, whereas ABA measurements in roots revealed significant differences between clones and treatments (Table 2). There were significant 'clone' \times 'treatment' and 'treatment' \times 'leaf-age' interactions for leaf ABA concentration, and 'clone' \times 'treatment' interactions for root ABA (Table 2).

$\delta^{13}C$ Values

Poli had more negative values than 58–861 (Fig. 4). Carbon isotope composition was altered by watering regimes in both clones (the interaction 'clone' \times 'treatment' was significant, Table 2). In 58–861, plants under moderate and severe water stress had more enriched $\delta^{13}C$ than control plants, whereas we found the opposite in Poli. According to the ANOVA analysis,

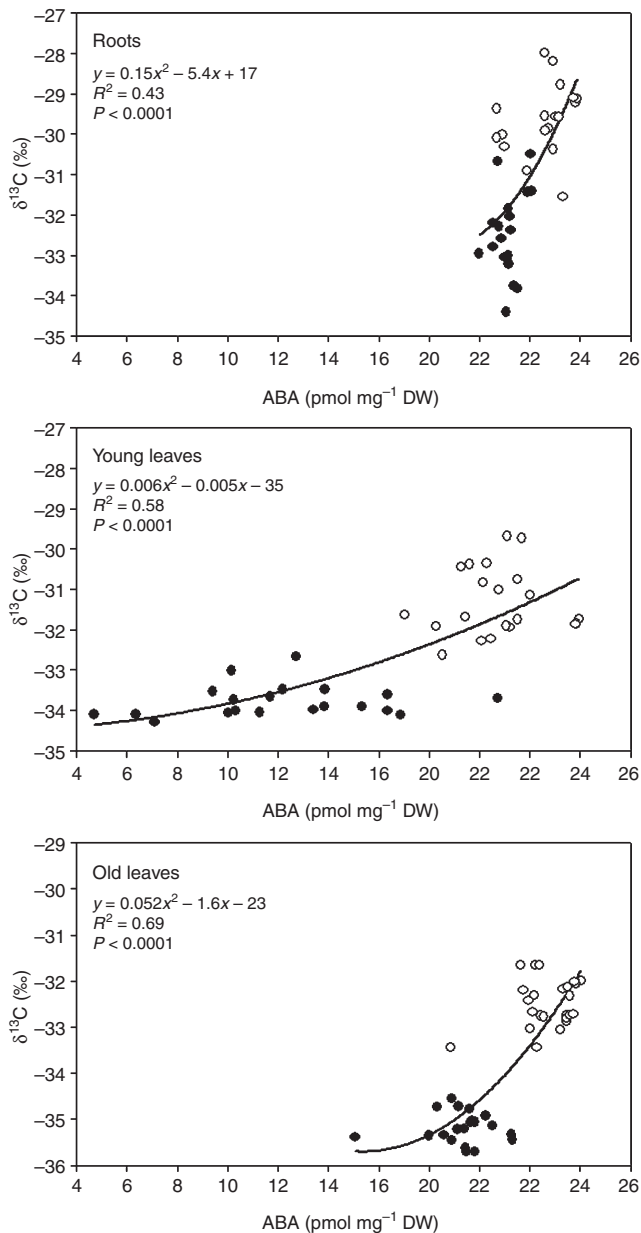


Fig. 4. Polynomial relationships between the ABA content and the leaf carbon isotope discrimination ($\delta^{13}\text{C}$), in the leaves and roots of Poli (black circles) and 58–861 (white circles).

there were significant clone and leaf-age differences for $\delta^{13}\text{C}$ in leaves and significant clone differences in roots. The interactions 'clone' \times 'treatment' and 'clone' \times 'leaf-age' were significant for leaf carbon isotope (Table 2), whereas the interaction 'clone' \times 'treatment' was not significant for root carbon isotopes (Table 2). The $\delta^{13}\text{C}$ values in the leaves and roots of Poli were more negative than in those of 58–861 by an average of 2.56‰ (Fig. 4). The relatively low $\delta^{13}\text{C}$ values in plant tissues (less than -30‰) suggested that greenhouse conditions enhanced the CO_2 concentration or the concentration was altered by CO_2 regulation in the chamber. This was considered negligible,

however, because we were interested in differences between genotypes and treatments.

Aquaporin genes

Aquaporin genes showed no consistent expression patterns in roots. Expression of NIP5–1 differed in the two clones. In 58–861, it was >2 -fold higher in control plants than in plants that were severely water stressed, whereas in Poli the expression was twice as low in the control plants as in severely water-stressed plants. In contrast, severe water stress induced the expression of PIP1–2 and TIP1–3 in 58–861, but downregulated the expression of these genes in Poli (Fig. 5).

Discussion

Because poplar hybrids grow fast, their demand for water increases as they grow larger. This may induce drought stress under dry conditions (Gebre *et al.* 1998). We chose highly contrasting parental clones (Gaudet *et al.* 2008), collected from divergent environments, to elucidate genotypic responses to water stress. Water stress resulted in a decrease in plant growth, leaf number, biomass and predawn water potential in both clones. All growth parameters decreased with reduced water availability. The variation in the pot water contents from the beginning to the end of experiment showed that 58–861 required more water than Poli in all treatments, probably because its biomass was greater on average. In both clones, water stress had a negative effect on root biomass (data not shown). Prolonging the water supply beyond the early developmental stages could result in progressively different growth rates between treatments and between clones, due to clone-specific drought sensitivity and biomass allocation. Higher values of Ψ_{soil} were recorded in well watered than in severely stressed pots, following temporal variation in soil water availability (Whalley *et al.* 2007). The decline in Ψ_{soil} and Ψ_{pd} throughout the experiment may also be related to declining growth rates (Arend and Fromm 2007).

Our data suggests that Poli is more reactive to water stress, whereas 58–861 is more tolerant of drought conditions. The intensity of drought stress was uniform over the whole experiment and significant differences between treatments were measured only at the end of experiment in both clones. In any case Ψ_{pd} dropped below -1 MPa (see also, Giovannelli *et al.* 2007). Therefore, tissue hydration was relatively stable under fluctuating environmental conditions, validating the isohydric behaviour of these poplar clones. Johnson *et al.* (2002) found that Ψ_{pd} decreased with increasing water stress conditions in poplar clones, whereas variations in midday Ψ were much less affected by drought.

Different growth traits between the two parental clones (e.g. stem height, leaf number) in unconstrained water conditions might indicate specific adaptation to native environmental conditions, affecting water consumption and early survival in a dry environment (e.g. Xu and Zhou 2006). During the experiment, only 58–861 showed senescence symptoms in the leaves of severely stressed plants (e.g. Bogeat-Triboulot *et al.* 2007). A clone-specific regulation of leaf number, and, thus, of transpiring surface, could indicate differences between genotypes in the skill of controlling water loss through foliage shedding under drought conditions.

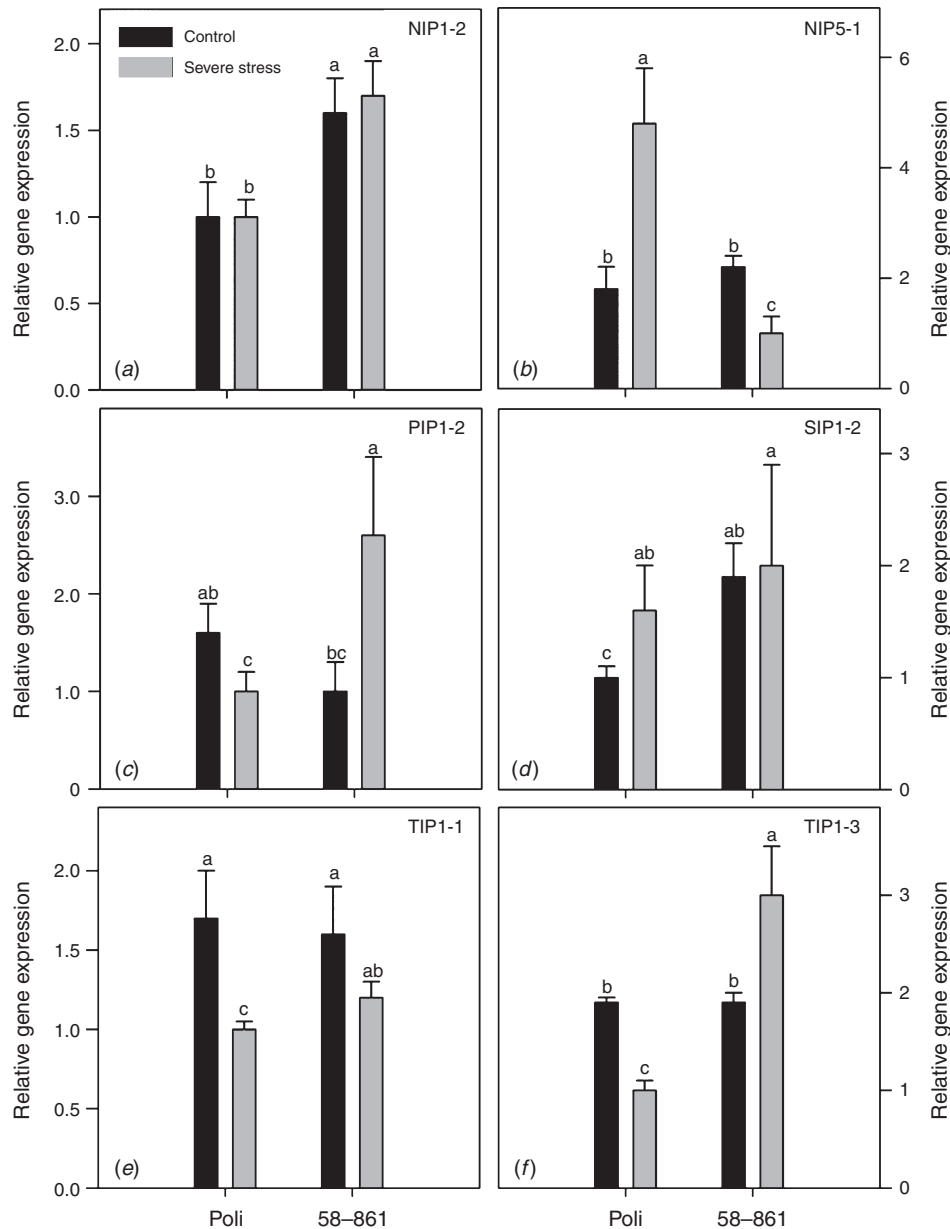


Fig. 5. Relative expression rates of aquaporin genes in roots of *Populus nigra* clones Poli and 58-861, obtained from quantitative real-time RT-PCR. Mean values \pm s.e. are given for three replicates. Values followed by the same letter for each gene are not significantly different ($P > 0.05$).

The genotypically developed ability to increase agronomic WUE (biomass production/water consumption) is considered a necessary characteristic to increase drought resistance. However, explaining the genotypic diversity of drought tolerance found among poplar hybrids as arising from their specific WUE may be misleading (Monclus *et al.* 2006).

At the end of experiment, the proline content did not appear to vary in the young leaves of either clone in response to drought. Proline generally accumulates in leaves of plants subjected to water stress (Ain-Lhout *et al.* 2001; Shvaleva *et al.* 2006; Ren *et al.* 2007). Inconsistent proline accumulation in leaves under

water stress (with rather high Ψ_{pd}) could be related to the lack of a marked response to short-term drought stress in both clones, and may be a feature only partially involved in early plant establishment. A low proline response to drought stress was found by Griffin *et al.* (1991) for *Populus tremuloides*. The influence of drought on proline contents in these clones, indeed, may reflect the duration of the water stress imposed during the experiment and the specific organ accumulation, taking leaf age into consideration. Differences found in young and old leaves could depend on age-specific responses (more-or-less fast) to stress conditions. In these poplar clones, proline was

found in lower concentrations than in other tree species subjected to water deficits, namely, *Pinus taeda* (Newton *et al.* 1986) and *Eucalyptus globulus* (Shvaleva *et al.* 2006).

One of the most fundamental roles of ABA in water-stressed plants is to directly induce stomatal closure and to prevent water loss, thereby slowing growth (Zhang and Davies 1990). The response to drought in these clones in terms of changes in ABA concentration was not, however, very strong even though it was statistically significant. Genotypic differences in the timing of ABA responses to water stress were nevertheless still found. Poli accumulated more ABA during the onset of water stress than in the second part of experiment, and the old leaves containing more ABA than young leaves. The absence of leaf abscission in this clone could be explained by its tolerance to ABA (Chen *et al.* 1997). In 58–861, the concentration of ABA in leaves was constantly high, regardless of leaf age. In this clone, the leaf shedding observed at the end of experiment was probably associated with the accumulation of ABA that restricted the transpiring surface in stressed plants. Temporal differences in ABA production could imply a genotypic sensitivity of roots to soil-water content (Chen *et al.* 1997). Roots of both clones might accumulate ABA as the soil dries (Zhang and Davies 1987). Yin *et al.* (2004) observed that foliar applications of ABA decreased dry-matter accumulation, specific leaf area and gas exchange, and increased dry-matter allocation to the root, endogenous ABA contents and $\delta^{13}\text{C}$ in poplar species. Ridolfi *et al.* (1996) also found that the drought control of stomatal conductance was ABA-independent in a hybrid clone of *Populus koreana* \times *trichocarpa*.

The two clones had different carbon isotope compositions. The southern clone (Poli), supposedly acclimatised to Mediterranean conditions, had lower $\delta^{13}\text{C}$ than the northern clone (58–861), from the temperate Piedmont area. Isotope values for 58–861 slightly increase with increasing water stress, which suggests that in this clone stomata closed earlier than in Poli in response to drought stress (Guehl *et al.* 1995) and also displayed slower growth rates. In Poli, conversely, $\delta^{13}\text{C}$ did not vary across treatments, suggesting that this clone could not perceive these conditions as involving marked water stress. Changes in $\delta^{13}\text{C}$ were observed in poplar species under drought-stress conditions by Yin *et al.* (2004). The comparison between the 'drought-tolerant' Poli and the 'drought-sensitive' 58–861 clones revealed that the latter clone used water with greater efficiency. An increase in $\delta^{13}\text{C}$ in 58–861, interpreted as an improvement in WUE in the Farquhar model, could be the result of either reduced stomatal conductance (at constant photosynthesis), or increased photosynthesis (at constant stomatal conductance). These poplar clones showed higher $\delta^{13}\text{C}$ values in younger leaves. Age-related effects on carbon isotope discrimination might be attributed to physiological changes related to structural development during leaf maturation (Marshall and Monsrud 1996).

Differences in the clone's ABA content and $\delta^{13}\text{C}$ composition might reasonably be ascribed to a contrasting stomatal behaviour. The ABA- $\delta^{13}\text{C}$ relationship highlighted clone-specific WUE. In fact, the lower (more negative) $\delta^{13}\text{C}$ in Poli might be explained by an increase in stomatal conductance and intercellular CO_2 concentration, with consequently lower WUE, which can also be inferred by the lower content of ABA in

leaves. The reduced water availability might have elicited the progressive enrichment in ^{13}C , related to a decrease in photosynthesis, and, indirectly, to an increase in WUE (Farquhar *et al.* 1989; Brugnoli and Farquhar 2000).

Transcripts of the aquaporins PIP1–2 and TIP1–3 were upregulated by water stress in 58–861 but downregulated in Poli. We assume that in poplar, these genes might be important for water transport, as we chose the closest homologues to genes which are known to be functional in *Arabidopsis*. However, because we analysed only about one tenth of the aquaporin genes annotated in the poplar genome, we cannot exclude the possibility that we missed some other genes that are also regulated during drought. In contrast, the expression of NIP5–1 was repressed in the roots of water-stressed 58–861 and induced in water-stressed Poli. In poplar, expression of aquaporins in the roots has been investigated only during root development (Kohler *et al.* 2003) and in the context of mycorrhiza formation (Marjanović *et al.* 2005). Until now, no research has been done on changes in aquaporin gene expression in the roots of poplar during drought, but for other plants there are conflicting descriptions of the role of aquaporins under drought stress. Several studies, of both annual and perennial plants, have shown that many of the aquaporin genes are downregulated by drought (Smart *et al.* 2001; Alexandersson *et al.* 2005; Secchi *et al.* 2007). This may reduce membrane permeability, allowing water conservation in the cells, especially when the soil-water potential is low. However, upregulation of some aquaporin genes during water stress has also been reported (Yamada *et al.* 1997; Montalvo-Hernández *et al.* 2008). Upregulation should enhance membrane permeability and facilitate water transport between cells. If expressed in the vascular systems of roots, aquaporins could support water transport to aerial tissues. It is possible that 58–861 and Poli might follow these contrasting strategies of optimising their water relations under water-limited conditions. 58–861 might increase the permeability of the vascular tissue by overexpressing aquaporins genes PIP1–2 and TIP1–3, probably in order to ease water transport, Poli might increase water conservation in root cells by downregulating these aquaporins. To test this hypothesis, these genes should be further characterised and the expression patterns analysed *in situ*.

This study was conducted to determine how two parental clones of *P. nigra*, from contrasting environments, differ in their drought-tolerance responses to different water-stress levels during early stages of plant establishment in an airconditioned greenhouse. Plants induced mechanisms of internal regulation in response to external conditions, and these were related to the morphological characteristics of the clone. When drought stress became severe, the 58–861 female clone shed leaves, probably due to its delay in recognising stress conditions and in order for it to adjust osmotically to cope with drought. Growth parameters showed a close link between stem height and leaf number, on the one hand, and plant development and water availability, on the other. Reduced growth could be a strategy to control energy dissipation so that the plant has enough energy to trigger drought-tolerance mechanisms. Since the degree of adjustment between well watered and severely stressed plants was ~ 0.4 MPa, we conclude that Poli responded promptly to stress conditions by activating drought-tolerance mechanisms, whereas 58–861 was unable to avoid damage in time, undergoing leaf shedding and

accumulating ABA in roots. The quick recognition of water stress in Poli could be related to the plant's strategy to reduce Ψ_{pd} early and to accumulate proline to preserve plants from drought damage. Proline protects cell membranes and proteins and enhances dehydration tolerance without the need to reduce stomatal activities, as shown by very negative values of $\delta^{13}C$ in Poli, even in severely stressed plants. Our results indicate different drought-tolerance mechanisms in these two clones, which may be used for breeding purposes.

Acknowledgements

Many thanks to Maurizio Sabatti (Università della Tuscia, Viterbo, Italy) for providing the *P. nigra* cuttings and to Silvia Dingwall for comments on the English language. The work was partially funded by the Swiss Secretariat for Education and Research, COST Action E38 (woody root processes, Grant No. C04.0256). The authors thank the two anonymous reviewers for their helpful comments on the previous version of the manuscript.

References

- Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G (2003) Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. *The Plant Cell* **15**, 439–447. doi:10.1105/tpc.009225
- Ain-Lhout F, Zunzunegui M, Diaz Barradas MC, Tirado R, Clavijo A, Garcia Novo F (2001) Comparison of proline accumulation in two mediterranean shrubs subjected to natural and experimental water deficit. *Plant and Soil* **230**, 175–183. doi:10.1023/A:1010387610098
- Alexandersson E, Fraysse L, Sjövall-Larsen S, Gustavsson S, Fellert M, Karlsson M, Johanson U, Kjellbom P (2005) Whole gene family expression and drought stress regulation of aquaporins. *Plant Molecular Biology* **59**, 469–484. doi:10.1007/s11103-005-0352-1
- Arend M, Fromm J (2007) Seasonal change in the drought response of wood cell development in poplar. *Tree Physiology* **27**, 985–992.
- Barta C, Loreto F (2006) The relationship between the methyl-erythritol phosphate pathway leading to emission of volatile isoprenoids and abscisic acid content in leaves. *Plant Physiology* **141**, 1676–1683. doi:10.1104/pp.106.083063
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline water-stress studies. *Plant and Soil* **39**, 205–207. doi:10.1007/BF00018060
- Betts RA, Boucher O, Collins M, Cox PM, Falloon PD, *et al.* (2007) Projected increase in continental runoff due to plant responses to increasing carbon dioxide. *Nature* **448**, 1037–1041. doi:10.1038/nature06045
- Bogeat-Triboulot MB, Brosche M, Renaut J, Jouve L, Le Thiec D, *et al.* (2007) Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiology* **143**, 876–892. doi:10.1104/pp.106.088708
- Brugnoli E, Farquhar GD (2000) Photosynthetic fractionation of carbon isotopes. In 'Advances in photosynthesis – photosynthesis: physiology and metabolism. Vol. 9'. (Eds RC Leegood, TD Sharkey, S von Caemmerer) pp. 399–434. (Kluwer: Dordrecht, The Netherlands)
- Ceulemans R, Impens I, Lemeur R, Moermans R, Samsuddin Z (1978) Water movement in the soil-poplar-atmosphere system. I. Comparative study of stomatal morphology and anatomy, and the influence of stomatal density and dimensions on the leaf diffusion characteristics in different poplar clones. *Oecologia Plantarum* **13**, 1–12.
- Chen S, Wang S, Altman A, Hüttermann A (1997) Genotypic variation in drought tolerance of poplar in relation to abscisic acid. *Tree Physiology* **17**, 797–803.
- Cochard H, Ridolfi M, Dreyer E (1996) Responses to water stress in an ABA-unresponsive hybrid poplar *Populus koreana* × *trichocarpa* cv. Peace. II. Hydraulic properties and xylem embolism. *New Phytologist* **134**, 455–461. doi:10.1111/j.1469-8137.1996.tb04362.x
- Cochard H, Casella E, Mencuccini M (2007) Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiology* **27**, 1761–1767.
- Davies WJ, Tardieu F, Trejo CL (1994) How do chemical signals work in plants that grow in drying soil? *Plant Physiology* **104**, 309–314.
- Dickmann DI (1971) Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* Bartr.). *Botanical Gazette* **132**, 253–259. doi:10.1086/336588
- Dix PJ, Pearce RS (1981) Proline accumulation in NaCl-resistant and sensitive cell lines of *Nicotiana sylvestris*. *Zeitschrift für Pflanzenphysiologie* **102**, 243–248.
- Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**, 121–137. doi:10.1071/PP9820121
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503–537. doi:10.1146/annurev.pp.40.060189.002443
- Gaudet M, Jorge V, Paolucci I, Beritognolo I, Scarascia Mugnozza G, Sabatti M (2008) Genetic linkage maps of *Populus nigra* L. including AFLPs, SSRs, SNPs, and sex trait. *Tree Genetics & Genomes* **4**, 25–36. doi:10.1007/s11295-007-0085-1
- Gebre GM, Kuhns MR (1991) Seasonal and clonal variations in drought tolerance of *Populus deltoides*. *Canadian Journal of Forest Research* **21**, 910–916. doi:10.1139/x91-126
- Gebre GM, Tschaplinski TJ, Tuskan GA, Todd DE (1998) Clonal and seasonal differences in leaf osmotic potential and organic solutes of five hybrid clones grown under field conditions. *Tree Physiology* **18**, 645–652.
- Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaro A (2007) Evaluation of drought response of two poplar clones *Populus* × *canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina' through high resolution analysis of stem growth. *Journal of Experimental Botany* **58**, 2673–2683. doi:10.1093/jxb/erm117
- Griffin DH, Schaedle M, Manion PD, Devit M (1991) Clonal variation in amino acid contents of roots, stems, and leaves of aspen *Populus tremuloides* Michx. as influenced by diurnal drought stress. *Tree Physiology* **8**, 337–350.
- Guehl JM, Nguyen-Queyrens A, Loustau D, Ferhi A (1995) Genetic and environmental determinants of water-use efficiency and carbon isotope discrimination in forest trees. In 'EUROSILVA – Contribution to forest tree physiology. Editions Colloques de l'INRA'. (Eds M Bonnet-Masimbert, H Sandermanns) pp. 297–321. (INRA: Paris)
- Hare PD, Cress WA (1997) Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Regulation* **21**, 79–102. doi:10.1023/A:1005703923347
- Harvey HP, van den Driessche R (1997) Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiology* **17**, 647–654.
- Hellemsans J, Mortier G, De Paeppe A, Speleman F, Vandensompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR. *Genome Biology* **8**, R19. doi:10.1186/gb-2007-8-2-r19
- Hong Z, Lakkinen K, Zhang Z, Verma DPS (2000) Removal of feedback inhibition of Δ^1 -pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiology* **122**, 1129–1136. doi:10.1104/pp.122.4.1129

- Johnson JD, Tognetti R, Paris P (2002) Water relations and gas exchange in poplar and willow under water stress and elevated atmospheric CO₂. *Physiologia Plantarum* **115**, 93–100. doi:10.1034/j.1399-3054.2002.1150111.x
- Kaldenhoff R, Bertl A, Otto B, Moshelion M, Uehlein N (2007) Characterization of plant aquaporins. *Methods in Enzymology* **428**, 505–531. doi:10.1016/S0076-6879(07)28028-0
- Katsuhara M, Hanba YT, Shiratake K, Maeshima M (2008) Expanding roles of plant aquaporins in plasma membranes and cell organelles. *Functional Plant Biology* **35**, 1–14. doi:10.1071/FP07130
- Kohler A, Delaruelle C, Martin D, Encelot N, Martin F (2003) The poplar root transcriptome: analysis of 7000 expressed sequence tags. *FEBS Letters* **542**, 37–41. doi:10.1016/S0014-5793(03)00334-X
- Liu Z, Dickmann DI (1992) Abscisic acid accumulation in leaves of two contrasting hybrid poplar clones affected by nitrogen fertilization plus cyclic flooding and soil drying. *Tree Physiology* **11**, 109–122.
- Marjanović Z, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiß M, Hampp R, Nehls U (2005) Aquaporins in poplar: what a difference a symbiont makes! *Planta* **222**, 258–268. doi:10.1007/s00425-005-1539-z
- Marron N, Delay D, Petit LM, Dreyer E, Kahlem G, Delmotte FM, Brignolas F (2002) Physiological traits of two *Populus × euramericana* clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. *Tree Physiology* **22**, 849–858.
- Marshall JD, Monsereud RA (1996) Homeostatic gas-exchange parameters inferred from ¹³C/¹²C in tree rings of conifers. *Oecologia* **105**, 13–21. doi:10.1007/BF00328786
- Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Le Thiec D, Brèchet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides × Populus nigra*. *New Phytologist* **169**, 765–777. doi:10.1111/j.1469-8137.2005.01630.x
- Montalvo-Hernández L, Piedra-Ibarra E, Gómez-Silva L, Lira-Carmona R, Acosta-Gallegos JA, Vazquez-Medrano J, Xoconostle-Cázares B, Ruiz-Medrano R (2008) Differential accumulation of mRNAs in drought-tolerant and susceptible common bean cultivars in response to water deficit. *New Phytologist* **177**, 102–113.
- Newton RJ, Sen S, Puryear JD (1986) Free proline changes in *Pinus taeda* L. callus in response to drought stress. *Tree Physiology* **1**, 325–332.
- Pih KT, Kabilan V, Lim JH, Kang SG, Piao HL, Jin JB, Hwang I (1999) Characterization of two new channel protein genes in *Arabidopsis*. *Molecules and Cells* **9**, 84–90.
- Ramakers C, Ruijter JM, Lekanne Deprez RH, Moorman AFM (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* **339**, 62–66. doi:10.1016/S0304-3940(02)01423-4
- Ren J, Dai W, Xuan Z, Yao Y, Korpelainen H, Li C (2007) The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. *Forest Ecology and Management* **239**, 112–119. doi:10.1016/j.foreco.2006.11.014
- Ridolfi M, Fauveau ML, Label P, Garrec JP, Dreyer E (1996) Responses to water stress in an ABA-unresponsive hybrid poplar *Populus koreana × trichocarpa* cv. Peace. I. Stomatal function. *New Phytologist* **134**, 445–454. doi:10.1111/j.1469-8137.1996.tb04361.x
- Rozen S, Skaletzki H (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology (Clifton, N.J.)* **132**, 365–386.
- Secchi F, Lovisolo C, Schubert A (2007) Expression of OePIP2.1 aquaporin gene and water relations of *Olea europaea* twigs during drought stress and recovery. *The Annals of Applied Biology* **150**, 163–167. doi:10.1111/j.1744-7348.2007.00118.x
- Shvaleva AL, Silva FCE, Breia E, Jouve L, Hausman JF, Almeida MH, Maroco JP, Rodrigues ML, Pereira JS, Chaves MM (2006) Metabolic responses to water deficit in two *Eucalyptus globulus* clones with contrasting drought sensitivity. *Tree Physiology* **26**, 239–248. doi:10.1093/treephys/26.2.239
- Sjödin A, Street NR, Sandberg G, Gustafsson P, Jansson S (2009) The *Populus* genome integrative explorer (PopGenIE): a new source for exploring the *Populus* genome. *New Phytologist* **182**, 1013–1025. doi:10.1111/j.1469-8137.2009.02807.x
- Smart LB, Moskal WA, Cameron KD, Bennett AB (2001) MIP genes are down-regulated under drought stress in *Nicotiana glauca*. *Plant & Cell Physiology* **42**, 686–693. doi:10.1093/pcp/pce085
- Souch CA, Stephens W (1998) Growth, productivity and water use in three hybrid poplar clones. *Tree Physiology* **18**, 829–835.
- Stewart CR, Larher F (1980) Accumulation of amino acids and related compounds in relation to environmental stress. In 'The biochemistry of plants'. (Ed. BJ Mifflin) pp. 609–635. (Academic Press: New York)
- Tschaplinski TJ, Tuskan GA, Gebre DE, Todd DE (1998) Drought resistance of two hybrid *Populus* clones grown in a large-scale plantation. *Tree Physiology* **18**, 653–658.
- Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, *et al.* (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**, 1596–1604. doi:10.1126/science.1128691
- Volts J, Serrano L, Hernández M, Pemán J (2006) Carbon isotope discrimination, gas exchange and stem growth of four euramerican hybrid poplars under different watering regimes. *New Forests* **31**, 435–451. doi:10.1007/s11056-005-0879-7
- Watanabe S, Kojima K, Ide Y, Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* *in vitro*. *Plant Cell, Tissue and Organ Culture* **63**, 199–206. doi:10.1023/A:1010619503680
- Whalley WR, Clark LJ, Take WA, Bird NRA, Leech PK, Cope RE, Watts CW (2007) A porous-matrix sensor to measure the matric potential of soil water in the field. *European Journal of Soil Science* **58**, 18–25. doi:10.1111/j.1365-2389.2006.00790.x
- Xu ZZ, Zhou GS (2006) Nitrogen metabolism and photosynthesis in *Leymus chinensis* in response to long-term soil drought. *Journal of Plant Growth Regulation* **25**, 252–266. doi:10.1007/s00344-006-0043-4
- Yamada S, Komori T, Myers PN, Kuwata S, Kubo T, Imaseki H (1997) Expression of plasma membrane water channel genes under water stress in *Nicotiana excelsior*. *Plant & Cell Physiology* **38**, 1226–1231.
- Yin C, Duan B, Wang X, Li C (2004) Morphological and physiological responses of two contrasting poplar species to drought stress and exogenous abscisic acid application. *Plant Science* **167**, 1091–1097. doi:10.1016/j.plantsci.2004.06.005
- Zhang J, Davies WJ (1987) Increased synthesis of ABA in partially dehydrated root tips and ABA transport from roots to leaves. *Journal of Experimental Botany* **38**, 2015–2023. doi:10.1093/jxb/38.12.2015
- Zhang J, Davies WJ (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. *Plant, Cell & Environment* **13**, 277–285. doi:10.1111/j.1365-3040.1990.tb01312.x

Manuscript received 18 June 2009, accepted 22 December 2009