Genotypic variation in drought tolerance of poplar in relation to abscisic acid

SHAOLIANG CHEN, SHASHENG WANG, ARIE ALTMAN and ALOYS HÜTTERMANN

Summary We investigated effects of water stress and external abscisic acid (ABA) supply on shoot growth, stomatal conductance and water status in 1-year-old cuttings of a drought-sensitive poplar genotype *Populus × euramericana* cv. I-214 (Italica) and a drought-tolerant genotype *P. × popularis* 35-44' (popularis). *Populus popularis* was more productive and maintained higher leaf water potentials throughout the drought treatment than cv. Italica. Supply of ABA to the xylem sap caused a greater decline in growth and more leaf abscission in shoots of cv. Italica than in shoots of *P. popularis*. Immediately after initiation of the drought treatment in *P. popularis*, the ABA concentration ([ABA]) of the xylem increased rapidly and stomatal conductance declined; however, stomatal conductance had returned to control values by the third day of the drought treatment, but the increase continued for 3 days at which time a tenfold increase in xylem [ABA] was observed. In contrast, xylem [ABA] of cv. Italica initially increased more slowly than that of *P. popularis* in response to the drought treatment, but the increase continued for 3 days at which time a tenfold increase in xylem [ABA] was observed that was followed by abscission of more than 40% of the leaves. We conclude that sensitivity of poplar roots to variation in soil water content varies by clone and that a rapid short-term accumulation of ABA in shoots in response to water stress may contribute to drought tolerance.

Keywords: ABA, plant water status, *Populus popularis*, shoot growth, stomatal conductance, water stress.

Introduction

There is much variation in drought tolerance among genotypes of the genus *Populus* (Ceulemans et al. 1978, Tschapliniski and Blake 1989, Rhodenbaugh and Pallardy 1993, Chen et al. 1996). The physiological basis of the genetic variation in drought tolerance of *Populus* has not been established. In general, a reduction in leaf water content or potential results in a decline in growth and stomatal conductance. However, recent studies have shown that shoot growth and stomatal conductance can also be suppressed in the absence of a coincident change in plant water status (Blackman and Davies 1985, Gowing et al. 1990). Based on observations that the abscisic acid concentration ([ABA]) of xylem is linked to soil water reserves (Tardieu et al. 1992), and that stomatal conductance and leaf growth are negatively correlated with xylem [ABA] (Zhang and Davies 1990), it has been suggested that roots sense soil water deficiency and communicate this information to shoots by a chemical signal, possibly ABA (Couts 1981, Blackman and Davies 1984, Gollan et al. 1986, 1992, Smit et al. 1990, Davies and Zhang 1991, Scurr et al. 1992).

Although the role of ABA in stress physiology has received much attention, attempts to correlate ABA production and drought tolerance in plants have yielded conflicting results. Drought-tolerant plants of several maize cultivars accumulate more ABA or increase production of ABA more rapidly than drought-sensitive cultivars (Larque-Saavedra and Wain 1974). Spring wheat cultivars with high accumulations of ABA produce higher yields than cultivars with low accumulations of ABA (Innes et al. 1984). Conversely, sorghum, which is more drought-resistant than maize, accumulates less ABA than maize (Beardsell and Cohen 1975) and maximal ABA contents are negatively correlated with drought resistance (Ilahi and Dorffling 1982). These conflicting studies on crop species indicate different mechanisms of drought tolerance in different species.

Stomatal response to drought differs among poplar genotypes (Schulte and Hinckley 1987a, 1987b) and clonal differences in leaf ABA accumulation have been observed under soil drying conditions (Liu and Dickmann 1992). However, the relationship between drought-induced ABA accumulation and drought tolerance in poplar has not been elucidated.

For more than 30 years, *P. popularis* has been used in preference to other fast growing but more drought-sensitive genotypes for afforestation in northern China. In an attempt to determine the physiological mechanism underlying the drought tolerance of *P. popularis*, we compared the endogenous [ABA] of the xylem of *P. popularis* with that of the drought-sensitive genotype *P. × euramericana* cv. Italica. We also examined how these two genotypes respond to drought and exogenous ABA.
Materials and methods

Plant materials
In April 1995, 20-cm cuttings of two poplar genotypes, *Populus × euramericana* cv. I-214 (cv. Italica) and *P. × popularis* 35-44 (P popularis) were planted in 25-liter pots containing loam soil. The cuttings were placed in a greenhouse, kept well watered and fertilized with 1 liter of Hoagland’s nutrient solution every 2 weeks. The experiments started in July when the cuttings were 1.4–1.8 m high and had 50–70 leaves.

Water stress treatment
Water stress was induced by withholding water until soil water content fell to between 30 and 40% of field capacity (FC) (soil water potential at 30% FC was −2.108 MPa, Figure 1) for 3 weeks. Cuttings in the control treatment were kept well watered by maintaining soil in the pots at 70% FC. To keep soil water content close to the target value, pots were weighed twice a day and watered as required. Height growth of four replicate trees per treatment was measured weekly for 3 weeks. Predawn water potential of upper mature leaves was obtained at 0300–0400 h with a pressure chamber. Gas exchange and stomatal conductance were measured at 0830–0930 h with an Li-6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE) under natural conditions. Photosynthetically active radiation (PAR) was approximately 1200 µmol m$^{-2}$ s$^{-1}$ and air temperature ($T_{air}$) was 32–34 °C. Following gas exchange measurements, the concentration of ABA ([ABA]) in the xylem was determined.

Determination of xylem [ABA]
Fifteen- to 20-cm apical growing tips (with 7 or 8 leaves each) were excised from water-stressed (30% FC) and well-watered (70% FC) plants, and the xylem sap was immediately expelled with a pressure chamber using a pressure of approximately 1.0 MPa over the balancing pressure. One to 2 ml of extruded xylem sap was collected from each tip and used for analysis of ABA by HPLC (Chen and Wang 1992).

ABA Treatment
On clear days, one 30-cm plastic tube (5 mm diameter) was attached with glue to the stem of each plant. After the glue had dried, holes (1 mm diameter) were drilled through the water-filled tubes to the middle of the xylem, and the holes in the tubes were immediately plugged to prevent leaking. Plants were monitored for water uptake over a 30-min period and plants with similar uptake rates were selected for the experiment. Ten ml of 200 µM ABA was fed into the xylem of each plant by way of the tube. When the feeding was completed, distilled water was added continuously to avoid air entering the xylem. Control plants were fed with distilled water only. The pHs of the ABA solution and distilled water were adjusted to that of the xylem prior to feeding (pH 6.8). Four well-watered plants and four mildly water-stressed (40% FC) plants of each genotype were selected for the ABA treatment.

Plant measurements
For 10 days following ABA application, exchange gas of upper mature leaves was measured at 1030–1130 h with a Li-Cor Li-6200 portable photosynthesis system under natural conditions where PAR was approximately 1200–1600 µmol m$^{-2}$ s$^{-1}$ and $T_{air}$ was 34–36 °C. Following the gas exchange measurements, midday water potential was measured with a pressure chamber on the leaves directly above and below the leaf sampled for gas exchange measurement. Height growth was determined every day and relative height growth rate was computed using the formula:

$$\text{Relative growth rate} = \left(\ln H_2 - \ln H_1 \right) / (t_2 - t_1),$$

where $H_2$, $H_1$ are the height growth variables at the beginning and the end of the time interval, and $t_2 - t_1$ is the time interval of interest.

Data analysis
The data were subjected to ANOVA and significant differences between means were determined by Duncan’s multiple-range test. Unless otherwise stated, differences were considered statistically significant when $P < 0.05$.

Results

Growth
Under conditions of optimum soil water content (70% FC), cuttings of cv. Italica maintained significantly higher relative height growth rates than cuttings of *P. popularis*, whereas *P. popularis* cuttings maintained significantly higher relative height growth rates than cv. Italica cuttings during the 3-week severe drought (30% FC) treatment (Figure 2).

After ABA was fed into the xylem of well-watered plants of both genotypes (70% FC), the relative height growth rate of cv. Italica plants was greatly reduced, whereas there was no observed decline in relative height growth rate of *P. popularis* plants over the sampling period, even though the relative height growth rate of control *P. popularis* plants was typically

Figure 1. Relationship between soil water potential and soil water content (% of field capacity, FC).
less than that of the control cv. Italica plants (Figure 3). Four days after ABA treatment, the relative height growth rate of cv. Italica plants began to recover, and by the sixth day after ABA treatment there was no significant difference in relative height growth rates between ABA-treated and control cv. Italica plants.

Leaf abscission

Compared with well-watered control plants (70% FC), ABA treatment of well-watered plants (70% FC + ABA) caused leaf abscission in both genotypes (Table 1). The supply of 10 ml of 200 µM ABA to mildly water-stressed plants (40% FC) increased the amount of leaves shed by 20–40% and cv. Italica plants exhibited greater leaf loss than P. popularis plants.

Table 1. Effects of soil water content and the introduction of 10 ml of 200 µM ABA into the xylem sap on leaf abscission of P. popularis and cv. Italica poplar genotypes. Values are means (± SE) of four measurements.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of leaves abscised per plant</th>
<th>% of leaves abscised</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% FC</td>
<td>cv. Italica 0</td>
<td>0</td>
</tr>
<tr>
<td>P. popularis 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% FC + ABA</td>
<td>cv. Italica 12 ± 2</td>
<td>17.5</td>
</tr>
<tr>
<td>P. popularis 6 ± 2</td>
<td></td>
<td>10.2</td>
</tr>
<tr>
<td>40% FC</td>
<td>cv. Italica 0</td>
<td>0</td>
</tr>
<tr>
<td>P. popularis 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30% FC</td>
<td>cv. Italica 26 ± 2</td>
<td>43.3</td>
</tr>
<tr>
<td>P. popularis 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40% FC + ABA</td>
<td>cv. Italica 24 ± 3</td>
<td>36.9</td>
</tr>
<tr>
<td>P. popularis 13 ± 2</td>
<td></td>
<td>24.7</td>
</tr>
</tbody>
</table>

Figure 2. Effects of soil water content on relative height growth rates of P. popularis and cv. Italica poplar genotypes. Each point is the mean of four measurements. Bars represent the standard error of the mean.

Figure 3. Effects of the introduction of 10 ml of 200 µM ABA to the xylem sap on relative height growth rates of well-watered P. popularis and cv. Italica poplar genotypes. Each point is the mean of four measurements. Bars represent the standard error of the mean.

Figure 4. Effects of soil water content on predawn leaf water potentials of P. popularis and cv. Italica poplar genotypes. Each point is the mean of four measurements. Bars represent the standard error of the mean.
The severe drought treatment (30% FC) caused 43.3% of leaves to abscise in cv. Italica plants, which was similar to the magnitude of leaf loss induced in the mildly water-stressed plants treated with ABA (40% FC + ABA). In contrast, no leaf abscission occurred in severely water-stressed (30% FC) plants of *P. popularis* over the 3-week study.

**Plant water status**

Predawn leaf water potential (Ψ<sub>p</sub>) of cv. Italica plants decreased to ~0.6 MPa on the first day of exposure to severe water stress (30% FC). The Ψ<sub>p</sub> gradually recovered to control values following abscission of old leaves on the fourth day of the drought treatment. The magnitude of drought-induced decline in Ψ<sub>p</sub> was much less in *P. popularis* plants than in cv. Italica plants (Figure 4). In general, water-stressed plants of *P. popularis* had a Ψ<sub>p</sub> that was about 0.1 MPa lower than that of well-watered control plants throughout the drought period.

**Gas exchange and plant water status after ABA treatment**

Introduction of ABA to the xylem sap greatly reduced stomatal conductance (C<sub>s</sub>), net photosynthetic rates (P<sub>n</sub>) and transpiration rates (E) of well-watered plants of both genotypes for the first 3 days after treatment (Figure 5). The ABA-induced decrease in E resulted in a 0.3–0.6 MPa increase in midday water potential (Ψ<sub>m</sub>) compared with that of the control plants (Figure 5). However, the effects of ABA on gas exchange and plant water status disappeared within 5 days of application, after

![Figure 5](image-url)
which time there were no significant differences in $C_s$, $P_n$, $E$ or $\Psi_m$ between control and ABA-treated plants.

**Xylem ABA concentrations and gas exchange**

Severe water stress (30% FC) caused significant increases in xylem [ABA] in both genotypes (Figure 6), but there were genotypic differences in the timing of the ABA response to water stress. On the first day of exposure to severe drought, *P. popularis* exhibited a 3.5-fold increase in xylem [ABA], whereas cv. Italica exhibited only a minor increase (0.7-fold) in xylem [ABA]. Thereafter, the xylem [ABA] in *P. popularis* decreased gradually, whereas the xylem [ABA] in cv. Italica continued to increase to reach a maximum on the third day of the drought treatment that was 10 times higher than that of the well-watered control plants.

In both genotypes, $C_s$, $P_n$ and $E$ were significantly decreased in response to the 30% FC treatment. However, coincident with the gradual decline in xylem [ABA] in *P. popularis* after the onset of water stress, $C_s$ and $P_n$ recovered to a large degree.

![Figure 6. Effects of soil water content on xylem [ABA], stomatal conductance ($C_s$), transpiration rate ($E$), and net photosynthetic rate ($P_n$) of *P. popularis* and cv. Italica poplar genotypes. Each point is the mean of four measurements. Bars represent the standard error of the mean.](http://www.heronpublishing.com)
There was no evidence of recovery of either $C_s$ or $P_n$ in the cv. Italica plants.

**Discussion**

In the 30% FC water-stress treatment, *P. popularis* plants maintained a higher relative height growth rate than cv. Italica plants, mainly because plants of the *P. popularis* genotype had a high photosynthetic rate (Figure 6), no leaf loss (Table 1) and a high leaf water potential (Figure 4). The maintenance of a high water balance in *P. popularis* plants appeared to be a consequence of the initial rapid increase in xylem [ABA] contributing to water conservation (Figure 5). Compared with cv. Italica plants, xylem [ABA] increased more rapidly in *P. popularis* plants in response to water stress (Figure 6). Similarly, O'Regan et al. (1993) found that a slow growing maize cultivar, which was drought resistant, accumulated more ABA during the onset of water stress than a faster growing cultivar. In contrast, cv. Italica plants accumulated ABA more slowly and over a longer period in response to the drought treatment, and plant water balance was reestablished only after a 43.3% leaf loss. The drought-induced loss of leaves was probably associated with the endogenous accumulation of ABA.

The difference in the timing and pattern of ABA production in response to the onset of water stress implies that the sensitivity of the root system to soil drying varies with genotype. The drought-induced increase in xylem ABA is thought to originate mainly from roots (Zhang and Davies 1987, Robertson 1990). This suggests that root tips of *P. popularis* provide an early indicator of soil drying to the shoot by synthesizing ABA, which triggers adjustments in the shoots that confer drought resistance. Compared with *P. popularis*, cv. Italica root tips appear to be less sensitive to soil drying and so the water balance of the plant is more severely affected by drought.

The direct introduction of ABA into the xylem sap may imitate the drought-induced increase in xylem [ABA]. Exogenously applied ABA exerted a significant influence on stomatal opening of both genotypes; however, shoots of *P. popularis* were more tolerant of exogenous ABA than shoots of cv. Italica, as shown by their lower leaf loss (Table 1) and a smaller ABA-induced decrease in height growth (Figure 3). Tolerance to ABA may explain the absence of leaf abscission in *P. popularis* during the period corresponding to the increase in xylem [ABA] at the onset of water stress. We conclude that the sensitivity of root tips to soil drying and the sensitivity of shoots to water stress signals, especially xylem [ABA], differ between drought-tolerant and drought-sensitive genotypes.

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**References**


