Some growth and chlorophyll fluorescence parameters of black and hybrid poplar clones under water stress

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Abstract: The effects of water stress on some growth and chlorophyll a fluorescence parameters were investigated in two native black poplar (Populus nigra L.) clones (Kocabey and Gazi) and in two hybrid poplar (Populus × euramericana (Dode) Guinier) clones (I-214 and I-45/51) to determine which among the four clones is the best adapted to water-stress conditions. The potted seedlings were grown under three watering regimes. As a result of the applied watering regimes, the predawn water potential ($\psi_{pd}$) of seedlings was maintained at about –0.3, –0.9, and –1.6 MPa in control, moderate, and severe water-stress treatments during the experiment, respectively. Drought treatment had significant effects on the morphology and growth characteristics of the clones but had generally insignificant effects on survival and the photochemical activity of photosystem II (PSII). Results obtained from the present study demonstrate that I-214 was the most successful in terms of its higher root/shoot ratio (R), maximum fluorescence yield ($F_m$), maximum quantum efficiency of PSII ($F_v/F_m$) values, and lower number of leaves among the examined four poplar clones under drought stress conditions. Consequently, the I-214 poplar clone may have the potential to be used in drought areas.

Key words: Chlorophyll a fluorescence, growth parameters, poplar, water stress

1. Introduction

Species and hybrids of Populus genus are important components of ecosystems due to their fast growth and they represent optimal species for production of biomass suitable as sources of fuel, fiber, lumber, and plywood. It is a diverse and widely distributed genus and it has been extensively cultivated and propagated (Yin et al., 2005a).

The annual volume of wood harvested from Turkish forests is about $19 \times 10^6$ m$^3$. Since annual wood consumption in Turkey is about $29 \times 10^6$ m$^3$ (OGM, 2015), about $10 \times 10^6$ m$^3$ wood of Turkey’s demand must be met by a combination of poplar wood production and wood imports. Poplar trees are becoming extremely important as a fast-growing species to address the increasing need for wood in Turkey (İşık and Toplu, 2004). Industries consuming poplar wood (furniture, packing, particle board, plywood, matches, etc.) have developed very quickly in recent years. These industries mostly use the wood of euramericana hybrid poplars. Nearly all wood production from hybrid poplars ($2.1 \times 10^6$ m$^3$) is consumed by the industries mentioned above. More than 80% of black poplar wood ($1.7 \times 10^6$ m$^3$) is utilized as round wood for rural construction purposes and for the daily needs of the rural people (Tunçtaner, 1998).

The high wood production of poplars is closely linked to water availability, which is normally assured by irrigation. However, increasing irrigation costs and water shortages have driven recent efforts to find clones that can grow rapidly even under drought conditions. In addition to that, it has been predicted that an increase of regional weather extremes, such as water restriction/drought, will occur in Europe and nearby regions according to global climate change scenarios (IPCC, 2014). This situation also indicates an urgent need to assess drought resistant clones.

Annual average rainfall in Turkey is around 630 mm, with 67% of it occurring during the winter and spring (Türkeş, 1996). However, soil moisture in Turkey is seldom adequate for optimum poplar growth, and watering is particularly necessary and expensive in some extensive semiarid regions. Since poplar requires abundant moisture during the growing season to maximize growth, species or hybrids need to be identified that grow well during drought periods in order to maximize the yield of Populus plantations. To sustain the extension of poplar cultivation
from flood plains and bottomlands toward upland areas where seasonal drought are severe, more drought-resistant clones are required.

Research on the growth performance of clones at different trial sites in Turkey identified a number of successful clones that were used in the establishment of new plantations, namely *Populus euramericana* clones I-214 and I-45/51, *Populus deltoides* clone I-77/51, and *Populus nigra* clones Gazi, Anadolu, and Kocabey (Poplar and Fast Growing Forest Tree Research Institute, 1994). Gazi, Anadolu, Kocabey, Geyve, and Beğiç are also registered clones based upon field experiments in Turkey by the International Poplar Commission. Kocabey has shown the best growth performance in the semiarid Central Anatolia region of Turkey (Tunçtaner, 1998). Responses to field conditions of these clones are well studied in Turkey, but to date there has been a lack of research to compare the effects of drought on these poplar clones.

Photosystem II (PSII) photochemical activity, which has a significant role in the response of photosynthesis to environmental stresses such as drought, cold, and salinity, has been extensively studied using chlorophyll a fluorescence (Baker, 1991; Colom and Vazzana, 2003; Lu et al., 2003; Oukarroum et al., 2009; Kalaji et al., 2016). Chlorophyll a fluorescence is frequently used as a screening method in order to determine the tolerance of plants against environmental stresses (Martinez-Ferri et al., 2000; Strasser et al., 2000, 2010; Boureima et al., 2012; Öz et al., 2014; Çiçek et al., 2015).

The aims of this study were to determine the effects of drought on poplar growth and morphology and photochemical activity evaluated by chlorophyll a fluorescence measurement of the poplar clones in order to identify clones with an enhanced capability to survive and grow during drought periods. Such knowledge is essential for poplar species or genotype selections and tree improvement under water-limited environments.

### 2. Materials and methods

#### 2.1. Plant material

The unrooted cuttings of two hybrid *Populus × euramericana* (I-214 and I-45/51) and two native *Populus nigra* (Kocabey and Gazi) clones were planted in March in 25-L pots filled with homogenized soil (clay-loam, pH 7). A total of 15 g of chemical fertilizer (15% N, 15% P, and 15% K) was added to each pot containing one cutting. From each clone, 120 seedlings were grown at the nursery during one growing season under natural conditions and regularly watered every second day to maintain soil moisture sufficient for the growth of the seedlings. At the beginning of the next growing season, 36 healthy 1-year-old seedlings of approximately equal height were chosen from each clone and transferred to the experimental gardens for further experimental investigations, which are described below.

#### 2.2. Water-stress treatment and experimental design

Water-stress regimes were imposed by controlled application of watering and by protection of the potted seedlings from natural rainfall, largely by placing clear plastic covers on PVC hoops over the seedlings during each rainfall event. The plastic covers were removed when rain ceased. During the experiment, plants were maintained in the experiment garden in open air.

After sprouting and growing for about one growing season, 1-year-old potted seedlings were installed in a completely randomized design with 12 replications in the experimental garden of the Central Anatolia Forestry Research Institute in Ankara. Seedlings from these four clones were subjected to three water-stress regimes (well-watered or control, moderate, and severe water stress) from 1 May to 30 August. The intensity of the water stress was evaluated by leaf water potentials that were measured at predawn. We aimed to apply 0.5, 1.0, and 1.5 MPa water stress to our seedlings. Timing and frequency of irrigation were based on the most recent mean of predawn leaf water potential (ψpd) of seedlings. It was measured by using a pressure chamber (Model 1000, PMS Instrument Company, Corvallis, Oregon, USA). Controls were irrigated daily or every 2 days to maintain ψpd at approximately –0.5 MPa throughout the experiment. In the stress treatments, the seedlings were irrigated on average every 15 and 25 days to allow ψpd to reach –1.0 and –1.5 MPa for the moderate and severe drought treatments. ψpd were assessed biweekly for each irrigation regime on a fully expanded leaf at around 0400 hours. As a result of these applied irrigation regimes, ψpd changed by about −0.3, −0.9, and −1.6 MPa in the control, moderate, and severe drought treatments, respectively (Figure 1).

#### 2.3. Growth measurements

After height growth measurements, all seedlings were harvested at the end of the experiment and divided into leaves, stem, and roots. Leaf area for each seedling was determined using a portable leaf area meter (CI-202, CID Inc., Camas, WA, USA). Biomass samples were dried (65 °C, 48 h) to a constant value and then weighed. The shoot height (Ht, cm plant⁻¹), total biomass (Tb, g plant⁻¹), number of leaves (NL, number plant⁻¹), total leaf area (La, cm² plant⁻¹), and root/shoot ratio (Rs) of each seedling were measured. Specific leaf area (Sla, cm² g⁻¹) was determined using the formula given by Larcher (1995) by dividing leaf surface area to leaf dry mass.

#### 2.4. Chlorophyll a fluorescence measurement

Chlorophyll a fluorescence transients were measured with a HandyPEA (Plant Efficiency Analyser, Hansatech Instruments, Norfolk, UK) according to Strasser et al.
At the end of July. Leaves were dark-adapted for at least 30 min before they were measured in the morning. Illumination consisted of a 1-s pulse of continuous red light (650 nm peak wavelength, 3000 µmol photons m⁻² s⁻¹ maximum light intensity), provided by an array of three light-emitting diodes focused on a leaf area with a diameter of 4 mm. Variable fluorescence was calculated as the difference between maximum fluorescence yield \( F_m \) and initial fluorescence yield \( F_o \). Maximum quantum efficiency of PSII \( (F_v/F_m) \) was used as an approximation of the maximum quantum efficiency of PSII.

### 2.5. Statistical analysis

Two-way ANOVA was performed in a completely randomized design with 12 replications. Twelve cuttings from each clone were exposed to each watering treatment, with a total of 144 one-year-old poplar seedlings used in the experiment. For each variable, Kolmogorov–Smirnov and Bartlett tests were applied to test the normality and homogeneity of variances. Duncan's multiple range test was used in the mean separation of groups, and the results from this test are displayed as letters associated with the mean. All variable values are expressed as the mean ± standard error of the mean (SE). The alpha level was set at 5%. The statistical software packages Minitab 17 (Minitab Inc., State College, PA, USA) and MSTAT-C were used.

### 3. Results

#### 3.1. Effects of water stress on growth properties

At the end of experiment, all plants had survived in all water-stress treatments. There were no significant clone × treatment interactions for Tb, Sla, and Rs, indicating that all clones were similarly impacted by the water-stress treatments \( (P > 0.05) \) (Table). According to the results from Duncan's test, Tb and Sla were reduced whereas Rs was increased in plants under moderate and severe water-stress treatments \( (P < 0.05) \). The Tb values of all clones were about 52% and 62% lower in moderate and severe water-stress conditions compared to well-watered plants. Moderate and severe water-stressed plants had 32% and 14% less Sla than well-watered plants. Water-stressed seedlings had 34% and 58% more Rs in moderate and severe treatments than control seedlings (Table).

Regardless of water-stress treatment, the clone factor significantly affected Sla \( (P < 0.05) \) and Rs \( (P < 0.01) \) (Table). The Sla of the Gazi clone was higher than those of the other clones \( (P < 0.05) \) (Figure 2a). In addition, the I-214 clone had greater Rs than the others \( (P < 0.05) \) (Figure 2b).

Water-stress treatment × clone interactions were significant for Ht \( (P < 0.05) \), Nl \( (P < 0.001) \), and La \( (P < 0.01) \); therefore, the differences among clones was evaluated for each water-stress regime (Table). While I-214 had the highest Ht among the control plants, the differences in Ht values of the clones were less distinct under moderate and severe water stress. The difference was only found to be significant between the I-214 and Gazi clones and the I-214 and Kocabey clones under moderate and severe water-stress treatments, respectively \( (P < 0.05) \) (Figure 3a). Severe water stress decreased the height growth of the Kocabey clone more than the others (Figure 3a).

The number of leaves was found to be significantly higher in the Gazi clone under all water regimes \( (P < 0.05) \). In addition, the Kocabey clone also had a higher number of leaves than the other two clones (I-45/51 and I-214) under severe water-stress conditions \( (P > 0.05) \) (Figure 3b). The Gazi and I-45/51 clones had significantly higher La than the others during the control treatment. La was similarly restricted by moderate and severe water-stress treatments in all clones \( (P > 0.05) \) (Figure 3c).
**Table.** Measured parameters and P-values of two-way ANOVA for some growth and chlorophyll a fluorescence parameters of four poplar clones subjected to different water-stress levels. Ht: shoot height; Tb: total biomass; Nl: number of leaves; La: total leaf area; Sla: specific leaf area; Rs: root/shoot ratio; Fo: initial fluorescence yield; Fm: maximum fluorescence yield; Fv/Fm: maximum quantum efficiency of PSII.

<table>
<thead>
<tr>
<th>Water deficit levels</th>
<th>Ht (cm plant⁻¹)</th>
<th>Tb (g plant⁻¹)</th>
<th>Nl (number plant⁻¹)</th>
<th>La (cm² plant⁻¹)</th>
<th>Sla (cm² g⁻¹)</th>
<th>Rs</th>
<th>Fo</th>
<th>Fm</th>
<th>Fv/Fm</th>
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<tbody>
<tr>
<td>Control</td>
<td>82.1 ± 3.6</td>
<td>212.6 ± 5.3 A</td>
<td>258.2 ± 16.8</td>
<td>6169 ± 22</td>
<td>166.9 ± 4.4 A</td>
<td>0.47 ± 0.02 C</td>
<td>581 ± 11</td>
<td>3060 ± 45 A</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>Moderate</td>
<td>27.7 ± 1.8</td>
<td>101.3 ± 3.2 B</td>
<td>127.5 ± 10.6</td>
<td>949 ± 59</td>
<td>110.3 ± 10.6 C</td>
<td>0.62 ± 0.03 B</td>
<td>593 ± 10</td>
<td>2957 ± 66 AB</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>Severe</td>
<td>19.4 ± 1.6</td>
<td>81.9 ± 2.3 C</td>
<td>95.19 ± 7.19</td>
<td>656 ± 50</td>
<td>139.1 ± 4.6 B</td>
<td>0.73 ± 0.03 A</td>
<td>602 ± 12</td>
<td>2861 ± 52 B</td>
<td>0.79 ± 0.05</td>
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<tr>
<td>P</td>
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<td>Watering, W</td>
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<td>0.000***</td>
<td>0.000***</td>
<td>0.000***</td>
<td>0.000***</td>
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<td>0.024*</td>
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<tr>
<td>Clones, C</td>
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<td>0.000***</td>
<td>0.000***</td>
<td>0.015*</td>
<td>0.000**</td>
<td>0.393</td>
<td>0.000***</td>
<td>0.015*</td>
</tr>
<tr>
<td>W × C</td>
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<td>0.269</td>
<td>0.000***</td>
<td>0.001**</td>
<td>0.535</td>
<td>0.787</td>
<td>0.104</td>
<td>0.276</td>
<td>0.274</td>
</tr>
</tbody>
</table>

*Statistically significant (P < 0.05); **statistically significant (P < 0.01); ***statistically significant (P < 0.001).
3.2. Effects of water stress on photochemical activity

Water-stress treatment had significant effects on $F_m$ ($P < 0.05$). The clone effect was also significant on $F_m$ ($P < 0.001$) and $F_v/F_m$ ($P < 0.05$) (Table 1). In the control plants, the mean of the $F_v/F_m$ ratio was 0.81, whereas the mean decreased to 0.79 in leaves exposed to water stress; however, this change was not significant ($P > 0.05$). Clones exhibited similar trends in $F_m$ and $F_v/F_m$. However, the I-214 clone had a significantly higher $F_m$ than the Gazi and I-45/51 clones and it also had higher $F_v/F_m$ values than only the Gazi clone ($P < 0.05$) (Figures 4a and 4b).

4. Discussion

While poplars are not considered drought-tolerant plants in general (Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1998), even under severe water-stress conditions, none of the plants examined in the present study died. The ability to acclimate to reduced soil moisture during moderate and even severe water-stress episodes would allow all clones to survive. In order to remain alive where water stress exists, plants typically possess morphological and physiological adaptations allowing increased uptake and reduced loss of water (Baquedano et al., 2008; Nahar et al., 2015).

In the present study, it was found that water stress caused a significant decrease in shoot height, total biomass, number of leaves, total leaf area, and specific leaf area, while the root/shoot ratio was significantly increased (Table; Figures 2 and 3). This is consistent with numerous studies finding alterations in morphology in poplar plants grown under water stress compared to plants growing with abundant soil moisture (Mazzoleni and Dickmann, 1988; Rhodenbaugh and Pallardy, 1993; Tschaplinski et al., 1998; Zhang et al., 2004; Yin et al., 2005b; Monclus et al., 2006).

The reduction of shoot height with water stress found in the present study was an expected result since decreased turgor can cause decreased height growth. In our experiment, severe water stress decreased Ht in clone Kocabey compared to moderate water stress, indicating that it is more susceptible to severe water stress (Figure 3a).
Limitation of the total leaf area in this study can be considered a defense against water stress. The smaller leaf area transpires less water, effectively conserving a limited supply from the soil for use over a longer period. Rivas et al. (2016) reported that the leaf area modulates water demand by plant canopy and then affects soil water availability and defines the onset of water stress for cowpea species. When soil water availability is limited, leaf expansion rates are commonly observed to decline in line with transpiration (Bacon, 1999). Leaf numbers in this study also followed this same pattern (Figure 3b). The number of leaves of the clones decreased by about 50% in moderate water-stress levels compared to their controls (Table). It was observed that after the leaves had matured, the older leaves dropped and only the youngest leaves at stem apexes remained until the experiment ended. This leaf area adjustment is considered a long-term change that improves the plant’s fitness in a water-limited environment (Kozlowski et al., 1991; Hopkins, 1995; Taiz and Zeiger, 1998). Reducing plant leaf area and leaf numbers conserves water during periods of limited water availability, reducing the transpiration rate. As long as the buds remain viable, new leaves will be produced when the water stress is removed (Hopkins, 1995).

The specific leaf area (Sla) decreased with increasing water-stress levels (Table). Similarly, Yin et al. (2005a) reported decreasing specific leaf area with increasing water stress in *Populus kangdingensis*. Those results suggest that poplar seedlings at high water-stress levels require low leaf area per gram of leaf weight to reduce the transpiration surface area, resulting in more accumulated dry matter per area or in the production of thicker leaves. Leaf thickness also has a significant effect on water use efficiency, with thinner leaves predicted to exhibit lower water use efficiencies than thicker leaves (Stanhill, 1986). Overall, smaller specific leaf areas can effectively reduce water losses (Larcher, 1995). In this context, the Gazi clone is more disadvantaged than the other clones (Figure 2a).

Root/shoot ratios (Rs) vary widely among species with age and environmental conditions, and they increase with greater water-stress levels (Kramer and Boyer, 1995). The root/shoot ratio of clones increased with increasing water stress in this study (Table), which is commonly observed when the soil water availability declines (Kozlowski et al., 1991). Our results support the established general hypotheses that roots are generally less sensitive than shoots to water stress and that one of the most effective safeguards against drought injury is a deep, extensively branched root system to absorb water from a large volume of soil (Kozlowski et al., 1991; Blum, 2011). Therefore, it may be suggested that I-214 has more advantages with a higher Rs ratio than the other clones for absorbing water (Figure 2b).

Previous studies have suggested that there are clonal variations in drought resistance within *Populus* species and hybrids (Pallardy and Kozlowski, 1981; Strong and Hansen, 1991; Tschaplinski and Tuskan, 1994; Tschaplinski et al., 1994, 1998; Brignolas et al., 2000). Similarly, in this study, considerable differences were found between clones in the shoot height, number of leaves, total leaf area, specific leaf area, and root/shoot ratio (Table). The I-214 clone showed significantly higher shoot height under all watering regimes than the other clones, with the lowest decrease found in the I-45/51 clone by 60%–70% under moderate and severe water stress, respectively (Figure 3a). Nevertheless, I-214 had a higher root/shoot ratio than the other three clones in both water-stress treatments (Figure 2b). Having a root system that enhances the ability of a plant to uptake water is an adaptation mechanism against drought. This result presumably reflects the conservative growth strategy of the I-214 clone. The I-214 and I-45/51 clones had a lower leaf number (Figure 3b). Lower leaf number and leaf area may drive lower transpiration rates under water-stress conditions. The lower leaf area also reduces the photosynthetic leaf area, but this disadvantage...
may be partially balanced by more highly productive leaves or higher root/shoot ratios in the I-214 clone. Additionally, I-214 had smaller specific leaf area (Figure 2a). Having smaller specific leaf area may provide another advantage for I-214 as Chaves et al. (2004) suggested that a decrease in specific leaf area normally results in increasing photosynthesis per unit leaf area.

Photosynthesis has been shown to be sensitive to water stress in higher plants, as well as in the growth and morphology of poplar clones. In particular, chlorophyll a fluorescence kinetics can give insights into the ability of a plant to tolerate environmental stresses and to detect its effects on the photosynthetic apparatus (Maxwell and Johnson, 2000; Lichtenthaler et al., 2005). The measurements of chlorophyll a fluorescence yields showed alterations in the photochemical activity of poplar clones in our present study, providing some evidence that some morphological traits may also have effects on the chlorophyll a fluorescence yields of clones. We observed insignificant alterations in initial fluorescence yield, \( F_o \), under different water-stress levels (Table). However, \( F_F \) decreased significantly under water stress and the I-214 clone had the highest value among all of the clones (Figure 4a; Table). It is generally accepted that \( F_m \) expresses the redox state of PSII, in which all \( Q_A \) molecules are in a reduced state (Maxwell and Johnson, 2000). The decline in \( F_m \) suggests a possible change in the ultrastructure of thylakoid membranes, affecting electron transport rates and reduction of the primary electron acceptor QA. Changes in the \( F/F_m \) ratio have often been used as a stress indicator and to describe the potential yield of photochemical reactions (Björkman and Demming, 1987). However, this parameter was not significantly affected by water stress (Table); therefore, the \( F/F_m \) ratio is not a sensitive parameter for assessing the effects of drought in poplar clones. The present results are also consistent with those of previous studies (Bota et al., 2001; Flexas et al., 2002). The results of this study, based on chlorophyll a fluorescence measurements, show that the photochemical efficiency of poplar clones was not significantly affected by water stress compared to the growth parameters. However, water-stress treatments in an earlier study led to substantial reduction in plant photosynthetic CO\(_2\) assimilation (Colom and Vazzana, 2003). The decrease in the photosynthetic rate of water-stressed plants largely results from stomatal closure, which decreases available CO\(_2\) concentrations and restricts water loss through transpiration (Cornic, 2000; Souza et al., 2004). In this context, the decline of shoot height caused by water stress was not accompanied by decreases in \( F/F_m \), showing that this parameter is less sensitive to water stress than some morphologic traits. As a result, significant differences among the poplar clones for the \( F/F_m \) fluorescence parameters in our study (Table; Figures 4a and 4b) may indicate interclonal variability.

In summary, we conclude that moderate (~0.9 MPa) and severe (~1.6 MPa) water-stress treatments have significant effects on poplar morphology and growth traits. However, the water-stress levels used in this study did not significantly damage PSII. These results may indicate that stomatal limitations are more effective than the photoinhibition of PSII under water-stress conditions in poplar. Maintenance of the efficiency of PSII photochemistry under moderate and severe water stress may also indicate that the poplar has a protection mechanism. The I-214 clone was most successful in terms of its higher \( R_s \), \( F/F_m \), and \( F/F_m \) values and lower NI compared to the other poplar clones under water-stress conditions. Consequently, in terms of various growth and chlorophyll fluorescence parameters, the I-214 poplar clone may have potential for use in areas with water stress.

Acknowledgment
We would like to thank Dr Craig D Allen of the US Geological Survey’s Fort Collins Science Center Jemez Mountains Field Station in Los Alamos for reviewing the manuscript.

References


