Growth Responses of *Milicia excelsa* (Iroko) Seedlings to Water Deficit: Implications for Provenance Selection in Ghana

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ABSTRACT

Response of growth, leaf water potential ($\Psi$), net photosynthesis ($P_n$), stomatal conductance ($Gs$), transpiration (E) to water stress were studied in seedling of *Milicia excelsa* from the three populations in Ghana. Two watering regimes were imposed: a control treatment (100% of field capacity), and a water stress treatment (25% of field capacity). Differences in the growth responses of the population to watering regimes were significant. Height, root length, total leaf area, dry matter production, $\Psi$, $P_n$, $Gs$, and E decreased under water stress in all the seedlings. The moist area populations (KK-6 and EMH-1) were the most sensitive to water stress compared to the AHW-1 from dry semi-deciduous forest zone. Water stress reduced height by 26%, 57% and 51% in AHW-1, KK-6, and EMH-1 seedlings respectively and dry total biomass (TB) by 40%, 78%, and 60% in AHW-1, KK-6 and EMH-1 seedlings respectively. Seedlings of AHW-1 also showed substantially lower values of $\Psi$ than seedlings KK-6 and EMP-1 even when well watered. These results suggest that AHW-1 population may have a more conservative water use behavior making the seedlings less vulnerable to drought than the other populations. On the other hand KK-6 and EMH-1 appear to be the most suitable for regularly irrigated conditions owing to their capacity to harvest the available water rapidly and thus maintain vigorous growth. In conclusion, the study premises were valid. Significant variations in phenotypic plasticity exist among the populations of Iroko suggesting that the mechanism underlying growth variation among population may be different. The populations of Iroko may use different structural as well physiological mechanisms to avoid or tolerate water stress at the seedling stage. The results underscore the need for choosing the appropriate population for improvement or nursery purposes.

KEY WORDS: Adaptation, Dry Weights, Iroko, Phenotypic Plasticity, Photosynthesis, Transpiration, Water Deficit

1. INTRODUCTION

*Milicia excelsa* (Welw. C. C. Berg) traded under the name Iroko is among the most valuable commercial timber species in Africa [30, 29, 32]. Iroko has wide natural distribution range in Africa covering both the dry semi-deciduous forests and the rain forest zones [43, 17, 29, 33, 32]. Excessive timber exploitation and forest clearance for agriculture have depleted Iroko populations. Currently, Iroko occur at less than one tree per ha [1]. Several studies suggest that climate change poses an even greater threat to the survival of tree species than land use in some ecosystems [36, 44], with many of them predicting an acceleration in seedling mortality in many valuable tree species such as Iroko due to rapid climate change [41, 14]. Global and regional climate models projects that climate warming will increase average temperatures as well change precipitation patterns including the introduction of frequent and prolong droughts. Drought is already one of the major factors limiting seedling establishment in plantations in Ghana. With the increasing probability of long dry periods due to climate change [23, 48, 34, 2] in many parts of Ghana [12], identifying the differential ability of species to cope with water limitations, particularly during the seedling establishment stages will be a key issue for community tree nursery practices. The initial growth of seedlings largely depends on availability of soil moisture and more during the first three years following transplanting [e.g.16, 8]. This is because water deficit has an adverse effect on seedlings growth processes at the initial growth in seedlings. Water stress can injure and may kill seedlings. At the initial stages of seedling development, water deficit can cause prolonged permanent wilting that usually kills seedlings. In
addition, stress seedlings are more vulnerable to insect and disease pests when compared to healthy seedlings.

Considering the fact that Iroko is distributed across a wide precipitation gradient, the populations are likely to produce ecotypic variation in drought tolerance and potentially, local drought adaptation. Thus, the populations of Iroko could exhibit differences in morphological and/or physiological responses to environmental stresses. These variations may reveal the competitive abilities and physiological tolerance of the populations to promote or worsen the species’ survival under environmental variability. Ecotypic variations in drought tolerance have been studied in many tree species. However, they are mostly on exotic and temperate trees [45, 20, 5]. The effect of an extended period of water deficit on seedlings of tropical forest trees has for the most part remain uninvestigated [49, 11] especially for local tree species such as Iroko that are native to Africa. Generating information on these strategies of seedlings to cope with drought will improve our understanding of carbon acquisition and growth of seedlings during water stress and consequently aid in provenance selection of the species for nursery practices and tree improvement. In particular the information will help reduce seedling mortality at the early stages of seedling establishment in plantation development by informing seed collectors on suitable populations’ sources and how they respond to precipitation change [e.g. 16, 8].

This study was undertaken to determine growth performance of Iroko populations under water stress at a seedling stage. The assumption was that (a) water stress will cause different growth and ecophysiological responses in Iroko populations at the seedling growth stage, and (b) differences in growth and ecophysiological responses should reveal drought adaptation strategies among the Iroko populations.

2. Theoretical framework in brief

To a large extent, morphological and physiological adaptations to drought determine growth in environments where rainfall is erratic or limited [13], and there are differences in the ability (physiological and structural adaptation) of tree species to withstand drought [e.g. 47, 21]. According to Levitt (1972, 1980) [19,18], the different strategies of plants to cope with soil water stress include drought avoidance by maximising water uptake [e.g. by tapping ground water by deep roots [6, 5] or minimising water loss (e.g., stomatal closure, small leaves, reduced rate of transpiration [28, 27] and drought tolerance (responses that allow a plant to tolerate desiccation).

Furthermore, similar trees grown in different conditions may look very different. In fact, forest management has been using the control of micro-environment to shape trees into desired phenotypes for centuries [38, 14]. For instance, by modifying light, water and nutrient availability through variations in stand density, one can direct growth to build tall, branchless poles or alternatively to enhance crown development for seed production and subsequent stand recruitment [10]. This ability of a genotype, i.e. of a single set of genes to generate a range of different phenotypes, depending on the environment that the developing organism must endure, is called phenotypic plasticity [37]. Phenotypic plasticity may take many forms, ranging from changes in physiology, to alterations of morphological structure and to shifts in behavioural repertoires [38, 14].

3. MATERIAL AND METHODS

3.1. Experimental Material

Seeds from the three main populations of M. excelsa (AHW-1, KK-6 and EMH-1) in Ghana were were obtained from the Forestry Research Institute of Ghana (FORIG) (Table 1). The seeds were germinated on a fertilised peat-sand mixture in a germination chamber at 20-22 °C, with a relative humidity of 70-80%. After one month of growth, germinated seedlings of uniform height were transferred into perforated plastic pots of 3.5 litres volume (16 cm deep x 20 cm diameter), and maintained in a greenhouse. The growth medium in the pots was the same as the peat-sand substrate used for germinating the seeds. One cubic meter of the substrate contained 0.8 kg of fertiliser (11% N, 4% P, and 21% K), and 4 kg of Mg-rich limestone powder. The substrate was packed into the seedling pot with a density of about 0.3g/cm³. The 3.5 litre pots were thoroughly watered and kept overnight in a basin partly filled with water to let them reach field capacity before transplanting. The pots were assumed to be at field capacity and weighed after they were removed from the water basin and allowed to drain. The seedlings were maintained in a greenhouse at temperatures between 26-32 °C. The photoperiod was maintained at 12/12 hours daily dark/light period and photosynthetic light intensity was maintained at 500-700 µmol m⁻² s⁻¹ on the seedling canopy.
Table 1. Description of the sources of *M. excelsa* (Iroko) seeds used in the study.

<table>
<thead>
<tr>
<th>Population Code</th>
<th>Forest Type</th>
<th>Co-ordinates</th>
<th>Number of Mother Trees</th>
<th>Temperature Max</th>
<th>Temperature Min</th>
<th>Annual Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHW-1</td>
<td>DSD</td>
<td>N07° 06.18.8 W001° 45.58.0</td>
<td>10</td>
<td>31.2</td>
<td>22.1</td>
<td>1200-1500</td>
</tr>
<tr>
<td>KK-6</td>
<td>MSD</td>
<td>N07° 08.55.3 W001° 44.23.1</td>
<td>12</td>
<td>32.2</td>
<td>22.1</td>
<td>1200-1800</td>
</tr>
<tr>
<td>EMH-1</td>
<td>WE</td>
<td>N05° 39.8 W02° 20.7</td>
<td>7</td>
<td>30.2</td>
<td>21.2</td>
<td>&gt;1750</td>
</tr>
</tbody>
</table>

DSD = dry semi-deciduous forest zone; MSD = moist semi-deciduous forest zone; WE = Wet evergreen forest zone.

3.2. Experimental design

A randomised complete block design with two factors (three populations and two watering regimes) was used. A total of 108 seedlings were maintained in the greenhouse in three separate blocks. Each block comprised 36 seedlings, and 12 seedlings in each block represented each population. The 12 seedlings in each block were equally divided between two watering regimes; i.e. control treatment (100% of field capacity), and water stress treatment (25% of field capacity). Seedlings were watered every second day. The water loss was measured every second day by weighing six selected pots in each population from the control treatment in each block. The measured water loss was completely compensated in the control treatment (well-watered). The drought stress treatment was then induced by compensating 25% (severe stress) of the amount of water replaced in the control treatment. This was expected to result in different levels of water stress in the seedlings between the three watering treatments. To avoid transpiration from the soil surface, the pots were covered with plastic bags tied to the stems of the plant. The mean soil water content measured at the end of the experiment was 70% and 17% for the well-watered and water stress treatment respectively.

3.3. Measurements

3.3.1. Growth traits

At five month of age all seedlings (whole-seedling) were harvested, and seedling height (SH), leaf area (LA), root length (RL), and total dry biomass per seedling (TB: root, stem, branch and leaf masses) were determined. SH was determined from the root collar to the terminal bud. The roots were washed with water to remove the substrate and RL (taproot length) was measured from the root collar to the tip of the root. Leaves were separated from the shoot by hand and enumerated per seedling. Fresh weights of roots, stems, branches and leaves per seedlings were immediately measured with a battery-operated scale, and dry weights were measured after oven-drying at 70°C for 24 hours. LA was determined with a LI-COR Model LI-3000A portable area meter.

3.3.2. Leaf water potential (Ψ)

Mid-morning Ψ was measured on two fully expanded leaves from six seedlings per treatment per population using the pressure chamber technique developed by Scholander et al. (1965). Pressure was raised at a rate of about 0.02 Mpa s⁻¹. Measurements were taken between 0900 and 1600h at a room temperature of 26°C and relative humidity varying between 70-80%.

3.3.3. Gas exchange

Net photosynthesis (PN), and transpiration (E), were determined on attached leaves at the end of the experiment with a Li-Cor portable photosynthesis system (CIRAS 2) equipped with a 250-ml cuvette, at photosynthetically active radiation (PAR) between 1300-1500 μmol m⁻²s⁻¹. The CO₂ concentration was between 326-328 ppm, leaf temperature was at 26°C, and the relative humidity was 70-80%. Measurement was made on one uppermost fully expanded leaf of six plants per treatment per population (3 measurements per seedling). Measurements were taken between 0900 and 1600 h and completed within one week.

3.4. Data analysis

Statistical analysis was carried using the analysis of variance (ANOVA) of the Statistical Package for the Social Sciences (SPSS) (FASW Statistics 17.0) software. Subsequent Tukey’s HSD post hoc tests were used for testing possible differences between the populations. The Mann-Whitney U-test was employed to detect the differences between treatments, separately for each provenance. Although the experimental design was a randomised complete block, preliminary analyses failed to detect significant (P<0.05) effects of blocks, which could therefore be ignored.
4. RESULTS

4.1. Seedling growth and productivity

Significant differences in seedling height (SH) (P < 0.05), total leaf area (TLA) (P < 0.01), root length (RL) (P < 0.000), and total biomass (TB) (P < 0.05) were detected among M. excelsa populations (AHW-1, KK-6, and EMH-1) under different watering treatments. All the growth traits tended to be lower for all seedlings under the water stress condition. For instance water stress reduced SH by 26% (AHW-1), 57% (KK-6) and 51% (EMH-1) and reduced TB by 40% (AHW-1), 78% (KK-6) and 60% (EMH-1) (Table 2).

Table 2: Growth traits of (M. excelsa) Iroko seedlings under water stress. Means (SE in parenthesis) are provided

<table>
<thead>
<tr>
<th>Trait</th>
<th>Watering Treatment</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25% field capacity</td>
<td>AHW-1</td>
</tr>
<tr>
<td>Seedling height (cm)</td>
<td></td>
<td>KK-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EMH-1</td>
</tr>
<tr>
<td>Total leaf area (cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root length (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass(g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Capital letters refer to differences in the populations over all treatment and small letters to the variation between treatments. Values annotated with the same letter are not statistically different at p < 0.05. Seeds were collected from the three populations in Ghana.

3.2. Mid-morning leaf water potential (Ψ)

There was large variation (P < 0.001) among the three populations of M. excelsa in seedling Ψ. There was significant effect of treatment and population-treatment interaction (P <0.001) on Ψ (Table 3). For all seedlings the values of Ψ were low under high water stress (i.e. 25% field capacity). However, Ψ of seedlings of population KK-6 decreased much more drastically than that of seedlings of AHW-1 and EMH-1 populations.

Table 3: Mean values of leaf water potential of (M. excelsa) (Iroko) seedlings (from three populations in Ghana) subjected to two watering regimes. SE in parenthesis; N= two leaves per six seedlings per treatment per population.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Watering treatment</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25% field capacity</td>
<td>AHW-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KK-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EMH-1</td>
</tr>
<tr>
<td>Leaf water potential (Mpa)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Capital letters refer to differences in the populations over all treatment and small letters to the variation between treatments. Values annotated with the same letter are not statistically different at p < 0.05.

3.3. Gas exchange

The populations of M. excelsa (AHW-1, KK-6 and EMH-1) exhibited significant differences in their net photosynthesis (Pₙ), stomatal conductance (Gs), and transpiration (E) (Table 4) under the different watering treatments. Lowest values of Pₙ, Gs, and E were observed for all seedlings under water stress condition. On average, seedlings of AHW-1 population had a lower E, Gs, Pₙ than KK-6 and EMH-1 populations.

3.4. Interdependence of growth and gas exchange traits

There were significant and positive correlations among seedling traits (i.e. TB, TLA, Pₙ, Gs, and E. (Table 5).
Some studies have shown that the more moist area population of *M. excelsa* may show significant variation in photosynthetic traits and gas exchange parameters. These results suggest that seedlings of AHW-1 population may have a more conservative water use behaviour than those from other populations [22]. They also showed the least sensitive of these traits to water stress. It is also noteworthy AHW-1 seedlings showed substantially lower values of Ψ than seedlings from the other population (KK-6 and EMP-1) even when well watered. In contrast, the moist area populations (KK-6 and EMH-1) had the highest biomass production and photosynthetic rates when water was abundant. These results suggest that seedlings of AHW-1 population may have a more conservative water use behaviour that makes the seedlings less vulnerable to insufficient water. As recorded, the AHW-1 seedlings also had deeper roots [commonly associated with good drought adaptation and enabling a plant to take up water effectively [6, 15] and a higher Ψ, which indicates that the AHW-1 seedlings were able to extract water quicker, thereby maintaining higher Ψ in the leaves. This result coincides with the fact that the origin of AHW-1 seedlings experiences significant annual variation in rainfall with sometimes prolongs periods without rain. Thus, the exhibited growth response may suggest significant variation in phenotypic plasticity among the population [e.g. 7].

On the other hand seedlings from the more moist were most sensitive to water deficit as shown in the pattern of responses. They appear to be the most suitable for regularly irrigated conditions owing to their capacity to harvest the available water rapidly and thus maintain vigorous growth. Some studies have shown that the more moist area population of *M. excelsa* have highest height growth and wider leaf size [e.g. 32, 33].

These results points to the fact that the mechanism underlying growth variation among population may be different. The population of *M. excelsa* may use different structural as well physiological mechanisms to avoid or tolerate water stress at the seedling stage. Assessing ecological structure and morphological variation in *M. excelsa* populations under field condition in Benin, Niger Delta, Nigeria, and Benin, West Africa should be considered in further studies.

5. DISCUSSION AND CONCLUSION

Table 4: Net photosynthesis (P$_N$), stomatal conductance (Gs), transpiration (E), (means and SE in parenthesis) of *M. excelsa* (Iroko) seedlings (from three populations Ghana) subjected to two watering regimes.

<table>
<thead>
<tr>
<th>Watering treatment</th>
<th>Population</th>
<th>P$_N$ (μmol m$^{-2}$ s$^{-1}$)</th>
<th>Gs (mol m$^{-2}$ s$^{-1}$)</th>
<th>E (mmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% field capacity</td>
<td>AHW-1</td>
<td>4.16 (0.25)</td>
<td>33.75 (2.42)</td>
<td>0.44 (0.05)</td>
</tr>
<tr>
<td></td>
<td>KK-6</td>
<td>5.40 (0.40)</td>
<td>36.00 (3.95)</td>
<td>0.63 (0.08)</td>
</tr>
<tr>
<td></td>
<td>EMH-1</td>
<td>3.93 (0.26)</td>
<td>32.14 (2.50)</td>
<td>0.44 (0.07)</td>
</tr>
<tr>
<td>100% field capacity</td>
<td>AHW-1</td>
<td>4.47 (0.21)</td>
<td>37.25 (2.41)</td>
<td>0.55 (0.06)</td>
</tr>
<tr>
<td></td>
<td>KK-6</td>
<td>8.55 (0.23)</td>
<td>92.66 (2.28)</td>
<td>1.16 (0.04)</td>
</tr>
<tr>
<td></td>
<td>EMH-1</td>
<td>7.75 (0.35)</td>
<td>81.25 (3.42)</td>
<td>1.04 (0.08)</td>
</tr>
</tbody>
</table>

F$_F$ = population effect, F$_W$ = watering effect, F$_F$*$F_W$ = watering/population effect. ** P < 0.01 *** P < 0.001.

Table 5: Pearson’s product-moment correlation coefficients (r) among photosynthetic traits and seedling growth traits of *M. excelsa* (Iroko).

<table>
<thead>
<tr>
<th>Trait</th>
<th>TB</th>
<th>TLA</th>
<th>P$_E$</th>
<th>Gs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLA</td>
<td>0.799***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P$_N$</td>
<td>0.742**</td>
<td>0.723***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.974**</td>
<td>0.972***</td>
<td>0.974**</td>
<td></td>
</tr>
<tr>
<td>Gs</td>
<td>0.758**</td>
<td>0.732**</td>
<td>0.977**</td>
<td>0.957***</td>
</tr>
</tbody>
</table>

TB= total dry biomass; TLA= total leaf area; P$_N$= net photosynthesis; E= transpiration; Gs= stomatal conductance; **= significant at P < 0.01.

Significant reductions in height, root length, leaf area, and dry matter production, plant water status were recorded under water stress. On average 45% and 59% reductions in height growth and biomass production, respectively, occurred under water stress. Photosynthetic rates, stomatal conductance, and transpiration rate were also markedly reduced in all the seedlings under water stress condition. Rao et al., (2008) also made similar observations. In their studies, seedling of *Leucaena leucocephala* (Lam.) reduced height by as much as 75.8% under severe water stress. Other studies reported similar trends for woody angiosperms [31], *Eucalyptus microtheca* [45, 20], *Hopea odorata* Roxb., *Mimusops elengi* Linn [50], some Mediterranean woody plants [42], African nightshades [26], and *Pistacia khinjuk* [40]. Reductions observed in the growth traits and gas exchange parameters of seedlings are common occurrences in water deficit plants [22], which are often exhibited as mechanisms to avoid drought [25].

AHW-1 seedlings had the lowest values for height growth, biomass productivity and gas exchange parameters under both water stress and well watered conditions. They also showed the least sensitive of these traits to water stress. It is also noteworthy AHW-1 seedlings showed substantially lower values of Ψ than seedlings from the other population (KK-6 and EMP-1) even when well watered. In contrast, the moist area populations (KK-6 and EMH-1) had the highest biomass production and photosynthetic rates when water was abundant. These results suggest that seedlings of AHW-1 population may have a more conservative water use behaviour that makes the seedlings less vulnerable to insufficient water. As recorded, the AHW-1 seedlings also had deeper roots [commonly associated with good drought adaptation and enabling a plant to take up water effectively [6, 15] and a higher Ψ, which indicates that the AHW-1 seedlings were able to extract water quicker, thereby maintaining higher Ψ in the leaves. This result coincides with the fact that the origin of AHW-1 seedlings experiences significant annual variation in rainfall with sometimes prolongs periods without rain. Thus, the exhibited growth response may suggest significant variation in phenotypic plasticity among the population [e.g. 7].

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Ouinsavi and Sokpon (30) also found morphological variation in M. excelsa populations as result of adaptive plasticity. The patterns of variation were strongly influenced by environmental factors such as soil texture, soil chemical characteristics, and annual rainfall.

In this study the response of the populations measured by the E, Gs, P were correlated positively with those measured by the seedling growth (SH, TLA, RL and TB). Generally morphological growth traits seem to have high correlation with physiological traits [e.g. 24, 47, 45, 20]. These results suggest strong phenotypic cline in drought tolerance of the three populations or ecotype. The observed relationships also suggest that both the growth traits and physiological traits can equally be used as selection criteria for adaptive traits.

Despite the limitations inherent to greenhouse experiments (e.g., limited soil volume for root development etc), for which seedling responses may not be fully representative of field grown seedling responses, it is noteworthy that the findings of this study are consistent with the trend reported for other species studied under field condition. This makes this current study more relevant and underscores the need for choosing the appropriate population of M. excelsa for improvement or nursery purposes.

Acknowledgement

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