ABSCISIC ACID (ABA) AND THE REGULATION OF SEEDLING GROWTH UNDER STRESS

by Siti Rubiah Zainudin$^1$ and Kamis Awang$^2$

Abstract. Xylem sap abscisic acid (ABA) and leaf ABA of Hopea odorata Roxb. and Mimusops elengi L. were measured under conditions of soil compaction and drought to investigate the role of ABA in the regulation of stomatal conductance. A rapid and substantial increase in xylem sap ABA concentration but not leaf ABA concentration was correlated with soil drying in both species. However, xylem ABA concentration was lower in M. elengi seedlings than in H. odorata seedlings. The increase in xylem sap ABA concentration observed at high soil bulk density was correlated with reduced stomatal conductance. These results suggest that xylem ABA may act as a stress signal in the control of stomatal conductance. Reduced xylem ABA levels may explain the lower urban stress tolerance M. elengi seedlings relative to H. odorata.

Key Words. Hopea odorata; Mimusops elengi; soil compaction; drought; stomata conductance; xylem sap abscisic acid; urban environment.

Stomatal conductance is a primary determinant of the net carbon balance and growth of plant species, and, because of its influence on transpirational water loss, stomatal conductance determines the water balance of a plant. Stomata impose a critical control mechanism over water loss and gas exchange between the atmosphere and leaf cells (Liang et al. 1996). Effective stomata control is important for plant growth and survival, especially when water supply is limited. Accumulated evidence has shown that inhibition of leaf growth and stomatal conductance are perhaps the first responses when root systems are exposed to stress conditions, such as drought, flooding, and soil compaction (Passioura 1988; Davies and Zhang 1991; Tardieu and Davies 1993; Hartung et al. 1994). Under these conditions, roots may respond by synthesizing and exporting chemical signals through the transpiration stream to shoots where physiological processes are regulated (Davies and Zhang 1991; Gowing et al. 1993).

Tardieu and Davies (1992) found that the abscisic acid (ABA) content of root tips increased when maize (Zea mays L.) was grown in compacted soil, while the application of 1µM ABA to unstressed seedlings promoted the growth of short, thick roots similar to those produced in response to mechanical impedance (Hartung et al. 1994). Stypa (1987) also observed increases in ABA content in both the stunted roots and shoots of maize plants grown in compacted soil under laboratory conditions. In contrast, Lachno et al. (1982) and More et al. (1988) failed to detect any increase in ABA concentration in mechanically impeded roots of maize.

Although the effects of soil compaction have been extensively studied, few experiments have examined the involvement of root-to-shoot signaling in mediating plant responses. Increased xylem sap ABA concentrations and associated reductions in stomatal conductance have been recorded in the shoots of maize plants growing in compacted soil under field conditions (Tardieu et al. 1991). Hartung et al. (1994) also reported increases in xylem sap ABA concentrations in plants subjected to mechanical impedance. It is therefore possible that a root-sourced signal such as ABA may be involved in plant responses to soil compaction.

This experiment was designed to examine the relationship among stomatal conductance, xylem sap ABA concentration, and leaf water potential in two species grown in compacted and unwatered soil.

MATERIALS AND METHODS

The experiment was a 2 × 2 × 2 factorial design with two species (Hopea odorata and Mimusops elengi), two soil compaction treatments (compacted and noncompacted), and two soil moisture treatments (well watered and at soil water potential ≥ −1.5 MPa). Seedlings were planted in a Tropicomp haplorthox soil series in a series of soil columns consisting of 70 cm (28 in.) long sections of polyvinyl chloride [with internal diameter of 10 cm (4 in.)]. Each column contained two layers of soil. The lower layer was noncompacted, with an approximate bulk density of 1.0 g/cm³, while the top layer was packed to a dry bulk density of 1.0 g/cm³ or 1.5 g/cm³ (Table 1). In total, eight different treatment combinations were used with 24-single plant replicates per treatment. Twelve days later, when the seedlings were well established (manifested by the appearance of new leaves), moisture stress was imposed by leaving half of the plants within each species unwatered, while the remaining half were watered daily. To determine soil water content, a 0 to 15 cm (0 to 6 in.) deep soil core was sampled with a punch 10 mm (0.4 in.) in diameter. Six samples were collected from each treatment. The soil core was weighed.
immediately and then oven dried at 105°C (221°F) to a constant weight. The soil water content was calculated as follows:

\[
\text{Soil water content (\%) = 100 \times \left( \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \right)}
\]

For each plant, physiological measurements were made on fully expanded leaves with a LiCor 6200 Photosynthesis System (Lincoln, NE, U.S.). Twelve measurements were carried out. Stomatal conductance was expressed as a percentage of the control (well-watered and noncompacted). Immediately after measurement, the water potential of the same leaf was measured. Leaf discs, 6 mm (0.24 in.) in diameter, were sampled and quickly placed into thermocouple chambers, which were connected to a Wescor HR-33T dewpoint microvoltmeter. A standard 2-hour incubation time at 25°C (77°F) was adopted before water potential readings were taken.

Table 1. Treatment combinations of bulk density and watering regimes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Descriptions of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>Well-watered at field capacity and noncompacted (control 1.0 g/cm³)</td>
</tr>
<tr>
<td>WC</td>
<td>Well-watered at field capacity and compacted at bulk density 1.5 g/cm³</td>
</tr>
<tr>
<td>UN</td>
<td>Unwatered and noncompacted (control 1.0 g/cm³)</td>
</tr>
<tr>
<td>UC</td>
<td>Unwatered and compacted at bulk density 1.5 g/cm³</td>
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</table>

Xylem sap was collected between 1400 and 1600 hours from detopped plants at the start of the experiment through the 12th day. Plants with intact root systems were taken out of PVC pipes, put in a large pressure chamber, and detopped about 20 cm (8 in.) from the base. The bark (with phloem tissues) 2 cm (0.8 in.) below the cut surface was removed carefully with a razor blade, and the cut surface was rinsed with distilled water. Collection pressures varied from 0.5 MPa (for well-watered plants) to 1.5 MPa (for unwatered plants). To avoid the contamination of the broken cells at the cut surface with phloem sap, the first 100 mm³ (0.006 in³) of sap were discarded, and the second 100 to 200 mm³ (0.006 to 0.12 in³) were collected and stored in Eppendorf tubes at −70°C (−94°F) pending ABA assay. After 12 days of drought, the collection of xylem sap was not carried out because the pressure needed to obtain xylem sap from unwatered plants exceeded the capabilities of the pressure chamber. Daily leaf samples for ABA analysis were also collected after measurements of stomatal conductance and water potential until the 21st day. Leaves were harvested and plunged into liquid nitrogen. After being freeze dried, samples were stored in a dessicator until assay. The concentration of ABA was measured by a modification of used by Loveys (1984, Figure 1*).

Data Analysis
The data were subjected to two-way analysis of variance (ANOVA) using an SAS statistical package to determine the significance of the various factors and their interactions. Treatment means were separated using Duncan’s new multiple-range test, at \( \alpha = 0.05 \) level of significance.

RESULTS AND DISCUSSION
Analysis of variance (Table 2) showed that the three main factors significantly \( (P < 0.001) \) influenced leaf water potential, stomatal conductance, and leaf xylem sap ABA of \( H. odorata \) and \( M. elengi \) seedlings. Stomatal conductance of stressed plants was significantly \( (P < 0.001) \) reduced three days after withholding water for both species (Figure 2a and b). With further decrease in soil water content, stomatal conductance decreased progressively to a minimum value of about 20% for \( M. elengi \) and 40% for \( H. odorata \) seedlings at the end of the experimental period relative to untreated control seedlings. With soil drying, significant reduction of stomatal conductance occurred earlier than the reduction of leaf water potential. Furthermore, the soil compaction treatment alone reduced stomatal conductance, indicating stomatal conductance is reduced in compacted soil, even without soil drying (Figure 3a and b).

When plants were grown in compacted but well watered soil, the leaf water potential and leaf ABA concentration were similar, but the ABA concentration in the xylem sap increased three- to fivefold, and stomatal conductance was significantly reduced (Figure 3c and d). Furthermore, xylem sap and bulk leaf ABA concentration were significantly highly correlated with stomatal conductance in both species \( (r^2 = 0.94 \text{ and } 0.99 \text{ for } M. elengi \text{ and } H. odorata, \text{ respectively}) \). However, leaf water potential and leaf ABA concentration were not significantly correlated \( (r^2 = 0.16 \text{ and } 0.03 \text{ for } M. elengi \text{ and } H. odorata, \text{ respectively}) \).

As a stress signal, ABA plays a central role in the communication between roots and shoots, especially when the root system is under stress conditions (Davies and Zhang, 1991; Davies et al. 1994). However, this hypothesis has been challenged by several recent reports (Munns and King 1988; Munns 1990; Trejo et al. 1995). When plants were grown in a compacted soil, stomatal conductance was significantly reduced. Hartung et al. (1994) in the laboratory and Tardieu et al. (1992) under field conditions showed an increase in ABA concentration in the xylem sap of maize plants growing on compacted soil and observed a significant relationship between leaf growth rate or stomatal conductance and xylem sap ABA concentration, but not between leaf growth rate and the current leaf water status or the concentration of ABA in the bulk leaf. The same situation has been
observed in the present study. When plants were grown in compacted but well-watered soil, the leaf water potential and leaf ABA concentration were almost unchanged, but the ABA concentration in the xylem sap increased three- to fivefold, and stomatal conductance was significantly lowered.

Mulholland et al. (1996) reported that changes in root and shoot growth of barley in compacted soil were accompanied by transitory increases in the xylem ABA during the early stages of growth. Similar responses were observed in this study, with the increase in the xylem sap ABA induced by compacted soil becoming most obvious at day 4. Xylem sap concentration, however, decreased in *M. elengi* but remained almost constant in *H. odorata* seedlings after day 8, suggesting that either the roots became increasingly adapted to the compacted soil or that there was a refractory period when the stimulus no longer promoted ABA biosynthesis and translocation (Knight et al. 1992).

Zhang and Davies (1990a, 1990b) have previously shown that stress-induced, root-sourced ABA may be detected more rapidly in the xylem sap than in the leaf extracts, a conclusion supported by this study, in which a substantial increase in xylem ABA was observed at high soil bulk density but by nonsignificant changes in foliar ABA. Mulholland et al. (1996) reported a subcritical bulk density that produced large differences in leaf expansion rates and leaf areas between two barley genotypes, Steptoe and Az34. The inability of Az34 to produce as much ABA compared to Steptoe in response to soil compaction may have been crucial to its failure to maintain near normal rates of leaf expansion at subcritical levels of compaction. A similar result was obtained from this study, whereby xylem sap ABA of *M. elengi* was comparatively lower than in *H. odorata* seedlings.

**CONCLUSIONS**

The increase in xylem sap ABA concentration for both species observed at high bulk density was correlated with reduced stomatal conductance. These results suggest that xylem ABA may act as a stress signal in the control of stomatal conductance. Xylem ABA concentration were lower in *M. elengi* seedlings than in *H. odorata* seedlings. This finding may explain why *M. elengi* seedlings have lower urban stress tolerance than *H. odorata*.

### Table 2. Analysis of variance on physiological parameters and ABA concentrations of the leaf and xylem sap of *Mimusops elengi* and *Hopea odorata* seedlings subjected to a factorial combination of water stress and soil compaction.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Leaf water potential</th>
<th>Stomata conductance</th>
<th>Leaf ABA</th>
<th>Xylem ABA</th>
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<tbody>
<tr>
<td><strong>Main factor</strong></td>
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<tr>
<td>Species</td>
<td>***</td>
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<td>***</td>
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<tr>
<td>Soil moisture*</td>
<td>***</td>
<td>***</td>
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<td>***</td>
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<tr>
<td>Soil compaction†</td>
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<td>N</td>
<td></td>
<td>***</td>
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<tr>
<td><strong>Two-way interactions</strong></td>
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<td></td>
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<tr>
<td>Species × watering</td>
<td>***</td>
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<tr>
<td>Species × compaction</td>
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<tr>
<td>Water × compaction</td>
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<td>**</td>
<td>NS</td>
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<td><strong>Three-way interactions</strong></td>
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<tr>
<td>Species × watering × compaction</td>
<td>***</td>
<td>**</td>
<td>NS</td>
<td>***</td>
</tr>
</tbody>
</table>

*Two soil moisture treatments (well-watered and soil water potential maintained at ≥ −1.5 MPa).†Two soil compaction treatments (compacted at 1.5 g cm⁻³ and noncompacted at 1.0 g cm⁻³, respectively).‡Significant differences at *P* < 0.05 (*), *P* < 0.01(**), *P* < 0.001(***), and nonsignificant (NS).

**LITERATURE CITED**


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Extract overnight in acetone with internal standards

\[ \downarrow \]

Evaporate to water phase \textit{in vacuo}

\[ \downarrow \]

Filter through nylon and adjust pH to 2.8

\[ \downarrow \]

Partition 3$\times$ with diethyl ether

\[ \downarrow \]

Reduce ether phase to dryness under N$_2$ stream and redissolve in 0.5 mL ethanol

\[ \downarrow \]

Pass through preconditioned C18 Sep-Pak, rinse with 5 mL H$_2$O, and elute with 5 mL methanol

\[ \downarrow \]

Concentrate methanolic extract to 1 mL under N$_2$ stream, analyze by HPLC/salicylate for GCMS

Figure 1. Schematic representation of sample preparation method used in this study for HPLC and GCMS analysis of ABA (after Loveys 1984).

Figure 2. Stomatal conductance relative to noncompacted, well-watered control plants of (a) \textit{Mimusops elengi} and (b) \textit{Hopea odorata} seedlings following treatments. [WW = well-watered at field capacity and noncompacted (control 1.0 g/cm$^3$), WC = well-watered at field capacity and compacted at bulk density 1.5 g/cm$^3$, UN = unwatered and noncompacted (control 1.0 g/cm$^3$), UC = unwatered and compacted at bulk density 1.5 g/cm$^3$]. Vertical bars indicate standard error of the mean ($n = 6$).
Figure 3. Changes in ABA concentration in the leaves and xylem sap of (a, c) *Mimusops elengi* and (b, d) *Hopea odorata* seedlings following treatments. [WW = well-watered at field capacity and noncompacted (control 1.0 g/cm$^3$), WC = well-watered at field capacity and compacted at bulk density 1.5 g/cm$^3$, UN = unwatered and noncompacted (control 1.0 g/cm$^3$), UC = unwatered and compacted at bulk density 1.5 g/cm$^3$]. Vertical bars indicate standard error of the mean (n = 6).
Résumé. L’acide abcisique contenu dans la sève du xylème et celle dans les feuilles de *Hopea odorata* Roxb. et de *Mimusops elengi* L. a été mesurée dans des conditions de sol compacté et de sécheresse, et ce afin de vérifier le rôle de l’acide abcisique dans la régulation de la conductivité stomatique. Un rapide et substantiel accroissement en acide abcisique dans la sève du xylème mais non dans les feuilles a été corrélé avec l’assèchement du sol chez les deux espèces. Cependant, les concentrations en acide abcisique dans la sève du xylème étaient plus faibles chez les semis de *M. elengi* que chez ceux de *H. odorata*. L’accroissement en concentration d’acide abcisique dans la sève du xylème qui était observée dans les sols très compactés a été corrélée avec une diminution de la conductivité stomatique. Ces résultats suggèrent que l’acide abcisique du xylème pourrait agir comme un signal de stress dans le contrôle de la conductivité stomatique. Des niveaux réduits d’acide abcisique dans le xylème pourraient expliquer la tolérance plus faible au stress urbain des semis de *M. elengi* par rapport à ceux de *H. odorata*.


Resumen. La savia del xilema ABA y de la hoja ABA de *Hopea odorata* Roxb. y *Mimusops elengi* L. fueron medidas bajo condición de compactación del suelo y sequía para investigar el papel de ABA en la regulación de la conductancia estomatal. Un rápido y sustancial incremento en la concentración de ácido abscísico del xilema (ABA), pero no en la hoja, estuvo correlacionado con la sequía del suelo en las dos especies. Sin embargo las concentraciones de ABA del xilema fueron más bajas en brinzales de *M. elengi* que en los de *H. odorata*. El incremento en la concentración de la savia del xilema ABA, observado a alta densidad aparente del suelo, estuvo correlacionado con la reducida conductancia estomatal. Los niveles reducidos de ABA del xilema pueden explicar las menores tolerancia de los brinzales de *M. elengi* en relación a *H. odorata*. 

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