

## Stomatal Conductance in Relation to Xylem Sap Abscisic Acid Concentration in *H. odorata* and *M. elengi* Seedlings\*

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### Introduction

Stomata impose a critical control over water loss and exchange of gases between the atmosphere and leaf cells (Liang *et al.*, 1996). Effective control is important for plant growth and survival especially when water supply is limited especially in the harsh urban environment. 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 *et al.*, 1988; Davies *et al.* and Zhang *et al.*, 1991; 1992a; Tardieu *et al.*, 1993; Hartung *et al.*, 1994). Under these conditions, roots may respond by synthesising and exporting chemical signals through the transpiration stream to shoots where physiological processes are regulated (Davies *et al.* and Zhang *et al.*, 1991). Much evidence has indicated that root-derived abscisic acid (ABA) in the xylem is involved, and ABA is the most likely candidate as the chemical signal involved in communication between root and shoot and in the regulation of leaf growth and stomatal conductance, especially under conditions of soil drought and compaction (Zhang *et al.* and Davies *et al.*, 1989; Gowing *et al.*, 1993). Tardieu *et al.*, (1992) found that the abscisic acid (ABA) content of root tips increased when maize (*Zea mays* L.) was grown in compacted soil, whilst the application of 1 $\mu$ M 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 *et al.* (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 the impeded roots of maize. Although the effects of soil compaction have been extensively studied, few experiments have examined the involvement of root-to-shoot signalling 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 relations between stomatal conductance, xylem sap ABA and leaf water potential in two species grown in compacted and unwatered soil, and to investigate whether ABA plays a similar role under these two different stresses in its regulation of stomatal conductance of these two species.

### Materials and Methods

The experiment was a 2x2x2 factorial design, i.e. two species, *H. odorata* and *M. elengi*, two soil compaction treatments (compacted and non-compacted), and two soil drying treatments (well-watered and at soil water potential  $\geq -1.5$ MPa). The soil used belongs to the Tropeptic haplorthox and the seedlings were grown in a series of soil columns consisting of 70-cm long sections of polyvinyl chloride (with internal diameter of 10 cm), with each column containing two layers of soil. The top layer was packed to a dry bulk density of 1.0 and 1.5 g/cm<sup>3</sup> to a depth of 20 cm and only the first 20 cm of the soil in the column was compacted to simulate urban environment.

In total, eight different treatments were used with 24 plants each as replicates. Twelve days later, when the seedlings were well-established (manifested by the appearance of new leaves) drought treatments was imposed by leaving half of the plants unwatered, while the remaining half were watered daily as the well watered treatments.

Soil sampling and determination of soil water content: A 0 - 15 cm depth of the soil was sampled with a punch (10 mm in diameter and 20 cm in length). Six samples were collected for each treatment. Fresh soil was weighed immediately after sampling and then over dried at 105°C to a constant weight. The soil water content was calculated based on the following equation:

$$\text{Soil water content (\%)} = 100 \times \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}}$$

Measurements of stomatal conductance and leaf water potential: For each plant, the physiological measurements were made on fully expanded leaves with a Li Cor 6200 Photosynthesis System (Lincoln NE, USA). Twelve measurements were carried out, and stomatal conductance was expressed as a percentage of the control (well-watered and non-compacted). Immediately after measurements, the water potential of the same leaf was measured. Leaf discs, 6mm in diameter, were sampled and quickly sealed into thermocouple chambers, which were connected to a Wescor HR-33T dew-point microvoltmeter. A standard 2-hour incubation time at 25 °C was adopted before water potential readings were taken.

Collection of xylem sap: Xylem sap was collected between 1400 and 1600h from detopped plants from the start of the experiment until the 12<sup>th</sup> day. Plants with intact root systems, taken out of PVC pipes using a plastic bag, were carefully put in a large pressure chamber and detopped about 20 cm

from the base. The bark (with phloem tissues) 2cm below the cut surface was removed carefully with a razor blade and the cut surface was then rinsed with distilled water. Collection pressures varied from 0.5MPa (for well-watered plants) to 1.5MPa (for unwatered plants). In order to avoid the contamination of the broken cells at the cut surface with phloem sap, the first 100 mm<sup>3</sup> of sap was discarded and the second 100-200mm<sup>3</sup> collected and stored in Eppendorf tubes at -70 °C 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 were too high. ABA assay of leaf and xylem sap samples: Leaf samples for ABA analysis were also collected after measurements of stomatal conductance and water potential everyday until the 21<sup>th</sup> day. Leaves were harvested and plunged into liquid nitrogen. After being freeze dried, samples were stored in a desiccator until assay. The concentration of ABA was measured by a modification of that of Loveys et al. (1984). The data were subjected to three-way analysis of variance (ANOVA) to determine the significance of the various factors and their interactions. The treatment mean values of each parameter were subjected to the Duncan's New Multiple Range Test (DMRT) to evaluate the differences among the treatments.

### Results and Discussion

**Effects on Leaf Water Potential and Stomatal Conductance:** After being transplanted, tree seedlings did not grow immediately owing to the disturbance to their root system during transplanting. They started to grow normally after about 7-9 days of recovery when new roots and leaves appeared. These new roots could have been exposed to the compacted soil environment (for soil compaction treatments) once they appeared. Soil drying treatment was applied 14 days after transplanting. After withholding watering, soil water content decreased rapidly to around 40 to 53% of initial soil water content after 12 days and this decrease slowed down somewhat afterwards. Compacted or non-compacted soil water content showed a similar decline. Soil drying resulted in the development of leaf water deficits in all treatments but the response differed between difference levels with

the decrease in leaf water potential of *M. elengi* took place much earlier than in *H. odorata*. Stomatal conductance of well-watered seedlings somewhat fluctuated throughout the experimental period, presumably as the result of small differences in ambient conditions. Three days after withholding water, there was a significant reduction ( $P < 0.001$ ) in stomatal conductance compared to well-watered controls for both species. With further decrease in soil water content, stomatal conductance decreased progressively to a minimum value of about 20% of the control for *M. elengi* and 40% of the control for *H. odorata* seedlings respectively at the end of the experimental period.

**Effects on Leaf and Xylem Sap ABA Concentrations:** For *M. elengi*, the increase in ABA concentration occurred after 4 days of soil drying in mature leaves and withholding watering resulted in more leaf ABA being produced under soil compaction alone. A similar trend was observed in *H. odorata*, in which the ABA concentration in the leaves increased with stress treatments particularly by withholding watering. There was no significant change in the leaf ABA concentration of the control plants for both species throughout the experimental period. The leaf ABA concentration of *M. elengi* seedlings fluctuated throughout the experimental period and for *H. odorata* seedling, a significant ( $P < 0.05$ ) increase of leaf ABA concentration was established on day 5, and this was followed by further decline on day 10. The ABA concentrations in the xylem sap of control plants fluctuated throughout the experimental period though not significantly. However, the ABA concentration in the xylem sap of the stressed plants increased significantly ( $P < 0.001$ ) on day 4 after withholding water relative to the control. The increase in ABA concentration in xylem sap of the two species was more sensitive to stress treatments than that of leaf ABA. However after day 8, the ABA concentration in the xylem sap of *M. elengi* seedlings declined sharply while the xylem sap of *H. odorata* remained almost constant thereafter. Within 12 day of withholding watering, the concentration of ABA in xylem sap under soil compaction and water stress increased three - to seven fold, with variation among species and treatments.

**Relationship between Stomatal Conductance, ABA in the Leaf and the Xylem Sap:** The increase in xylem sap ABA concentration was associated with decline in stomatal conductance of water-stressed and compacted seedlings. When stomatal conductance of water-stress plants and compacted plants was plotted against xylem sap ABA and ABA, stomatal conductance showed a highly significant ( $r^2 = 0.94$  for *M. elengi* and  $r^2 = 0.98$  for *H. odorata*,  $P < 0.001$ ) correlation with the xylem sap ABA, while it exhibited only a low relationship with the bulk leaf ABA ( $r^2 = 0.17$  for *M. elengi* and  $r^2 = 0.03$  for *H. odorata*).

### Conclusions

With soil drying, significant reduction of stomatal conductance occurred much earlier than the reduction of leaf water potential and furthermore, the soil compaction treatment also reduced stomatal conductance. These results suggested that, at least at the initial state of both treatments, stomatal conductance was controlled by something, other than leaf water potential. Further analysis of xylem sap and bulk leaf ABA indicated that there existed a tight relationship between stomatal conductance and xylem ABA concentration in both species but not with the leaf water status or with the concentration of ABA concentration in the leaf. When plants 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 about three-to fivefold, and stomatal conductance was significantly inhibited. Therefore, as in the case of soil drying, xylem ABA could act as a positive stress signal to control the shoot physiology when plants are grown in compacted soil and also control the gas exchange of plants under field conditions. A substantial increase in xylem ABA was observed at high bulk density and accompanied by smaller, non-significant changes in foliar ABA. Mulholland *et al.*, (1996b) reported a subcritical bulk density, which produced large differences in leaf expansion, rates and leaves 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 ex-

pansion at sub critical level of compaction. A similar result was obtained from this study whereby, xylem sap ABA of *M. elengi* was comparatively lower than *H. odorata* seedlings. And this account for its failure to maintain normal growth rate compared to *H. odorata* seedlings.

To conclude, stomatal conductance is very much controlled by ABA concentration in the xylem. These results suggested that ABA has a positive role as a root to shoot signal in maintaining leaf expansion and aiding seedling establishment at this threshold level of soil compaction.

### Benefits from the study

Understanding the importance of ABA in the xylem sap in controlling stomatal conductance and as a growth hormone and aiding seedling establishment at certain level of soil compaction.

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None.

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