

## THE ROLE OF ROOT TO SHOOT SIGNALLING IN COORDINATING RESPONSES TO SOIL COMPACTION

S.L. Aphale<sup>1\*</sup>, T.S. Stokes, C.R. Black, I.B. Taylor and J.A. Roberts

### ABSTRACT

Soil compaction imposes physical and hypoxic stresses on roots, promoting consequent reductions in stomatal conductance and shoot growth. Ethylene and ABA are recognized long distance signals, mediating responses to various stresses including soil drying, flooding and compaction. Wildtype and mutant genotypes of tomato with a reduced capacity to produce ABA or ethylene were grown in a novel growth system in which ballotini beads were used to simulate sub-critical compaction stress. The objective was to establish the role of ethylene and ABA in mediating plant growth under compacted conditions. Their role as long-distance messengers mediating responses to soil compaction at the gene and whole plant level is discussed.

### INTRODUCTION

The pressure which roots must exert to grow through soil is determined largely by soil water content and bulk density; as the soil dries, its resistance to root penetration rises, increasing the stress imposed on the root tip (Passioura, 1988). Soil compaction affects root and shoot growth and may reduce crop yield (Clark *et al.*, 2003). Decreases in stomatal conductance and leaf growth may occur in the absence of any change in foliar water status, suggesting that root-sourced signals may be responsible for eliciting these responses, as occurs when roots are subjected to drought (Davies and Zhang, 1991).

Innovative split-pot systems developed to investigate responses to compaction in sensitive species such as tomato (Mulholland *et al.*, 1996) have shown that reductions in stomatal conductance are correlated with increases in xylem sap ABA concentration (Hussain *et al.*, 1999). In a detailed review of the evidence obtained from split-pot and root excision studies using wildtype and ABA-deficient mutants of tomato, Roberts *et al.* (2002) concluded that root-sourced ABA has a central role in mediating stomatal responses to compaction.

Similar approaches have been used to investigate the role of ethylene or its biosynthetic precursors, particularly 1-aminoacyclopropane-1-carboxylic acid (ACC), in mediating responses to compaction (He *et al.*, 1996). Hussain *et al.* (1999) noted that reductions in leaf growth were closely correlated with increased ethylene evolution from leaf tissue, while Roberts *et al.* (2002) reported that leaf growth was suppressed when wildtype plants were grown on compacted soil, but not in the ACO1<sub>AS</sub> mutant, which has a reduced ability to produce ethylene. This observation suggests a role for ethylene in mediating reductions in leaf growth. The role of crosstalk between ABA and ethylene in mediating compaction responses has also been investigated. In experiments in which wildtype, ethylene-deficient and ACO1<sub>AS</sub> and ABA-deficient mutant *notabilis* genotypes of tomato were grown in a split-pot system, Hussain *et al.* (2000) showed that wild-type levels of ABA restricted the increase in ethylene production which normally occurs when plants experience sub-critical soil compaction.

In the present study, an aerated hydroponic culture system containing ballotini beads placed was used to examine the impact of mechanical impedance to root growth on wildtype (Ailsa Craig) and ABA-deficient mutant (*notabilis*) genotypes of tomato. Effects on stomatal conductance and shoot and root growth were examined in the context of temporal and spatial

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<sup>1</sup>Plant Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LEICS, LE12 5RD, U.K

changes in the expression of genes involved in the biosynthesis of ABA (9'-*cis*-epoxycarotenoid dioxygenase; NCED) and ethylene (ACC synthase; ACO1).

## **MATERIALS AND METHODS**

### **Germination and seedling establishment**

Seeds of Ailsa Craig and *notabilis* were germinated and propagated until 22 days after emergence; they were then transferred to an aerated hydroponic system containing modified Hoagland's nutrient solution. Sub-critical mechanical impedance was simulated by adding glass ballotini beads (2.85 mm diameter) to the nutrient solution. The plants were arranged in a randomised block design; data were analysed using Genstat version 5.

### **Plant growth measurements**

Leaf area and leaf and root dry weights were determined at each harvest. Daily increments in leaf area were calculated from non-destructive measurements of leaf length using an allometric equation; leaf expansion rate was calculated for each treatment by linear regression analysis.

### **Gas exchange measurements**

A portable CIRAS-2 infrared gas analyser (PP Systems, Hitchin, UK) was used to make daily instantaneous gas exchange measurements (stomatal conductance and net photosynthetic rate) for the youngest fully expanded leaf (terminal leaflet of leaf 3).

### **Leaf and root tissue RNA extraction and two step RT-PCR**

At each harvest, leaves and roots from plants grown on nutrient solution containing ballotini (compacted treatment) or without ballotini (uncompacted) were snap-frozen using liquid nitrogen. RNA extracted using the Qiagen RNeasy® plant mini kit was treated with DNase using RQ1 RNase-free DNase (Promega) and cleaned up for downstream RT-PCR using the RNA cleanup protocol described in the Qiagen RNeasy® plant mini kit. First strand synthesis was carried using the ABgene Reverse-i1™ 1<sup>st</sup> strand synthesis kit to generate high yields of full-length cDNA. Oligo-dT primers were used in the RT step and gene-specific primers for NCED and ACO1 for the amplification. 35 cycles of PCR were carried out before analysing a 10 µl aliquot of the RT-PCR reaction medium using agarose gel electrophoresis.

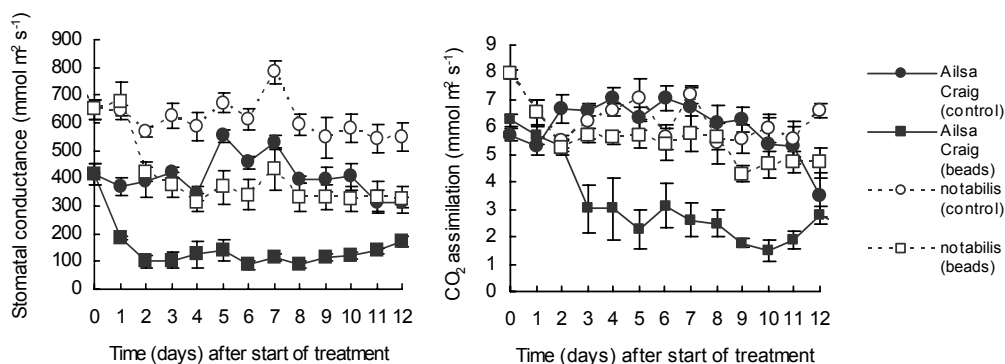
### **Experimental treatments**

Two experiments were carried out. In Experiment 1, the effects of sub-critical compaction on stomatal conductance, leaf area and dry weight were examined for the wildtype Ailsa Craig and ABA-deficient mutant *notabilis* of tomato over a 12 day period. In Experiment 2, changes in the expression of key genes regulating ABA and ethylene biosynthesis were examined by harvesting root and leaf tissue of Ailsa Craig at defined times during the experimental period; these samples were analyzed using RT-PCR to identify effects on gene expression. These were correlated with changes in gas exchange and leaf growth.

## **RESULTS**

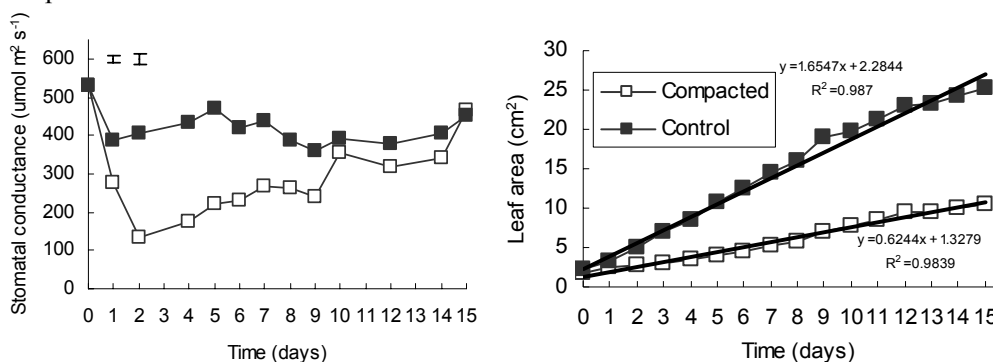
In Experiment 1, stomatal conductance decreased significantly after one day of compaction stress in both the wildtype Ailsa Craig and ABA-deficient *notabilis* (Fig. 1a) relative to uncompacted control plants, although the effect was greater extent in the wildtype ( $P < 0.001$ ). Net CO<sub>2</sub> assimilation (Fig. 1b) declined relative to uncompacted controls in Ailsa Craig ( $P < 0.001$  but not in *notabilis*). Leaf area and dry weight (data not shown) were reduced by

compaction in both genotypes ( $P < 0.001$ , but to a greater extent in the wildtype plants ( $P < 0.001$ )).



**Figure 1.** (a) Stomatal conductance and (b) net CO<sub>2</sub> assimilation rate for Ailsa Craig and *notabilis* plants subjected to control and sub-critical compaction stress. Values are the mean  $\pm$  double standard error for five plants per treatment

In Experiment 2, stomatal conductance declined in the compacted treatment within two days of imposing compaction stress (Fig. 2a; ( $P < 0.001$ )), but recovered to the control level by the end of the experimental period. Leaf expansion rate (Fig. 2b) was greatly reduced within one day of imposing compaction stress ( $P < 0.001$ ), an effect which was maintained throughout the experimental period. RT-PCR analysis (data not shown) revealed that NCED1 expression in the roots increased within one day of imposing stress but decreased again on the following day. Further analysis is required to elucidate changes in ACO1 expression in the leaves and roots.



**Figure 2.** (a) Timecourses of stomatal conductance and (b) leaf area in Ailsa Craig plants subjected to compaction stress. Vertical bars in (a) show standard errors of the difference between means for comparing treatments at specific times and changes with time.

## DISCUSSION

The initial reduction in stomatal conductance induced by compaction was greater in the wildtype Ailsa Craig than in the ABA-deficient mutant *notabilis* (Fig. 1; ( $P < 0.001$ )), probably because its inability to produce wildtype ABA concentrations limits its stomatal responses (Roberts *et al.*, 2002). The reduced stomatal conductance in the wildtype was accompanied by increased NCED1 expression in the roots, suggesting that ABA biosynthesis increased following exposure to compaction. However, this increase might have resulted from handling stress rather than *de novo* synthesis induced by compaction. The observed

stomatal closure in plants exposed to impeded rooting conditions may have resulted from increased root to shoot transport of ABA, or alternatively from redistribution between internal ABA pools (Wilkinson and Davies, 2002).

The observation that the reduction in leaf area induced by compaction was smaller in *notabilis* than in Ailsa Craig suggests that this effect may have resulted from its lower endogenous ABA concentration. By contrast, Roberts *et al.* (2002) reported that the reduction in leaf growth was greater in *notabilis* than in Ailsa Craig and concluded that wildtype ABA levels were necessary to limit the increase in ethylene concentrations in plants grown on compacted soil and prevent the ethylene-induced reduction in leaf growth seen in *notabilis*. Further genetically-based studies are required to elucidate whether differences in ethylene biosynthesis between *notabilis* and Ailsa Craig are causally linked with observed differences in ABA biosynthesis and leaf growth.

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