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Plant responses to environmental change  
with special emphasis on anatomical and  
physiological adaptations of stomata  
across an altitudinal gradient

A thesis submitted for the Degree of Doctor of Philosophy

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## Abstract

The sessile nature of plants requires them to continually adapt to variations in their environment and stomata are vital for this function. This work focused on the response of stomata to environmental gradients, including increasing carbon dioxide concentration ( $[CO_2]$ ) and temperature. The variation in stomatal characters across an altitudinal gradient was of particular interest due to the associated gradients of  $[CO_2]$  and temperature with increasing altitude. *Arabidopsis thaliana* ecotypes originating across an altitudinal gradient and *Camellia sinensis* seedlings were grown under elevated (800ppm) and ambient (400ppm)  $CO_2$  concentrations in controlled environment chambers. In the elevated temperature experiment the growth chambers were maintained at  $28^{\circ}C/18^{\circ}C$  and  $31^{\circ}C/28^{\circ}C$  day/night temperatures for *A. thaliana* and *C. sinensis* respectively while  $21^{\circ}C/18^{\circ}C$  day/night temperatures were maintained in the control chamber. A field based study was conducted on mature bushes of *C. sinensis* cultivars across an altitudinal gradient in Sri Lanka. Stomatal density (SD), epidermal density (ED), guard cell length (GCL), stomatal index (SI) and potential conductance index (PCI) were determined alongside physiological and leaf anatomical traits. A majority of the *A. thaliana* ecotypes increased their SD in response to elevated  $CO_2$ . Stomatal size, measured as GCL, was not as plastic as stomatal numbers in response to both elevated  $CO_2$  and temperature. However, ecotypes with a higher GCL were more responsive to elevated  $CO_2$ , while those with a higher SD were more responsive to elevated temperature. The  $^{13}C:^{12}C$  isotope ratio, as measured by the  $\delta^{13}C_p$  value, of *A. thaliana* ecotypes became more negative (i.e. reduced) in response to elevated temperature showing a decrease in water use efficiency (WUE). A majority of stomatal characters did not show a clear linear response to the altitudinal gradient. The stomatal dimensions of *C. sinensis* were more responsive to  $CO_2$  as the GCL decreased while the SD did not vary with increasing  $[CO_2]$ . However, stomatal conductance decreased by 25%, suggesting an aperture level control. Elevated  $CO_2$  increased the net photosynthetic rate of *C. sinensis* despite a reduction in leaf nitrogen content. Increased leaf thickness probably contributed to this response. Both palisade and spongy parenchyma thickness increased in response to elevated  $CO_2$  and cell expansion was the major contributing factor to this response. The  $10^{\circ}C$  increase in temperature stimulated growth of *C. sinensis* seedlings while  $\delta^{13}C_p$  and WUE decreased. The latter decrease was probably caused by the overall increase in SD and PCI of *C. sinensis* under elevated temperature. The *C. sinensis* cultivars in the field study showed a cultivar based response to altitude in their stomatal numbers and dimensions, though stomatal size was

a more conserved trait. While leaf thickness increased with altitude, the highest palisade:spongy parenchyma ratio (PSR) was shown by the cultivars sampled at the lowest altitude while the estate cultivars showed the lowest PSR at each altitude. The stomatal characters showed a significant relationship with leaf anatomical characters. The present work, which is based on two contrasting plants species, highlighted the complex nature of the internal and external environmental controls that determine stomatal traits of plants.