Upside down, outside in: Extreme drought and the evolution of ‘reverse transpiration’ in Lithops stomata.

Background

CO₂ uptake through stomata (microscopic pores on leaf surfaces) for plant photosynthesis is inextricably linked with water loss through transpiration. For plants in the driest habitats on Earth, this major challenge can cause cessation of photosynthesis and death. Southern African ‘Living Stones’ (Lithops) can partially mitigate this conflict between CO₂ uptake and water loss through crassulacean acid metabolism (CAM). Unlike most plants, CAM plants close their stomata during the day, minimising water loss and allowing them to survive. Lithops’ habitats frequently experience almost no rainfall per year, leading to a key research question – how do Lithops gain enough water from their environment to stay alive? Namibian desert grasses intercept fog and direct dew run-off to the roots [1]. In other drought tolerant plants dew uptake may even by-pass roots, perhaps entering the plant directly as vapour via specialised stomata [2,3]. Lithops above-ground stomata may be specialised toward water- rather than CO₂- capture using a ‘reverse transpiration’ mechanism.

Lithops stomatal distribution suggests this unusual function. Above-ground stomata confined to the face are not associated with photosynthetic tissues, suggesting a limited role in CO₂ uptake (Figures Ai & iii and Bi & ii, adapted from Field et al., 2013). These stomatal “islands” contribute to the variation in Lithops surface patterning (Figure C). Below-ground Lithops stomata (Figure Biii & iv; Bv & vi), however, are associated with photosynthetic tissues. Lithops’ unusual stomatal distribution represents a knowledge gap in drought-tolerant plant physiology, and represents a research area with potentially far-reaching impacts. A recent publication by Field et al. (2013) demonstrates regional specificity of photosynthetic mechanisms in Lithops. However, it does not address the role stomata play in photosynthesis or in water relations. In collaboration with Dr Field (APS), this SURE project will shed new light on the mechanisms and regional specificity of stomatal function in Lithops.

Plants capture over 120 Giga tonnes of atmospheric carbon per year through stomata [4]. CO₂ levels are rising in excess of this [5], increasing the frequency of global drought episodes and threatening global food security. The SURE scheme is the ideal opportunity to explore the unknown mechanisms by which Lithops stomata influence extreme drought tolerance. This research may have important novel applications for future crop improvement.
Hypotheses

H_1. The function of *Lithops* above-ground stomata is for water uptake while below-ground stomata are for CO_2 assimilation.

H_2. *Lithops* face stomata are capable of absorbing moisture from fogs and night dew.

H_3. *Lithops* from environments subject to fogs and night dew have more above-ground islands of stomata than *Lithops* from environments with strong seasonal rainfall. This causes the observed geographical variation and distribution in stomatal patterning of *Lithops* ecotypes.

Aims/objectives

Obj_1. Determine whether *Lithops* face stomata are capable of water uptake (H_1 and H_2)

Obj_2. Investigate whether *Lithops* can absorb air-borne moisture through above-ground stomata (H_2)

Obj_3. Identify climatic correlates of *Lithops* face stomatal ‘islands’ (H_3)

Research Plan

Plant material: Plants (4 species, n = 15 plants) will be collected from the UK National *Lithops* Collection (Matlock, Derbyshire) which has granted permission to use species of known provenance for experiments. Plants will be transferred to a controlled environment chamber (Conviron BDR16, Conviron, Canada) under conditions designed to mimic Namib Desert dry-season (12/12hr day/night, 30/15°C, 10 % RH and 440 ppm CO_2). Plants will be established within chambers three months prior to project commencement to ensure completion within the timeframe.

Stomatal uptake of water: Water uptake will be approximated using dyes (fluorescein diacetate) to determine stomatal absorption in above- and below-ground *Lithops* tissues (n=6 plants) over a time course of 8 hours (Obj_1) and between different *Lithops* species (Obj_2). Four species will be compared: two with face stomata, e.g. *L. localis*, and two without, e.g. *L. aucampiae*. Excised tissue will be observed using fluorescence microscopy.

Water vapour absorption through stomata (Obj_2): Half of the experimental plants (n = 6) will be exposed to a nightly fog treatment, whereas supplemental water will be withheld from the other plants beyond ambient humidity control, and masses measured daily. ‘Drought’ will be imposed one month before project commencement.

Variation of stomatal patterning among species according to local climate (Obj_3): Pattern analysis of face, above-ground, and below-ground surfaces of species from the National *Lithops* collection (n = 6) will be performed using image analysis software (Image j). Regional climatic data will be obtained from resources including the South African Department of Water Affairs and Forestry (http://www.dwaf.gov.za/bi/), and key variables (e.g. precipitation, cloud cover) will be correlated with the pattern analyses.

Research Timetable

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