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Photosynthetic characteristics and the response of stomata to environmental determinants and ABA in *Selaginella bryopteris*, a resurrection spike moss species

Devendra K. Soni [a], Sanjay Ranjan [a], Ruchi Singh [a], Prem B. Khare [b], Uday V. Pathre [a], Pramod A. Shirke [a,*]

[a] *Plant Physiology Division, CSIR[1]-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, India*
[b] *Pteridology Division, CSIR[1]-National Botanical Research Institute, Lucknow, India*

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ABSTRACT

Selaginella bryopteris is a spike-moss lycophyte species with resurrection capability. These plants have small sized stomata that occur in higher density than in other fern species. The diurnal gas-exchange studies under natural conditions showed a bell shaped net photosynthesis curve. The effective quantum yield of PSII ($\Delta F/F_{m'}$) showed an inverse relationship with light and recovered to its maximum at sunset. This suggests that there was a complete recovery of PSII efficiency during the late evening hours. *S. bryopteris* displayed broad temperature optima for net photosynthesis from 28 °C to 37 °C. The stomatal sensitivity in response to vapor pressure deficit (*VPD*), was maximum at 25 °C temperature while at temperatures from 30 to 35 °C it was low. Our study demonstrates that *S. bryopteris* plants show a very poor mechanism for its stomatal regulation in response to high light, high temperature, high *VPD*, high CO_2 and to ABA treatment. At the same time they show a high stomatal conductance leading to unrestricted rates of transpiration and a lack of capacity to optimize water use efficiency (*WUE*).

1. Introduction

Selaginella bryopteris is a known resurrection, perennial spike-moss plant. It grows in shallow soils on rocky out crops of slopes of small hills under direct sunlight in humid tropical regions. *S. bryopteris* is periodically exposed to harsh conditions of high light and temperatures, and consequently stronger evapotranspiration and water loss. During summer *S. bryopteris* undergo extreme desiccation, the fronds curl and turn brownish and remain quiescent until the onset of monsoon [1,2]. Recently the genus *Selaginella* has attracted a lot of attention from the evolution point of view. Banks and Nishiyama [3] have reported the genome sequence of *Selaginella moellendorffii*, the first for a nonseed vascular plant. Brodribb and McAdam [4] showed that the complexity that characterizes stomatal control in seed plants is absent in early-diverging vascular plant lineages. They observed that lycophytes, including *Selaginella kraussiana* and ferns have stomata lacking pore closure response to abscisic acid (ABA). Hanada et al. [5] have observed presence of genes related to ABA metabolism in *S. moellendorffii*, however, they are unclear of the physiological function of these ABA-related genes. Ruszala et al. [6] reported that stomatal response of *Selaginella uncinata* to ABA and CO_2 are directly comparable to the seed plant *Arabidopsis*. However, very little information is available on the physiology of *S. bryopteris*.

The genus *Selaginella* is a prominent member of the lycophytes and is one of the oldest genera of vascular plants and includes some 750 species occurring mainly in tropical zones [7]. Phylogenetic re-constructions indicate that the lycophytes are the sister group to the ferns and the seed plants [3,8]. *Selaginella* species have small leaves or microphylls with a single unbranched vascular bundle and show considerable variation in the numbers, pattern and distribution of their stomata [9–11]. Stomata first appeared in

Abbreviations: $\Delta F/F_m$, effective quantum yield of PSII [$\Delta F/F_{m'} = (F_{m'} - F_s)/F_{m'}$; *A*, net photosynthesis; A_{LCP}, light compensation point for net photosynthesis; A_{LSP}, light saturation point for net photosynthesis; A_{max}, maximum net photosynthesis rate; *AQE*, apparent quantum use efficiency; *CE*, carboxylation efficiency; C_i, leaf intercellular CO_2 concentration; *E*, transpiration; *ETR*, apparent electron transport rates through PSII [$ETR = \Delta F/F_{m'} \times PPFD \times 0.5 \times 0.84$, where by 0.5 is the division factor as absorbed *PPFD* is equally distributed between PSII and PSI and 0.84 is the *PPFD* absorption factor]; F_0, minimal fluorescence emission of a dark adapted plant with primary quinone acceptor Q_A, oxidized and non-photochemical quenching inactive; F_m, maximal fluorescence emission of a dark adapted plant exposed to a short pulse of a strong light leading to a transient reduction of Q_A without induction of non-photochemical quenching; $F_{m'}$, maximum fluorescence in light adapted state; F_s, steady state fluorescence; F_v/F_m, maximum quantum yield of PSII photochemistry; F_v, variable fluorescence ($F_v = F_m - F_0$); g_s, stomatal conductance; J_{max}, maximum rate of electron transport driving RuBP regeneration; *NPQ*, non-photochemical quenching [$NPQ = (F_m - F_{m'})/F_{m'}$]; *PPFD*, photosynthetic photon flux density; PS, photosystem; q_P, photochemical quenching [$q_P = (F_{m'} - F_s)/(F_{m'} - F_{0'})$]; R_d, rate of respiration in dark; *TPU*, triose phosphate utilization; $V_{c,max}$, maximum rate of RuBP carboxylation; *VPD*, vapor pressure deficit; *WUE*, water use efficiency; φ, index of stomatal sensitivity; Γ, CO_2 compensation point in presence of mitochondrial respiration.

* Corresponding author. Tel.: +91 522 2297928; fax: +91 522 2205847.
E-mail address: pashirke@nbri.res.in (P.A. Shirke).
[1] Council of Scientific and Industrial Research.

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terrestrial plants about 400 million years ago as a possible adaptation to new and variable environments [12]. The stomata play a central role in the pathways for both the loss of water from plants and the exchange of CO_2. Consequently both processes, transpiration (E) and net photosynthesis (A) are essential for plants. Transpiration is required for nutrient absorption as well as maintaining the temperature of plants while photosynthesis assimilates CO_2 into photosynthates. When water availability for plants is scarce, stomata will close to minimize the water loss. The closure of stomata reduces transpiration and conserves water but restricts CO_2 exchange, adversely affecting photosynthesis. Through evolution plants have developed sophisticated strategies and mechanisms to achieve a compromise between photosynthesis and transpiration. Among these, stomatal behavior, frequency, and size play central roles in controlling water and CO_2 fluxes and thus stomata are one of the key factors affecting water use efficiency (*WUE*) of plants [13,14].

Under natural conditions major environmental factors that influence the stomatal regulation include light, temperature and vapor pressure deficit (*VPD*) [15]. It has been shown in several plants that there is a reduction in steady-state stomatal conductance (g_s) with an increase in *VPD* [16,17]. This is interpreted as a means by which plants can reduce water loss [15,18]. However, recent researches on stomata in the early-diverging vascular plant lineages have shown that the stomata of these plants have weak or no response to increased CO_2 concentration in both light and dark [19,20], blue light [21], low light [22], or the phytohormone, ABA [4]. Therefore it was of interest to study the stomatal response of *S. bryopteris* to the environmental determinants, light, temperature, *VPD* and ABA, which are known to regulate stomatal closure. The influence of irradiance, temperature and *VPD* on the photosynthetic performance of *S. bryopteris* an ecologically important species were studied under simulated conditions. Diurnal studies were performed under natural environmental conditions during optimum growth phase of the plants from July to September [2].

2. Materials and methods

S. bryopteris (L.) Bak. plants (Fig. 1A) were collected from Mirzapur district, Uttar Pradesh, India: latitude 23°52′–25°32′ N and longitude 82°7′–83°33′ E and transferred to 0.5 L pots containing a mixture of garden soil and neopeat planting material. The plants were maintained at Institute's fern house under natural sunlight (>1000 μmol m^{-2} s^{-1} photosynthetic photon flux density, *PPFD*) and humid conditions (approximately 60–70% during the peak *PPFD* hours). The potted plants were shifted in the open with the onset of monsoon in early July and subjected to the ambient environmental conditions. The plants showed a healthy growth during the monsoon months (July–September). All the experiments were carried out during these months at our Institute at Lucknow. Plants were irrigated regularly with tap water and supplemented with Hoagland nutrient solution.

2.1. Microscopy studies

The fronds of *S. bryopteris* were rinsed with 80% ethyl alcohol for 3 h, to partially remove the chlorophyll and to increase the visibility of the stomata. The microphylls were detached from the fronds and mounted in glycerol and examined with Leica DM 500 microscope, mounted with Leica EC-3 Camera (Leica Microsystems Ltd., Heerbrugg, Switzerland). Stomatal dimensions were measured using a 40× objective lens and 10× eyepiece (total magnification 400-fold). The guard cell length, width and pore length were measured according to Lawson et al. [23] with a precalibrated ocular micrometer, in at least 20 stomata of the central and lateral microphylls. The stomatal frequency was measured in an area of 0.166 mm^2 and 50 such measurements were made in central and lateral microphylls from fronds of different plants.

2.2. Measurements of gas exchange and fluorescence parameters

The gas exchange and chlorophyll '*a*' fluorescence parameters were measured diurnally on clear and different days under natural conditions with an open infrared portable gas-exchange fluorescence system (GFS-3000; Heinz Walz GmbH, Germany) equipped with a clear top cuvette, "Standard Measuring Head 3010-S" with "Leaf Area Adapter 3010-2 × 4" and "PAM-Fluorometer 3050-F" fiberoptics probe, under ambient temperature, *VPD*, and *PPFD*. During gas exchange measurements the flow rate of air through the cuvette was maintained such that conditions in the cuvette approximated ambient CO_2 (which ranged between 375 and 385 μmol mol^{-1}).

The various chlorophyll fluorescence parameters of the effective quantum yield of PSII ($\Delta F/F_{m'}$), apparent electron transport rates through PSII (*ETR*) photochemical quenching (q_P) and non-photochemical quenching (*NPQ*) and the maximum quantum yield of photosystem II (PSII) F_v/F_m were calculated as mentioned by Maxwell and Johnson [24].

The gas exchange studies of influence of *PPFD*, temperature, *VPD* and ABA treatment on the photosynthetic performance of *S. bryopteris* and AC_i studies were carried out inside the laboratory using the GFS-3000 system attached with the "LED-Array/

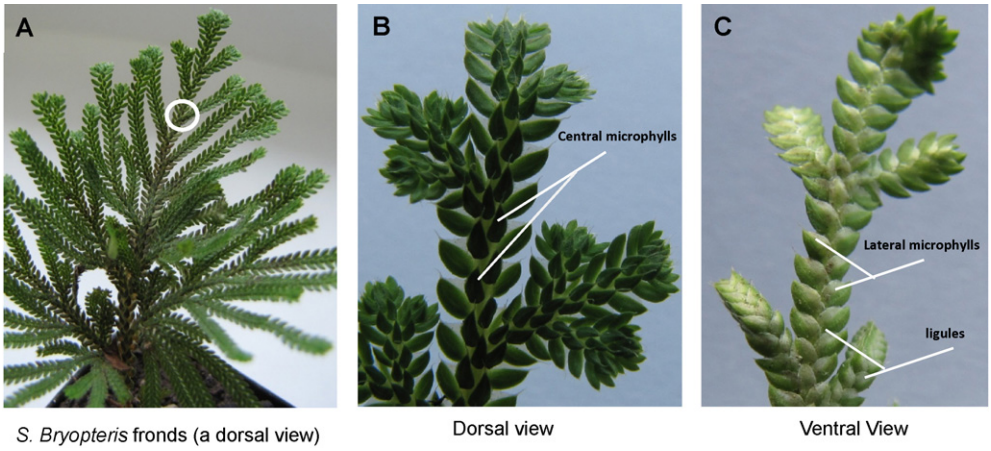

Fig. 1. Fronds of *S. bryopteris* growing in a pot (A). Close dorsal view of the frond showing the alternate arrangement of the microphylls on the axis (B). Ventral view of the frond showing the lateral microphylls and the transparent ligule at its base (C).