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Evaluating guard cell photosynthesis in intact green leaves using chlorophyll fluorescence imaging

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Introduction

Stomatal aperture determines plant gas exchange and therefore influences water loss and overall plant productivity. The opening and closure of stomata are brought about through changes in turgor within the guard and subsidiary cells. These change in turgor requires energy for the net accumulation or loss of K+ ions and the parallel loss or accumulation of organic solutes, such as sucrose and malate (e.g. Willmer and Fricker, 1996). In most species guard cells are the only cells to have chloroplasts in the epidermis. These tend to be smaller, less numerous and have fewer grana than the adjacent mesophyll chloroplasts (Willmer and Fricker, 1996). Previous fluorescence studies have established the existence of both PSI and PSII associated with linear electron transport, along with photophosphorylation. Photophosphorylation, on a chlorophyll basis, has been reported to be as high as 80 % of that of the mesophyll cells (Shimazaki and Zeiger, 1985). Although guard cell chloroplasts have between 25 -100 fold lower chlorophyll compared with mesophyll cells, they are also considerably (10 fold) smaller, so their chloroplasts could represent a significant energy source for ion transport. There is still considerable debate regarding the role of the Calvin cycle for CO_2 fixation in the guard cells with respect to stomatal movements (Outlaw, 1989; Outlaw, 1996), however, rubisco could function as part of an environmental sensing system (Outlaw, 1989; Goh et al, 1999). Previous measurements of chlorophyll fluorescence from guard cells have been largely restricted to epidermal peels (e.g. Melis and Zeiger, 1982), or guard cell protoplasts (Goh *et al.* 1999). Cardon and Berry (1992) were the first to study guard cells in intact tissue, however, they were only able to compare changes in the steady-state fluorescence signal (*F'*) from guard cell chloroplasts located within white regions of variegated leaves of *Tradescantia* with those from mesophyll chloroplasts within green regions of the same leaves. Although guard cells from the white regions of leaves are capable of opening, they are obviously not subject to the same influence of mesophyll cell activity as in green areas (e.g. Scarth and Shaw, 1951).

In this study a unique, high resolution chlorophyll *a* fluorescence imaging system is used to determine photosynthetic performance from guard cell chloroplasts in intact green leaves separately from that of the underlying mesophyll. The responses of mesophyll and guard cell PSII efficiency, estimated from *Fq'/Fm'* to changes in PPFD, [CO_2], [O_2] and humidity were determined in parallel with stomatal aperture.

Materials and methods

Plant material

Seeds or cuttings of a number of species were grown in a peat and loam based compost (F2, Levington, Horticulture Ltd, Ipswich, UK) either in a controlled environment chamber (SGC066, Fitotron, Sanyo Gallenkamp, Leicester, UK), or in the glasshouse during summer. Plant were used after 6-7 weeks. The chamber air temperature was maintained at 18°C at night, and 22°C through the day. Light was provided by halogen quartz iodide lamps (Neutralweiss, Germany) from 0600 - 2100 h, at a constant PPFD of 530 µmol m^{-2} s^{-1}. Relative humidity was maintained at 70% through the day and 65% at night. All plants were kept well-watered using capillary matting.

The imaging system

The optical part of the instrument used in these experiments is the same as that described previously (Baker et al., 2001). Chlorophyll fluorescence was defined by using a 680 nm bandpass filter (Coherent, Watford, UK). The filter was located within a filter wheel, between the camera and microscope, along with the 630 nm shortpass filter used for reflected light images (Oxborough and Baker, 1997a). A purpose designed microscope cuvette attached to a portable photosynthesis system (CIRAS2, PP Systems, Hitchin, Herts., UK) was used to control [CO_2] and VPD. All images were taken from the abaxial surface of leaves using a 40 x objective. Chloroplasts within guard cell pairs were isolated from images using the ends-in search and other editing tools (e.g. Fig. 1) described in Oxborough and Baker (1997a). The fluorescence terminology used here is described in greater detail by Baker et al. (2001). Briefly, the fluorescence parameter *Fq'/Fm'* provides an estimate of the quantum efficiency of PS II photochemistry. This parameter is the product of , *Fv'/Fm'* and *Fq'/Fv'*. *Fv'/Fm'* provides an estimate of the maximum quantum efficiency of PS II photochemistry, i.e. PS II operating efficiency when all centers are in the open state at the point of measurement, therefore its value is largely regulated through down-regulation and non-photochemical quenching. *Fq'/Fv'* relates the PS II maximum efficiency to the PS II operating efficiency, and is non-linearly related to the proportion of PS II open centers and therefore indicates the level of photochemical quenching of chlorophyll fluorescence. Images of *Fv'/Fm'* and *Fq'/Fv'* were generated from images of *Fo*, *Fm* and *Fm'*. This is achieved through production of a virtual image of *Fo'* (in computer memory), calculated as described previously (Oxborough and Baker, 1997b). Stomatal movements between the dark and light-adapted states often make it difficult to match up the locations of chloroplasts within all three required images. Although this can prevent the generation of images of *Fv'/Fm'* and *Fq'/Fv'*, it is still possible to generate mean values of *Fo*, *Fm* and *Fm'* for the guard cell chloroplasts within each image, which can

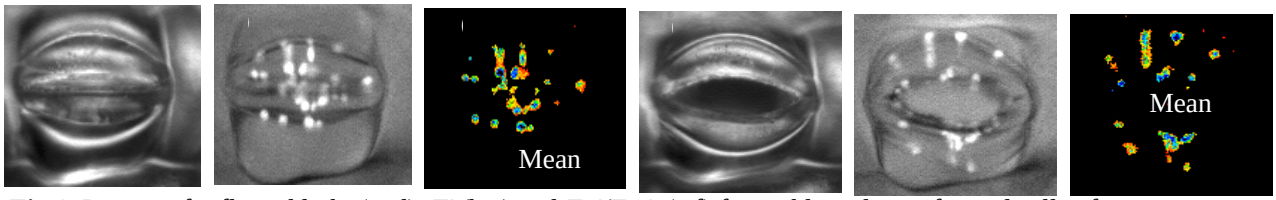

Fig.1. Images of reflected light (a, d), *F'* (b,e) and *Fq'/Fm'* (e,f) from chloroplasts of guard cells of an attached leaf of *Commelina communis*.

then be used in the calculation of mean values of *Fv'/Fm'* and *Fq'/Fv'*.

Results

Responses to changing PPFD

Changes in *Fq'/Fm'* with increasing PPFD were calculated from the images and were similar for both guard and mesophyll cells (Fig. 2). These data show significantly lower values of *Fq'/Fm'* in guard cells within both green and white areas of leaves, compared with mesophyll cells, at PPFDs above 20 µmol m^{-2} s^{-1}. Values of *Fq'/Fm'* from guard cell chloroplasts in the white area were lower than from guard cell chloroplasts in the green area at PPFDs between 46 µmol m^{-2} s^{-1} and 446 µmol m^{-2} $s^{-1.}$ The lower values of *Fq'/Fm'* from guard cell chloroplasts in the white area appeared to result from lower values of *Fv'/Fm'* (data not shown), suggesting a higher level of down regulation in these cells. The differences in *Fq'/Fm'* between the mesophyll and guard cell chloroplasts in green areas were due primarily to higher values of *Fq'/Fv'* within the mesophyll cells (data not shown). *Fq'/Fm'* of guard cells in green areas was always lower that for mesophyll cells at any given PPFD. There was a linear relationship between *Fq'/Fm'* for guard and mesophyll cells over the PPFD range (data not shown), suggesting that the response of the photosynthetic apparatus to changing PPFD was similar in both cell types.

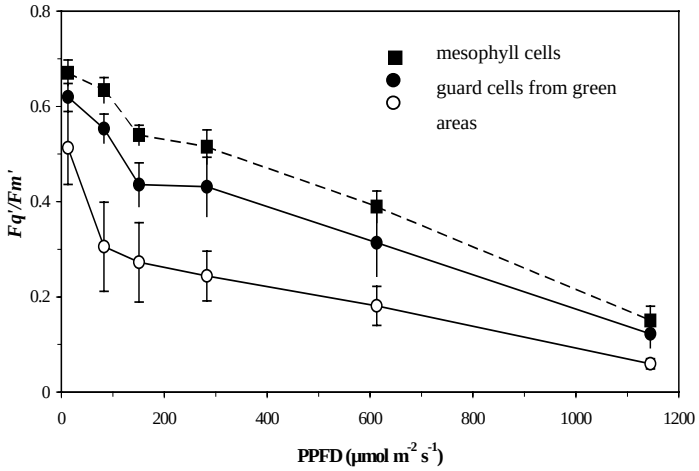

Fig.2. Responses of the fluorescence parameters F_q'/F_m' of a variegated tradescantia leaf to increasing PPFD. Measurements were made at 25°C and a C_a of 360 µmol mol^{-1}. Data are the means of 6 replicates ± SE.

Responses to changing CO_2 concentrations

The response of the guard and mesophyll cells to changes in ambient CO_2 concentration were studied in commelina (Fig.3). *Fq'/Fm'* increased with Ca up to approximately 350 µmol mol^{-1}, after which the response curves flattened out in both the guard and the mesophyll cells.

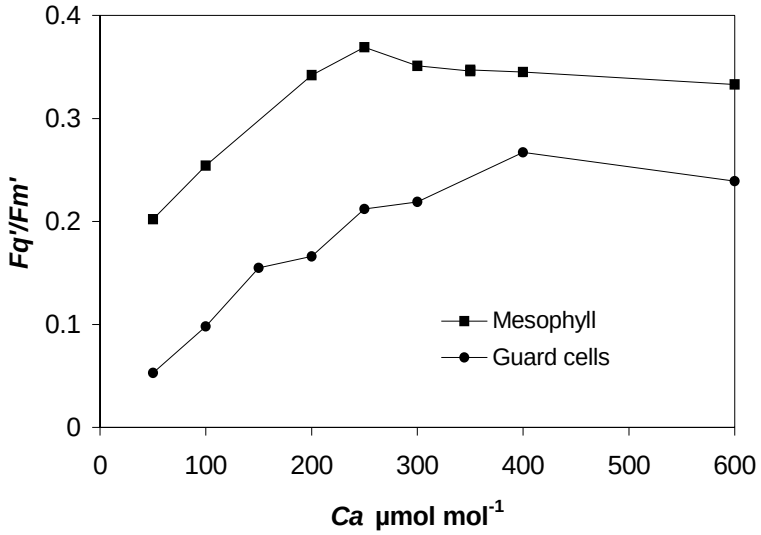

Fig.3. Response of F_q'/F_m' of mesophyll and guard cells to increasing C_a in the green areas of a commelina leaf. Measurements were made at a PPFD of 275 µmol m^{-2} s^{-1} and an ambient temperature of 25°C.

Fq'/Fm' of guard cells was always lower than that from the underlying mesophyll cells over the Ca range. However, these data strongly suggest that the same photosynthetic mechanism exists in both cells types.

Effect of O_2 concentration

We investigated the effects of varying concentration of O_2 at high and low CO_2 concentrations. *Fq'/Fm'* in both guard and mesophyll cells responded to changes in $[CO_2]$ and $[O_2]$ in both *Polypodium* and *Vicia* (Fig. 4), and a number of other C3 species studied (data not shown). However, in *Amaranthus* a C4 species, no mesophyll response to varying O_2 concentrations was observed, however the guard cells behavior similar to the C3 species. The decrease in *Fq'/Fm'* was largely due to decrease in *Fq'/Fv'* ('photochemical') rather than in *Fv'/Fm'* ('non-photochemical') (data not shown). *Fq'/Fm'* of both cell types was lower at 2% O_2, compared to 21% O_2, at a Ca of 150 µmol mol^{-1}. As Ca increased, the difference in *Fq'/Fm'* at the two O_2 concentrations decreased, and became close to zero above 350 µmol mol^{-1}.

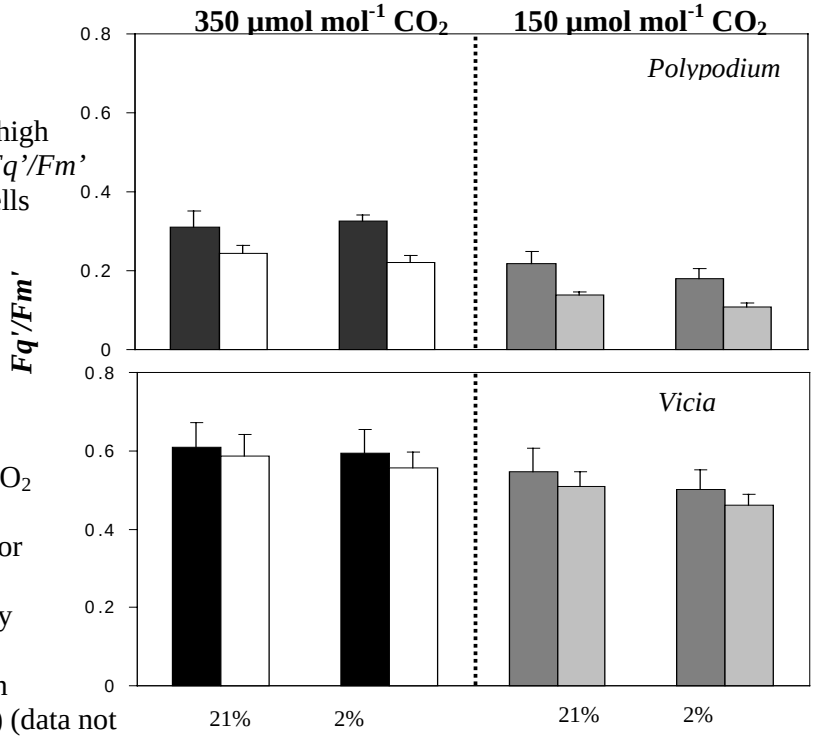

Fig.4. The effect of CO_2 and O_2 on chlorophyll fluorescence in mesophyll (□, ▒) and guard cells (■ , ▒)

Responses of stomata and Fq'/Fm' to changing humidity

The effects of changing VPD on stomatal aperture and the corresponding change in *Fq'/Fm'* of both mesophyll and guard cells in a tradescantia leaf at a Ca of 80 µmol mol^{-1} is shown in Fig. 5. Stomatal closure was stimulated by rapidly decreasing the humidity in the leaf cuvette so that VPD increased from ca. 1.0-1.5 to 3.0-3.5 kPa. The initial effect of the increase in VPD was a hydropassive opening of the stomata over ca. 10 min, due to changes in the balance between epidermal and guard cell turgor pressures (see Willmer and Fricker, 1996). The initial increase in stomatal aperture was followed by a slower decrease (over 1 h), which was accompanied by a decrease in *Fq'/Fm'*. When VPD was decreased to the original level, both stomatal aperture and *Fq'/Fm'* increased to within a few percent of their original values (over a period of ca.1- 2h). When such experiments were repeated at a higher Ca of 200 µmol mol^{-1}, the same increase in the VPD induced a similar stomatal response, but with little effect on *Fq'/Fm'*.

Discussion

This is one of the first studies to show the effect of changing environmental conditions on PS II photochemical efficiency (measured by *Fq'/Fm'*) within guard cells and mesophyll cells of intact, green leaves. From the differences shown by guard cells from white areas of tissue in the PPFD response curve (Fig.2) we suggest that the responses of the guard cells in white areas of leaf cannot be considered to be indicative of the responses of those in green tissues. We observed no difference in dark adapted *Fv/Fm* of guard and mesophyll cells, which is consistent with Goh *et al*. (1999) who worked with guard cell protoplasts of *Vicia* and *Arabidopsis*. However, in low light we

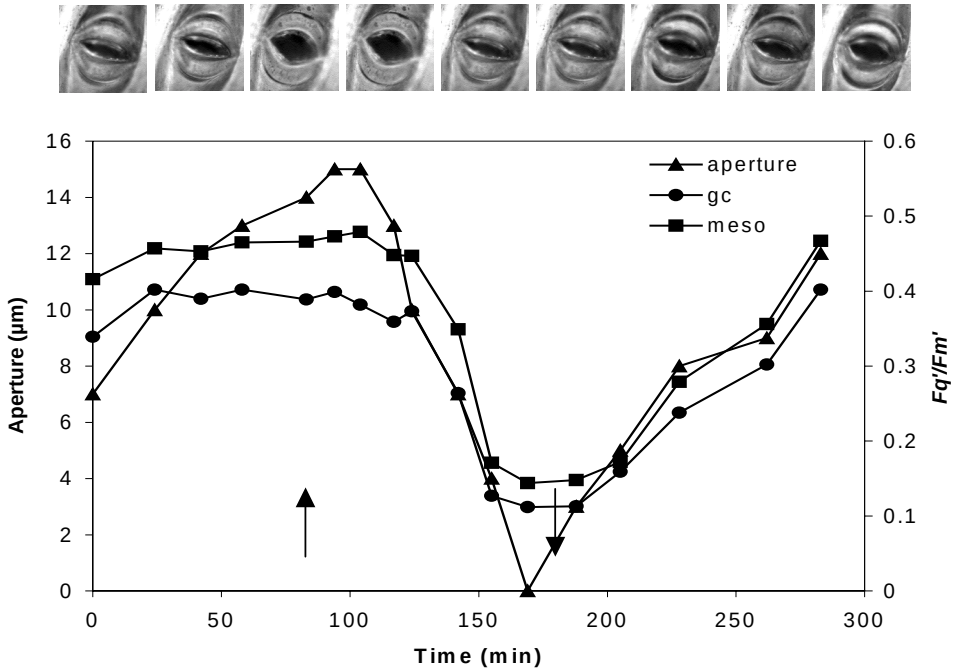

Figure 5. The effects of changes in VPD on stomatal aperture and F_q'/F_m' from mesophyll and guard cells in a commelina leaf at a C_a of 180 µmol mol^{-1}. Stomatal aperture was determined from reflected light images. Measurements were made at a PPFD of 265 µmol m^{-2} s^{-1} and an ambient temperature of 25°C. ↑indicate the time at when VPD was increased from 1.0-1.5 kPa to 3.0-3.5 kPa; ↓ indicate the time when VPD was decreased back to 1.0-1.5 kP

found a decrease in *Fq'/Fm'* in guard and mesophyll cells which corresponded to a decrease in *Fv'/Fm'* which supports Goh et al. (1999) who suggested substantial light induced non-photochemical quenching in guard cell protoplasts. Such an increase in non-photochemical quenching would indicate that some zeaxanthin is maintained in the dark, which has also been suggested by Frechilla *et al*. (1999). *Fq'/Fm'* was consistently lower in guard cell chloroplasts than underlying mesophyll in intact green tissue, suggesting that guard cell photosynthetic electron transport rates per unit chlorophyll are likely to be between 70 - 80% of that in the mesophyll. We suggest that even though guard cells are somewhat less photosynthetically efficient than mesophyll cells, the guard cell chloroplasts are clearly capable of producing ATP and NADPH, which may (Cardon and Berry, 1992) or may not (Outlaw, 1989) be sufficient for carbon fixation. We have demonstrated a very clear co-dependence of F_q'/F_m' in guard cells on CO_2 and O_2 concentration (Fig. 3 & 4). These results are strong evidence for oxygenase activity and indicate that rubisco in guard cells is a sink for a similar proportion of the products of photosynthetic electron transport as it is in the mesophyll. The effect of stomatal behaviour on changes in *Fq'/Fm'* shown in Fig. 5 indicates that *Fq'/Fm'* of both the

mesophyll and guard cells is strongly affected by CO_2 supply through the stomatal aperture. When a higher Ca concentration was used, no decrease in *Fq'/Fm'* was observed with a decreasing aperture (data not shown). Similarly, when the internal CO_2 concentration was changed by manipulation of Ca changes in *Fq'/Fm'* were observed (Fig. 3). Fig.1 also shows the effect of restricted CO_2 diffusion through open and closed stomata, as the isolation of *Fq'/Fm'* in guard cells gave the lowest value for the closed stoma. The simplest conclusion is that the change in guard cell *Fq'/Fm'* as aperture changed was not due to any change in guard cell metabolism but solely due to the change in CO_2 diffusion through the stomatal pore, as there was no change in *Fq'/Fm'* at high Ca even though aperture changes were as large as those observed at low Ca..

Conclusion

This work has shown that guard cells have a 20-30% lower photosynthetic efficiency than mesophyll cells. The photosynthetic efficiencies of these two cell types exhibited similar responses to changes in light, CO_2 and O_2. We infer that photosynthetic electron transport rate (on a chlorophyll basis) in guard cell chloroplasts are of similar magnitude to those in mesophyll cells, and that the Calvin cycle and rubisco are active in guard cells of the species examined, which include the C3 species *Tradescantia albiflora, Vicia faba, Nicotiana tabacum, Polypodium vulgare* and *Commelina communis* and the C4 species *Amaranthus caudatus*.

Acknowledgements

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