The Use of Imaging of the Efficiency of Photosystem II Electron Transport to Visualise the Effect of Dry Storage on the Photosynthesis and Stomatal Closure of Cut Rose Stems

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Abstract
Following their harvest, cut roses are generally stored dry prior to and during transport and only rewetted once they near the end of the chain. This treatment results in overall dehydration of the rose shoots and to the development of emboli within the xylem of the stems. A major consequence of this dehydration event will be stomatal closure as a result of the water stress that develops in the leaves. In addition to reducing water loss from the leaves, stomatal closure will also have major effects on leaf photosynthesis. Quantitative chlorophyll fluorescence imaging of leaves (or any other photosynthetic tissue) permits the visualisation of how efficiently light is being used to drive photosynthetic electron transport. Stomatal closure affects photosynthesis and thus photosynthetic electron transport. So, chlorophyll fluorescence imaging can be used to visualise the responses of leaves to the water stress imposed by cutting and the relief of water stress by rewetting.

Results show that the degree of recovery of stomatal opening is generally only partial and that in addition to a persistent limitation of stomatal opening, there is an effect on photosynthetic electron transport due to processes acting at the level of the mesophyll. The results obtained illustrate the usefulness of chlorophyll fluorescence imaging to rapidly and effectively visualise and measure the effect of water stress on cut flowers and to quantify their recovery from this stress.

INTRODUCTION

In common with all other cut flowers, cut rose stems usually develop reduced relative water contents and water potentials, leading to water stress in the post-harvest phase. This is due to the combined effects of being severed from the vascular system of the parent plant on the one hand, and the vapour-pressure deficit between the leaf’s internal gaseous phase and the external atmosphere on the other. The consequences of these changes in water status are various, with some, notably gas emboli in the xylem, being strongly associated with serious loss of post-harvest quality. The ability to detect the development and distribution of water stress and its alleviation by post-harvest treatments and procedures is necessary if water-stress induced loss of quality is to be better understood, detected and managed. To date, the techniques available for the detection of water-stress induced post-harvest problems are largely focussed upon the measurement of water uptake and transpiration by the stem as a whole. Though these techniques have proved their usefulness over many years and are simple to apply, they suffer from some drawbacks; as commonly applied they offer no means to describe spatial variability of the stress or its alleviation, they are relatively time-consuming in application, and they require climate-controlled rooms for their quantitative application. A significant consequence of water stress, and one that is intimately related to the control of transpiration, is an increase in stomatal resistance, leading ultimately to stomatal closure. This response has consequences for the processes of photosynthesis which offer a means of imaging stomatal closure and thus water stress. Using chlorophyll fluorescence it is possible to measure the quantum efficiency of light-use by photosystem II electron transport ($\Phi_{\text{psII}}$), which is also the quantum efficiency of linear photosynthetic electron transport (Genty
and Harbinson, 1996). It is possible to produce images of $\Phi_{ PSII}$, which should in principle allow images of stomatal resistance to be made (Genty and Meyer, 1994). The application of this technique requires, however, a detailed understanding of the relationship between linear photosynthetic electron transport and photosynthetic metabolism.

**MATERIALS AND METHODS**

**Principles Underlying the Measurement Procedure**

As a process, photosynthesis is based upon the coupling of photosynthetic electron transport with numerous metabolic processes. Of these, it is the processing of the products of the carboxylation of RuBP by CO$_2$ (the first step of CO$_2$ fixation) or of its oxygenation by O$_2$ (the first step of photorespiration) that are the most significant metabolic processes for photosynthetic tissues in air (Genty and Harbinson, 1996). The coupling of photosynthetic metabolism with photosynthetic electron transport is both intricate and incompletely understood, but it is clear that it is dependent upon the operating point of several fundamental photosynthetic processes. The hydrogen ion potential difference that develops across the thylakoid membrane, which provides the driving force for ATP synthesis, is comprised of both a transthylakoid voltage and proton concentration difference. The proton concentration difference is largely generated by a decrease in the intrathylakoid pH, and decreases in the intrathylakoid pH will restrict linear electron transport. The magnitude of the intrathylakoid pH is dependent on the ATP/ADP.Pi ratio, which is dependent upon the demand for ATP by photosynthetic metabolism. Additionally, the redox state of the stroma, a reflection of the ratio of reduced to oxidised ferredoxin, or the the NADPH/NADP ratio, also appears to be involved in the regulation of linear transport. The stromal and intrathylakoid pH changes associated with the development of the $\Delta \mu_{H^+}$ are also important in the activation of enzymes in the stroma associated with photosynthetic metabolism. The combined roles of carboxylation and photorespiration in regenerating ADP, Pi, and oxidised ferredoxin has major implications for the response of photosynthetic electron transport to partial or complete stomatal closure. Though increases in stomatal resistance will produce a decrease in the intercellular CO$_2$ concentration and thus a decrease in the rate of carboxylation, this will not produce a proportional response in the rate of electron transport. This is because the normal atmospheric O$_2$ concentration of 20% results in an intercellular O$_2$ concentration that is not significantly affected by stomatal closure and the continued activity of photorespiration also supports electron transport. This interference from photorespiration can be almost completely eliminated by placing the photosynthetic tissue (normally a leaf) in an atmosphere with an O$_2$ concentration of 2% or less (Genty et al., 1990). Thus, a measurement of $\Phi_{ PSII}$ made in a low CO$_2$ atmosphere may be used to reveal areas of closed stomata because of the effect that these will have on the movement of CO$_2$ into the leaf and because under low CO$_2$ conditions the potentially complicating effects of photorespiration are removed.

One possible problem with the use of $\Phi_{ PSII}$ measurements used in conjunction with low O$_2$ atmospheres is the possible down-regulation of photosynthetic activity, and thus electron transport chain activity and $\Phi_{ PSII}$, that can occur in leaves subjected to non-photorespiratory conditions. This down-regulation is most likely due to a feedback limitation, or product inhibition of photosynthesis due to the potentially large increase in the rate of CO$_2$ fixation produced under the nonphotorespiratory conditions (Harbinson, 1994). A reduction of $\Phi_{ PSII}$ due to this biochemically mediated regulatory process could easily be mistaken for a limitation due to a stomatal limitation. These two conditions may be distinguished by exposing the leaf to a CO$_2$ concentration sufficiently high to overcome any effect of stomatal limitation or closure, while using to low O$_2$ concentration to prevent any photorespiration. By this means the effect of stomatal restriction of photosynthesis and thus $\Phi_{ PSII}$ can be distinguished from biochemical restrictions of photosynthesis.

In this article we shall illustrate the use of images of $\Phi_{ PSII}$, made by means of
chlorophyll fluorescence, in conjunction with changes in the composition of the gaseous phase, to map stomatal closure over the surface of rose leaves. The stems to which these leaves are attached will be subjected to drought treatment and then rewetted.

Treatment of the Rose Stems

Single stems of Rosa hybrida 'First Red' were obtained from a local grower. The stems were harvested at the normal stage for commercial harvesting, and immediately placed in ice-cold water prior to transport to the laboratory. There, the lowest 10 cm of the stems were removed under water, and the stems placed in tap-water and stored in a cold-room at 4 °C. The terminal leaflet of the youngest mature leaf was used for the measurements of \( \Phi_{\text{PSII}} \).

For the production of images of \( \Phi_{\text{PSII}} \), the leaflet was enclosed in a gas-tight cuvette that was placed in the imaging area of a commercial imaging fluorimeter (Fluorocam, Photon Systems International, Brno, Czech Republic). This instrument permitted the exposure of the leaf to a constant irradiance during which images of \( \Phi_{\text{PSII}} \) could be made. During the imaging process the leaflet was exposed to an actinic irradiance of 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and allowed to reach steady-state efficiency by maintaining the leaf at this irradiance for 20 min in an atmosphere of normal air. That the leaf had in fact reached steady-state was verified by using a measuring protocol for \( \Phi_{\text{PSII}} \) that made two consecutive images separated by 120 s. If these two images differed from each other the leaf was considered not to have reached steady-state. Once the image of \( \Phi_{\text{PSII}} \) had been made for the leaf in air, the gaseous phase was changed first to 2% \( \text{O}_2 \), 350 ppm \( \text{CO}_2 \), remainder \( \text{N}_2 \), and then to 2% \( \text{O}_2 \), 5% \( \text{CO}_2 \), remainder \( \text{N}_2 \). After each change the leaf was allowed to reach steady-state before an image of \( \Phi_{\text{PSII}} \) was made. The 2% \( \text{O}_2 \), 350 ppm \( \text{CO}_2 \) treatment was used to eliminate photorespiration and thus reveal areas of the leaf with possibly closed stomata, and the 2% \( \text{O}_2 \), 5% \( \text{CO}_2 \), remainder \( \text{N}_2 \) treatment was used to overcome any stomatal limitation upon the rate of \( \text{CO}_2 \) fixation while at the same time preventing any significant photorespiration, thus allowing regions of a leaf with a down-regulation in the rate of \( \text{CO}_2 \) fixation due to reasons other than stomatal resistance to be identified. An area of about 1 cm diameter on the underside of each leaf was covered with petroleum jelly in order to block the stomata in this area and partly simulate stomatal closure.

RESULTS AND DISCUSSION

The images of the non-droughted leaf (Fig. 1) show little difference between the different gaseous treatments and little variation of \( \Phi_{\text{PSII}} \) within each image. There are some small areas of low efficiency around the margins of the leaf, and these are evident in all three gaseous treatments, suggesting that they are due to a internal, cellular restriction of photosynthesis and not a stomatal effect. The lack of any significant differences between the air and 2% \( \text{O}_2 \), 5% \( \text{CO}_2 \) treatments implies that the leaf was not sink-limited - had this been so then the 2% \( \text{O}_2 \) treatment would have produced a large fall in \( \Phi_{\text{PSII}} \). In comparison to the image made in air and the image made in 2% \( \text{O}_2 \), 5% \( \text{CO}_2 \) treatments, the image made in 2% \( \text{O}_2 \), 350 ppm \( \text{CO}_2 \) showed a slightly lower \( \Phi_{\text{PSII}} \) (more yellow and less orange in the image), implying a small non-cellular limitation on photosynthesis that could possibly be due to a stomatal limitation on gaseous diffusion into the leaf.

After 4 hours of dry storage the pattern of \( \Phi_{\text{PSII}} \) distribution was radically altered (Fig. 2). When measured in air \( \Phi_{\text{PSII}} \) was, if anything, slightly higher than in the control image (Fig. 1) implying a continued turnover of the photosynthetic electron transport chain at a rate that was slightly higher than in the control leaf. However, under conditions where photorespiration was prevented using 2% \( \text{O}_2 \), 350 ppm \( \text{CO}_2 \) it is evident that photosynthetic efficiency has been reduced, especially in the distal half of the leaf. That this decrease in efficiency is at least partly due to stomatal closure can be deduced by comparing the images made under 2% \( \text{O}_2 \), 350 ppm \( \text{CO}_2 \) and 2% \( \text{O}_2 \), 5% \( \text{CO}_2 \). The increased \( \text{CO}_2 \) concentration largely restores photosynthetic efficiency over the leaf area, though not to the levels observed in the air treatment nor the control treatments. This
implies two things. First, there is a stomatal restriction acting upon photosynthetic electron transport, this accounts for the difference between the 2% O₂, 350 ppm CO₂ treatment and the 2% O₂, 5% CO₂ images. Second, in addition to the effect of drought on the stomata, there is also a non-stomatal restriction on carbohydrate metabolism that acts to restrict electron transport in the image obtained in a 2% O₂, 5% CO₂ atmosphere compared to the droughted leaf measured in air. Notably, drought has no negative effect on photosynthetic electron transport measured in air, at least not at the low irradiance employed in this measurement protocol. Though the difference between the 2% O₂, 5% CO₂ image and that made in air implies a limitation on photosynthesis acting at a metabolic level, the restriction may have its origins elsewhere, for example in the leaf carbohydrate transport pathway. Whatever its origins, the limitation evident in the 2% O₂, 5% CO₂ image indicates a non-stomatal drought-induced limitation of photosynthesis in this leaf.

After one day recovery in tap-water, the \( \Phi_{PSII} \) images reveal a deterioration in the condition of the leaf (Fig. 3). This can be seen by comparing the 2% O₂, 350 ppm CO₂ image of (Fig. 2) with the 2% O₂, 350 ppm CO₂ of (Fig. 3). Since the end of the drought treatment on the previous day the area of depressed photosynthetic electron transport has increased and now covers the entire leaf. On the other hand, the images of \( \Phi_{PSII} \) made in air and 2% O₂, 5% CO₂ are scarcely unaltered compared to the measurement made at the end the drought stress. The conclusion that may be drawn from these changes is that the stomatal restriction that developed during the drought stress has increased the area over which it exerts an effect, so there has been no recovery in the leaf’s stomatal response to drought. Otherwise the photosynthetic function of the leaf appears unaltered compared to that at the end of the drought stress.

Finally, after 3 days recovery in tap-water it appears from the 2% O₂, 5% CO₂ image (Fig. 4) that the underlying photosynthetic integrity of the leaf is unaltered from day 2. The image made in 2% O₂, 350 ppm CO₂ is likewise very similar to that made on day 2, with a minor increase in the area of the leaf that lacks any stomatal restriction (the yellow areas - compare with the overall efficiency of the image made in 2% O₂, 5% CO₂). The \( \Phi_{PSII} \) image made in shows a slight decrease in the efficiency of PSII electron transport, but this change is minor. Even after 3 days, therefore, the leaf had not significantly recovered from the drought stress.

**Literature Cited**


Figures

Fig. 1. Images of $\Phi_{PSII}$ of an attached rose leaf measured in different gaseous phases at an irradiance of 100 $\mu$ mol m$^{-2}$ s$^{-1}$. Prior to the measurements the cut-rose stem was recut under water and stored in a cold room at 4 °C. The colour bar above the images shows the colour mapping used for $\Phi_{PSII}$ (with this measurement technique the maximum relative efficiency obtained for PSII electron transport is about 0.82).

Fig. 2. Images of $\Phi_{PSII}$ of an attached rose leaf measured in different gaseous phases at an irradiance of 100 $\mu$ mol m$^{-2}$ s$^{-1}$. Prior to the measurement the cut-rose stem (used to produce images in fig. 1) was droughted for 4 hours.
after 4h dry storage on day 1, measured on day 2

Fig. 3. Images of $\Phi_{\text{PSII}}$ of an attached rose leaf measured in different gaseous phases at an irradiance of 100 $\mu$ mol m$^{-2}$ s$^{-1}$. Prior to the measurement the cut-rose stem (used to produce images in figs. 1 & 2) was allowed to rehydrate by being stored in tap-water for 24h.

Fig. 4. Images of $\Phi_{\text{PSII}}$ of an attached rose leaf measured in different gaseous phases at an irradiance of 100 $\mu$ mol m$^{-2}$ s$^{-1}$. Prior to the measurement the cut-rose stem (used to produce images in figs. 1, 2 & 3) was allowed to rehydrate by being stored in tap-water for 3 days.