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Research article

Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging

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Abstract

Spatial-temporal changes were examined by imaging chlorophyll (Chl) *a* fluorescence in four leaf areas, two central and two external of rose plants (*Rosa* x *hybrida*) cv. Grand Gala for 9 days, under progressive water stress. New fluorescence parameters based on the lake model have recently been used to determine Q_A redox state and excitation energy fluxes in order to gain a better understanding of the mechanisms that occur under drought stress. Chlorophyll fluorescence images showed a spatial variation in the leaves. The lower values for F_o, F_M, φ_2, q_P and q_L were found in the internal leaf area while higher values of non-photochemical quenching calculated from Stern–Volmer quenching (NPQ) and φ_{NPQ}. φ_{Po} were more homogeneous throughout leaf. Temporal changes were also observed during the experiment, a 10% decrease in relative water content (RWC) (between day 1 and 2), led to a decrease in photochemical quenching and an increase in non-photochemical processes. Chlorophyll fluorescence parameters were more or less constant till day 8. At the end of the experiment (day 9), energy dissipation by downregulation, electron transport and Q_A redox state, decreased and φ_{NO} increased to compensate the change. Chlorophyll fluorescence parameters based on the lake model q_L, φ_{NPQ} and φ_{NO} have been found more appropriate for estimating the fraction of open centres, the quantum yield of regulated energy dissipation in photosystem II (PSII) and the quantum yield of non-regulated energy dissipation in PSII, respectively. The F_s/F_o ratio is strongly correlated with NPQ and φ_{NPQ} up to a RWC of 20%. This coincides with a greater decrease in photochemical quenching and non-photochemical quenching and an increase in φ_{NO}.

Keywords: Chlorophyll fluorescence; Imaging; Non-photochemical quenching; Photochemical quenching; Rose; Water stress

1. Introduction

Plants are organisms that are exposed to a wide variety of abiotic stresses (light, temperature, water stress, atmospheric pollutants), which can directly or indirectly affect their photosynthetic function. Photosynthesis is a core function in the physiology of a plant, which captures light energy to produce both energy and reductor power, via photochemical processes. Absorbed light energy not used for photosynthesis, but is re-emitted as chlorophyll (Chl) *a* fluorescence [24] or dissipated as heat [13]. These are competing processes and, therefore, changes in Chl *a* fluorescence are reflected by a change in the photosynthetic function. Under stress conditions, the photosynthetic quantum conversion declines whereas heat emission and Chl fluorescence increase [25].

Remarkable progress has been made in measuring fluorescence through the Chl fluorescence imaging technique. Leaf imaging has revealed that photosynthetic activity within leaves can be heterogeneous (see [1]). The technique can be used to screen many plants simultaneously when fluorescence parameters obtained from image areas exceed 100 cm^2 [2]. There are spatiotemporal variations in fluorescence parameters, caused by different stress factors, which can be visualised

Abbreviations: Chl, Chlorophyll; F_M, maximum Chl fluorescence yield obtained with a dark-adapted sample; F'_M, maximum Chl fluorescence yield in illuminated samples; F_o, minimum Chl fluorescence yield in the dark-adapted state; F_s, Chl fluorescence yield during actinic illumination; F_v, ($F_M - F_o$) variable Chl fluorescence in the dark-adapted leaf; NPQ, non-photochemical quenching calculated from Stern–Volmer quenching; PAR, photosynthetically active radiation; q_P, photochemical quenching based on a puddle model for the photosynthetic unit; q_L, estimating the fraction of PSII centres in open states based on a lake model for the photosynthetic unit; PSII, photosystem II; RWC, relative water content; $\varphi_{;NO}$, quantum yield of non-regulated energy dissipation in PSII; φ_{NPQ}, quantum yield of regulated energy dissipation in PSII; φ_{Po}, maximum quantum yield of PSII photochemistry (noted too F_v/F_M); φ_2, effective quantum yield of photochemical energy conversion in PSII (noted too φ_{PSII}).

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through Chl fluorescence imaging, but cannot be detected by conventional fluorometers. The potential of fluorescence imaging as a powerful method to solve heterogeneity in the photosynthetic function across a leaf has been demonstrated by efficiently operating photosystem II (PSII) images of leaves, that have been subjected to the following stresses: fed with herbicides [2,11]; after pathogen infection [5,35]; during a sink-source transition [28]; with heterogeneous stomatal responses [30,40]; in response to changing CO_2 concentration [8]; under changing photon flux density [14]; under low growth temperature [31]; during change in photosynthesis induction [32] or screening of algal mutant colonies [4].

Many useful fluorescence parameters have been developed from saturation pulse induced fluorescence analysis (e.g. φ_{Po}, φ_2, q_P, NPQ, φ_p) by Genty et al. [16], Bilger and Björkman [6], Schreiber et al. [37], Krause and Weis [24], Rohácek [34] and used as proxies of photosynthetic activity under actinic light. Recently, Kramer et al. [23] has defined new fluorescence parameters, q_L, φ_{NPQ} and φ_{NO} based on the Stern–Volmer approach using a lake or connected model (see for review, Lazár [21]). The lake model assumes that a photosynthetic unit may consist of a relatively larger number of reaction centres, embedded in a common matrix of antenna, with high connectivity of PSII units, where all open reaction centres compete for excitation in the pigment bed (see [21,23]). The q_L parameter is appropriate for estimating the fraction of open centres; φ_{NPQ} and φ_{NO} parameters measure the fraction of excitons dissipated via two competing non-productive pathways, induced by activation of downregulatory processes versus other non-photochemical losses [23]. These parameters are calculated on the basis of more realistic models of the photosynthetic unit, like the lake model, as opposed to a puddle model, where each PSII centre possesses its own independent antenna system.

These new fluorescence parameters are incorporated in the new imaging PAM fluorometer software, where they can be measured and images can be obtained through induction fluorescence kinetics or under light curve responses.

In controlled greenhouse conditions and precision agriculture, information on plant response and growth measurement by non-invasive and non-destructive techniques can improve plant production [36]. Chlorophyll fluorescence measurements can be useful in rapidly evaluating the effects of climatic factors, or water and nutrient regimes on plant performance [1]. Application of Chl fluorescence techniques, in particular Chl fluorescence imaging, can be used to study plant response to dynamic climate control, because image information is the most intuitive, easily comprehensible, and provides useful information on plant status [29]. Water availability in the greenhouse is one of the most important limitations to photosynthesis and plant productivity in many regions of the world [15,40]. Proper monitoring of water stress in plants is essential to develop suitable and sustainable irrigation programs for crop production in semiarid areas [33].

In this study, we report on spatiotemporal variations of Chl *a* fluorescence parameters and image fluorescence parameters, under slow and progressive water stress in rose plants. We discuss spatial variations under four areas of interest (AOI) in leaves, two central areas and two lateral areas. Temporal changes in leaves have been measured over 9 days under progressive water stress. We also discuss the new fluorescence parameters described by Kramer et al. [23] (q_L φ_{NPQ} and φ_{NO}) compared to fluorescence parameters obtained using a puddle model (q_P, NPQ).

2. Results

2.1. Chlorophyll fluorescence parameters in control leaves

Chlorophyll *a* fluorescence parameters in control leaves (optimum water availability) are shown in Table 1. φ_{Po} ratio was higher in AOI 2 and 3 with significant differences between AOI 3 and 1. F_o and F_M were higher in the internal part of leaves near the main vein, the lowest values were obtained in the external leaf part, with significant differences compared to internal AOI. The ratio F_s/F_o, is a water stress sensitive parameter for C3, C4 and CAM plants and is inversely correlated with NPQ [15]. The ration F_s/F_o was lower in the central area (AOI 1) differing significantly from the rest of AOI. The AOI 1 gave lower values of φ_2 and q_P compared to other AOI. The q_L was similar in all AOI in contrast to q_P. Photochemical quenching, q_L, showed lower values than q_P. Non-photochemical quenching φ_{NPQ} or NPQ showed significant differences in AOI 1 versus other OAI. The quantum yield of non-regulated energy dissipation in PSII, φ_{NO}, was similar in all AOI.

External AOI (2 and 3) showed higher photosynthetic activity than internal AOI in the control rose leaves.

Chl *a* fluorescence parameters in control leaves were measured throughout the experiment (9 days) but there were no significant differences between days.

Table 1
Chlorophyll *a* fluorescence parameters in control leaves of rose plants after steady-state kinetics induced for four AOI, two internal (1 and 4) and two external (2 and 3) in the leaf. F_o, minimum Chl fluorescence yield obtained with dark-adapted leaf; F_M, maximum Chl fluorescence yield obtained with dark-adapted leaf; F_s, Chl fluorescence in the steady-state during actinic illumination; φ_{Po}, maximal quantum efficiency; φ_2, effective quantum yield of photochemical conversion in PSII; q_P, photochemical quenching; q_L, photochemical quenching calculated according Kramer et al. [23]; φ_{NPQ}, quantum yield of regulated energy dissipation in PSII calculated according to Kramer et al. [23]; φ_{NO}, quantum yield of non-regulated energy dissipation in PSII [23]; NPQ, non-photochemical quenching. Values are means of 10 samples. For comparison of means, ANOVA followed by LSD test, calculated at 95% confidence level, were performed. Values followed by the same letter indicate no significant differences

AOI	F_o	F_m	F_s/F_o	φ_{Po}	φ_2	q_P	q_L	φ_{NPQ}	φ_{NO}	NPQ
1	0.147a	0.735a	1.49b	0.802b	0.409b	0.608b	0.338a	0.313a	0.289a	0.988a
2	0.103c	0.547c	1.71a	0.810ab	0.463a	0.643a	0.335a	0.232b	0.287a	0.676b
3	0.097c	0.531c	1.69a	0.815a	0.459a	0.641a	0.336a	0.237b	0.285a	0.704b
4	0.120b	0.628b	1.65a	0.807ab	0.443a	0.629ab	0.334a	0.260b	0.289a	0.776b