FLUORESCENCE IMAGING AND PIGMENT DETERMINATION OF POPULUS BY THE PHOTOSYNTHETIC APPARATUS IN DAJT AREA

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Abstract

The high resolution multi-colour Chl fluorescence imaging techniques for whole leaves have been developed over the last years and successfully applied to stress detection on plant. These techniques offer the possibility to study the distribution and patchiness of fluorescence signatures over the whole leaf area. Many environmental and stress factors will effect either directly and indirectly the rate of photosynthesis. It is established that the rate of photosynthetic quantum conversion and the health state of the photosynthetic apparatus can be measured by the non-destructive in vivo chlorophyll fluorescence. The aim of this paper is to assess changes of photosynthetic activity and pigments determination of the plants by in the presence different stress as humidity, temperature through chlorophyll fluorescence imaging technique. Plants of Populus are taken in consideration in 2011 year two different season.

Fluorescence images of leaves were measured using the FluorCam 700MF imaging system that offers the possibility to study the distribution and patchiness of fluorescence signatures over the whole leaf area.

Keywords*: spontaneous plants, chlorophyll fluorescence imaging, induction kinetics, photosynthetic apparatus*

Introduction

Dajt area characterized by optimal psysiological conditions. In nature plants are repeatedly exposed to a variety of stressors which affect growth, physiological functions and yield. The higher temperature and high irradiance combined with water stress are the most damaging environmental stresses in Dajt area. Stressors affect either directly or indirectly the photosynthetic performance of leaves and can modify their optical and fluorescence properties. The chlorophyll (*Chl*) fluorescence signatures of plants have been applied as an efficient tool to describe and investigate the photosynthetic light processes and quantum conversion at physiological conditions as well as to detect stress effects in the photosynthetic apparatus (Lichtenthaler 1996, Buschmann and Lichtenthaler, Krause and Weis, 1991; Lichtenthaler and Miehé, 1997; Lichtentaler and Babani, 2004; Schreiber1986). Various parameters and ratios of the Chl fluorescence determined from the induction kinetics (Kautsky effect) can be used as indicators of the functional state or stress damage of the photosynthetic apparatus and photosynthetic electron transport (Babani and Lichtenthaler, 1996; Buschmann and Lichtenthaler, 1998; Govindjee 1995, 2004).

The objective of the presented research is the evaluation of damage by different plants in two different season on spontaneous plants characterizing the photosynthetic performance by imaging of chlorophyll fluorescence signature. Imaging of Chl fluorescence kinetics correctly screening the

emission heterogeneity reflects localized biotic or abiotic stress or heterogeneous metabolism. Offering the possibility to study distribution and patchiness of fluorescence signatures over the whole leaf area these techniques were developed as invaluable tool for determining the photosynthetic performance of plants. Observed differences on imaging of chlorophyll fluorescence signature and photosynthetic pigment metabolism of leaves allowed to characterize effect of environmental factors on photosynthetic performance as well as to estimate the variations among endemic plants in stress conditions.

Materials and Methods

Plant material

Endemic-spontaneous plants ***Populus x canadensis*** and **Cercius Siliquastrum** grown in different periods pollution conditions were analyzed. Study area were chosen in: Dajti area characterized by optimal physiological conditions in 2011 year in periods May and August.

Chlorophyll fluorescence imaging of induction kinetics

Chlorophyll (Chl) fluorescence induction kinetics were measured using the FluorCam 700MF kinetics imaging system constructed by Photon Systems Instrumentto capture kinetics and 2-dimensional maps of key fluorescence parameters. The fluorescence emission is induced by two sets of 325 super-bright orange light emitting diodes (LED's) (wavelength 605nm) that provide excitation flashes or a continuous actinic irradiance controlled by defined protocol. Fluorescence images are captured by CCD camera. The images are taken at 12-bit resolution in 512 x 512 pixels of CCD chip. The size of an analyzed object is smaller than 10 × 13 cm.

Chlorophyll fluorescence images of parameters as F_0 and $F_{0'}$(minimum fluorescence in the dark and in the light-adapted states), Fm and Fm' (maximum fluorescence in the dark and in the light-adapted states), Fp, (initial fluorescence increase caused by the actinic light exposure) and Fs, (steady-state fluorescence in actinic light exposure) were recorded. Images of various Chl fluorescence ratios obtained by pixel to pixel arithmetic operations performed by FluorCam software were: maximum quantum yields of Photosystem II Fv/Fm and Fm/Fo; effective quantum yields of Photosystem II Fv'/Fm'andFm'/Fo'; fluorescence decline ratio in steady-state which assess plant vitality Rfd=(Fp-Fs)/Fs; non photochemical quenching coefficients NPQ=(Fm-Fm')/Fm and qN=(Fv-Fv')/Fv; where Fv=Fm-Fo and Fv'=Fm'-Fo'.

Kinetics of the fluorescence transient over the leaf area was performed by FluorCam software where each data point represents one image. The represented induction kinetics are the mean curves of six different kinetics from six different leaves grown in every years.

Pigment determination

The leaf pigments were extracted with 100% acetone using a mortar. Chlorophylls (*Chla* and *Chlb*) and total carotenoids (*x+c*) were determined spectrophotometrically (SQ-4802 Double Beam

Scanning UV/Visible Spectrophotometer) and calculated using the re-evaluated equations of Lichtenthaler. The values represent the mean of 6 separate extracts

Results and Discussion

Fluorescence images and fluorescence image ratios of leaves of *Populus x canadensis*

The images of the measured Chl fluorescence intensity were obtained on false colour, whereby black is the lowest (zero) and red the highest fluorescence intensity

Images of all analyzed leaves of Figure1 an Figure 2, exhibited almost the same distribution as well as the same level of fluorescence signal over the whole leaf area showing no irregularities and nearly uniform distribution of fluorescence signatures

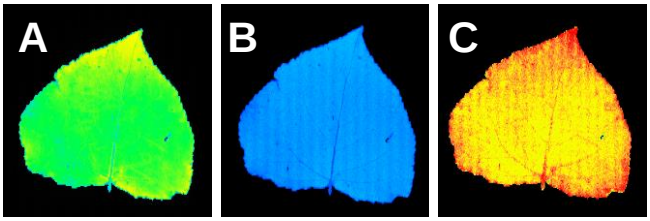

Figure 1. Images at the maximum fluorescence in the dark Fm (A), maximum fluorescence in the Light Fo (B) in (pseudoscale 0-800) and ratio Fm/Fo in (pseudoscale 0-5) of a Populus leaf grown in optimal in May physiological conditions.

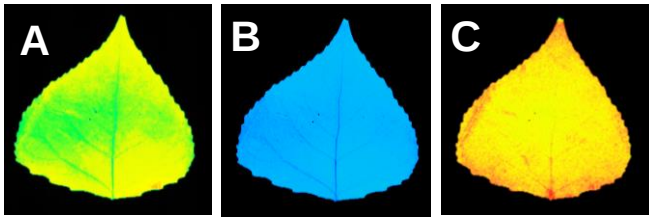

Figure 2. Images at the maximum fluorescence in the dark Fm (A), maximum fluorescence in the Light Fo (B) in (pseudoscale 0-800) and ratio Fm/Fo in (pseudoscale 0-5) of a Populus leaf grown in optimal in August.

Images at Fm of leaves grown in plant steel air pollution conditions Figure 2 showed a different distribution of the fluorescence emission over the leaf area as compare to the leaves grown in optimal conditions Figure 1.

Fluorescence images displayed at the same pseudoscale clearly showed changes of the values of this parameter and their distributions related to the heterogeneity over leaf area between leaves grown in different conditions.

Induced fluorescence image parameters

Table 1. Induced fluorescence image parameters of leaves of *Populus x canadensis* grown in area of optimal (May) and plan steel air pollution conditions (August) (mean of six leaves).

Populus x canadensis							
Image Fluorescence parameters		**Fo**	**Fm**	**Fv**	**Fo'**	**Fm'**	**Fv'**
May	mean	**128.8**	**563.4**	**434.6**	**124.9**	**180.0**	**55.1**
	std	***3.64***	***17.02***	***14.88***	***4.04***	***9.35***	***6.45***
August	mean	**101.7**	**432.7**	**331.0**	**136.0**	**182.0**	**46.0**
	std	***2.95***	***15.06***	***12.44***	***2.34***	***6.55***	***4.57***

The mean values of image fluorescence parameters demonstrated the differences between leaves grown in different conditions.

The values of standard deviations of fluorescence parameters of leaves grown in optimal conditions in August show no significant differences between analyzed leaves.

The observed increase of the values of standard deviations from optimal growth conditions to pollution conditions can be illustrated the increase of the variability through the leaves as the effect to pollution exposure in August.

Image fluorescence ratios

Table 2. Image fluorescence ratios of leaves of *Populus x canadensis* grown in May and August (mean of six different leaves).

Populus x canadensis						
Image Fluorescence ratios		**Fm/Fo**	**Fv/Fm**	**Fm'/Fo**	**Fv'/Fm**	**Rfd**
May	mean	**4.37**	**0.77**	**1.44**	**0.31**	**2.66**
	std	***0.11***	***0.01***	***0.05***	***0.02***	***0.10***
Augustr	mean	**4.25**	**0.77**	**1.34**	**0.25**	**2.12**
	std	***0.06***	***0.00***	***0.03***	***0.02***	***0.13***

Rfd values showed a considerable reduction of the activity of the photosynthetic apparatus of the leaves grown in August than of leaves grown in Dajt area as plants were grown in severe stress-pollution conditions

The values of Rfd ratios as plant vitality indicator demonstrated that full green leaves can be characterized by the high photosynthetic activity, as reflect by the mean values the ratio 2.66.

The fluorescence ratios that allow to estimate maximum and effective quantum yields of Photosystem II (Fv/Fm, Fm/Fo) indicated a decrease of these ratios from optimal growth conditions to stress-pollution conditions in August 2.12.

Increase of the standard deviation values were observed in all calculated fluorescence ratios to growth pollution conditions in August compare to optimal conditions in May. Observed increase can be illustrated the raise of the variability through the leaves as the effect to pollution exposure.

Pigment determination

***Populus x canadensis* (Plepi)**

Tabela 3. Photosynthetic pigment or *Populus x canadensis* leaf in periods May and August

Months	Kla (mg/dm^2)	Klb (mg/dm^2)	x+c (mg/dm^2)	Kl(a+b) (mg/dm^2)
May	5.827	1.589	2.172	7.416
August	4.799	1.306	1.868	6.105

The total Chl ($a+b$) content and total carotenoids ($x+c$) content were significantly higher in leaves of both endemic plants grown in optimal conditions – Dajti area than of plants grown in stress conditions (August).

The decrease of chlorophylls was faster than that of carotenoids

Results

- Values fluorescence images parameters in the leaves of endemic plant *Populus x Canadensis* grown in optimal conditions (May) exhibited a high photosynthetic activity as is demonstrated by the values of fluorescence ratios which evaluate the plant vitality and quantum yield of photosynthetic apparatus: Rfd=2.66, Fm/Fo=4.37.

- Activity of photosynthetic apparatus of leaves grown in plant steel air pollution conditions (August).was generally lower than activity of plants grown in optimal conditions: Rfd=2.12, Fm/Fo=4.25.
- The photosynthetic pigments, chlorophylls and carotenoids, could be considered functionally organized in plants grown in optimal conditions (Dajti area).
- The reduce of pigment content observed in both endemic plants grown in stress conditions (August) as well as in stress-pollution conditions compared to optimal conditions in May indicated a possible modifications in pigment composition during stress events.

- Observed differences on imaging of chlorophyll fluorescence signature and photosynthetic pigment content of spontaneous plants allowed to characterize the photosynthetic performance in order to evaluate the damage by plant in October related to stress of season in comparison with the period of August.

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