Effect of steel plant pollution on photosynthetic apparatus of some spontaneous plants by chlorophyll fluorescence imaging

EriolaZhuri Hida1, VeledinÇako2,FatbardhaBabani3,Theodhor Karaja4

1University 'AleksanderMoisiu', Durres, Albania,

2 Department of Physics, University 'Ismail Qemali',Vlora, Albania,

3 Biotechnology Department, University of Tirana, Tirana, Albania,

4Physics Department, University of Tirana, Tirana, Albania,

E-mails: *eri_zhuri@yahoo.com, v.cako69@hotmail.com, fbabani@yahoo.com, theodhor.karaja@yahoo.com*

Abstract

Environmental pollution by the steel plant in Elbasan, the largest metallurgical complex in Albania causes serious problems. Although the blast furnaces and basic oxygen converters closed in 1991, small scale steel production from scrap metal continued. This steel plant releasing chemicals and particulates into the atmosphere is considered a source of air pollution.

Industrial siderurgic air pollution as other stressors affects growth and physiological functions of plants. Pollution has effects on the photosynthetic performance of leaves and can modify their optical and fluorescence properties either directly or indirectly. The chlorophyll (Chl) fluorescence provides ample information on the performance of photosynthetic apparatus. Chl fluorescence signatures of leaves as an efficient tool and a nondestructive method for the in vivo analysis of plant stress is applying to describe and investigate the photosynthetic light processes and quantum conversion at physiological conditions as well as to detect stress on the photosynthetic apparatus. The chlorophyll fluorescence images were measured on leaves using the FluorCam 700MF imaging system (Photon Systems Instrument) as a techniques that offer the possibility to study the distribution and patchiness of fluorescence signatures over the whole leaf area.

Chl fluorescence images were measured on the leaves of some spontaneous plants grown in sites with different level of steel plant air pollution assessed on base of different distances and different directions from the source of the pollution. Efficiency of photosynthetic apparatus of analyzed plants was evaluated via chlorophyll fluorescence images during induction kinetics and various fluorescence ratio images. The images of Chl fluorescence ratios, acquired by pixel to pixel arithmetic operations performed by FluorCam software, were aplied as indicators of the functional state or the damage of the photosynthetic apparatus. The potential and effective quantum yields of photosynthetic electron transport were estimated too.

Observed differences on imaging of chlorophyll fluorescence signature and photosynthetic pigment content of some spontaneous plants allowed characterizing of the photosynthetic performance in order to evaluate the damage by plant steel air pollution.

Keywords: chlorophyll fluorescence imaging, induction kinetics, plant steel air pollution, photosynthetic apparatus, potential/effective quantum yield, spontaneous plant.

1.INTRODUCTION

Environmental pollution by the steel plant in Elbasan, the largest siderurgical complex in Albania causes serious problems. In this area operate some plants as steel plant operator "Kurum“, cement factory "ECF", Establishment of Ferro-Chrome "ACR“.

This steel plant releasing chemicals and particulates into the atmosphere is considered a source of air pollution. Monitored elements in the Elbasan area are:

- solids suspended in the air (LNP), rigid particles with dimensions smaller than 10 microns (PM10)

- lead (Pb), sulfur dioxide (SO2), nitrogen dioxide (NO2), ozone (O3) in the air.

Siderurgic air pollution as other stressors affects growth and physiological functions of plants. Pollution has effects on the photosynthetic performance of leaves and can modify their optical and fluorescence properties either directly or indirectly. 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 to detect pollution effects on the photosynthetic apparatus. Image analysis of the Chl fluorescence signals offers the possibility to study the function of photosynthetic apparatus and to detect early stress damage in plants by screening the fluorescence emission over the leaf area as well as the gradients and local irregularities in fluorescence emission and ratios (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). 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 (Buschmann and Lichtenthaler 1998, Lichtenthaler and Babani 2000; Lichtenthaler et al., 2000,Lichtenthaler and Babani 2004, Lichtenthaleret al., 2007, Nebdal et al. 2000, Schreiber 2004).

The objective of the presented research is the evaluation of damage by plant steel air pollution 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

2.MATERIALS AND METHODS

2.1. Plant material

Endemic-spontaneous plant Cercius siliquastrum grown in different steel plant pollution conditions were analyzed. Study areas were chosen in three different locations: Dajti area (site 1) characterized by optimal physiological conditions, Krrabe area (site 2 2.6 km) and Elbasan area(site3- 6 km)characterized by plant steel air pollution.

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.

The chlorophyll (Chl) fluorescence images and induction kinetics were measured on pre-darkened leaves (30 min) using the FluorCam quenching protocol. 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.

Chlorophyll fluorescence images of parameters as F0 and F0'(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 study area.

3.RESULTS AND DISCUSSION

3.1.Image Fluorescence parameters

Fully green leaves of Cercius siliquastrum grown in optimal physiological conditions (Dajti area-Site 1) from six different branches were analyzed. Images of the maximum fluorescence in the dark Fm of all analyzed leaves exhibited almost the same distribution as well as the same level of fluorescence signal over the whole leaf area. The images represented in Figure 1 (A) that belong to one of these leaves showed no irregularities and nearly uniform distribution of fluorescence signatures.

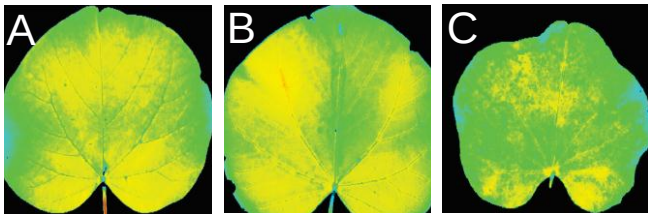

Figure 1. Images at the maximum fluorescence in the dark Fm of a leaf grown in optimal physiological conditions A-site 1, and plan steel air pollution conditions B-site 2, C- site 3 (pseudoscale 0-700).

Images at the maximum fluorescence in the dark Fm of leaves grown in plan steel air pollution conditions showed a different distribution of the fluorescence emission over the leaf area as compare to the leaves grown in optimal conditions, especially leaves grown in site 3 (Figure 1, B, C). Fluorescence images at Fm 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.

The shape of the fluorescence induction kinetics of green leaves grown in optimal conditions demonstrated the healthy physiologically state of these leaves (Figure 2, A). While the shape of the induction kinetics exhibiting an increase of the fluorescence signal at the steady state of fluorescence demonstrated the effect of pollution to which were exposed leaves during their growth (Figure 2, B, C).

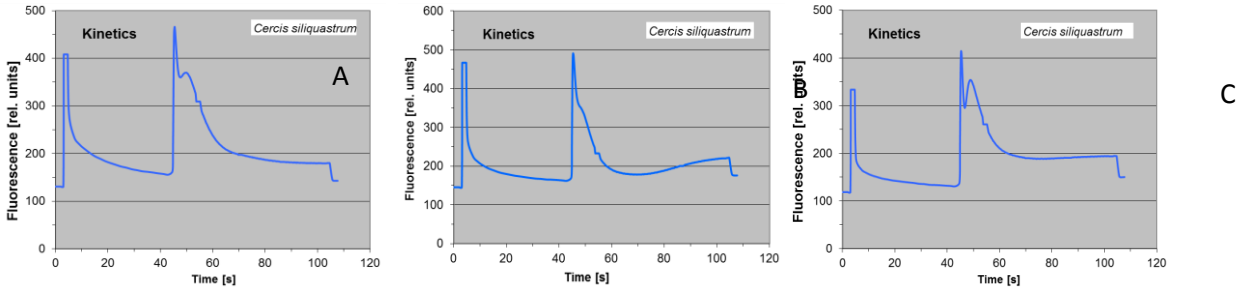

Figure 2.Induced fluorescence kinetics of leaves of Cercius siliquastrumgrown in optimal physiological conditions A-site 1, and plan steel air pollution conditions B-site 2, C- site 3.

Image fluorescence parameters of every leaf were calculated by FluoCam software as the mean of the fluorescence signals of all pixels over the leaf area. The represent values of these parameters (Table 1) correspond to a mean of six different leaves. 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 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 stress exposure.

Table 1. Induced fluorescence image parameters of leaves of Cercius siliquastrum grown in area of optimal and plan steel air pollution conditions (mean of six leaves).

Cerciussiliquastrum							
Image Fluorescence parameters		Fo	Fm	Fv	Fo'	Fm'	Fv'
Site 1	mean	130.8	406.8	276.3	141.7	181.4	40.0
	std	(3.36)	(2.62)	(5.39)	(3.92)	(4.87)	(1.88)
Site 2	mean	140.5	484.4	343.9	161.3	209.5	48.3
	std	(4.35)	(39.60)	(39.62)	(16.5)	(18.29)	(5.54)
Site 3	mean	118.4	333.2	214.8	149.9	193.4	43.4
	std	(5.54)	(64.12)	(61.32)	(26.76)	(23.52)	(13.49)

3.2.Image Fluorescence ratios

Images of the fluorescence decline ratio Rfd of a green leaf grown in optimal conditions showed almost no irregularities and a uniform distribution of the values of this ratios over the leaf area (Figure 3 A). The values of Rfd ratios as plant vitality indicator demonstrated that these full green leaves can be characterized by the high photosynthetic activity, as reflect by the mean values the ratio 1.63 (Table 2).

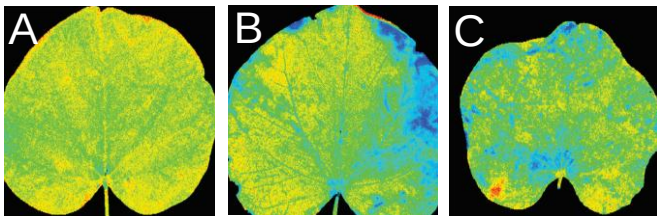

Figure 3. The fluorescence decline ratio image Rfdof leaves of Cerciussiliquastrum grown in optimal physiological conditions A-site 1, and plan steel air pollution conditions B-site 2, C-site 3 (pseudoscale 0-3).

The images of the fluorescence decline ratio Rfd of leaves grown in pollution conditions (Figure 3 B, C) showed a non uniform distribution and increase of irregularities of the values of this ratios over the leaf area.

Table 2. Image fluorescence ratios of leaves of Cercius siliquastrum grown in area of optimal and plan steel air pollution conditions (mean of six leaves).

Cerciussiliquastrum							
Image Fluorescence ratios		Fm/Fo	Fv/Fm	Fm'/Fo'	qN	NPQ	Rfd
Site 1	mean	3.15	0.684	1.27	0.856	1.240	1.63
	std	(0.09)	(0.009)	(0.02)	(0.005)	(.053)	(0.10)
Site 2	mean	3.09	0.66	1.12	0.826	1.732	1.26
	std	(0.29)	(0.02)	(0.04)	(0.007)	(0.142)	(0.18)
Site 3	mean	2.81	0.64	1.29	0.798	0.723	1.14
	std	(0.65)	(0.080)	(0.036)	(0.145)	(0.308)	(0.377)

The values of Rfd ratios represented a lower mean values of the ratio comparing to the optimal conditions, mean values of 1.26. The values of Rfd ratios in pollution growth conditions represented the lowest mean values of the ratio comparing to two other growth conditions, mean values of 1.14 (Table 2, Figure 3).

The distribution of the Rfd values over the leaf area as well as the values of the ratio demonstrated a lower activity of photosynthetic apparatus of the leaves grown in plan steel air pollution growth conditions in comparison of leaves grown in optimal conditions (Dajti area). The observed decrease of activity of photosynthetic apparatus in pollution conditions seem to be related to the distance of the source of pollution, (Figure 3, Table 2).

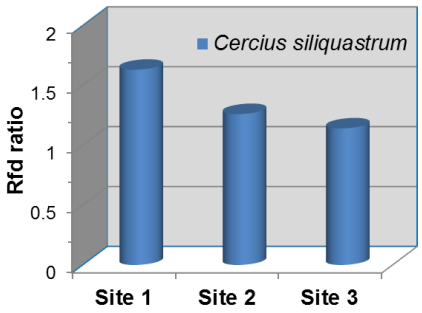

Figure 4.Fluorescence decline ratio Rfd of leaves in different growth conditions (mean of six leaves).

The distribution of the Rfd values showed a considerable reduction of the activity of the photosynthetic apparatus of the leaves grown in area of site 3 than of leaves grown in two other areas 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 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 (Table 2).

Non photochemical quenching coefficient qN have been decreased in growth pollution conditions (Table 2).

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

4.CONCLUSIONS

Fluorescence images measured during induction kinetics, shape of the Chl fluorescence kinetics and the values of the fluorescence parameters in the leaves of endemic plant Cerciussiliquastrum grown in optimal conditions (Dajti area - site 1) 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=1.63, Fm/Fo=3.15.

Activity of photosynthetic apparatus of leaves grown in plant steel air pollution conditions (site 2).was generally lower than activity of plants grown in optimal conditions: Rfd=1.26, Fm/Fo=3.1.

Activity of photosynthetic apparatus of leaves grown in plant steel air pollution conditions (site 3) -Elbasan area) demonstrated reduction compare to other areas as is expressed by the lowest values of fluorescence decline ratio (Rfd=1.14,Fm/Fo=2.81); increased non-uniformity distribution and heterogeneity of signal of fluorescence over the leaf area and shape of induction kinetics.

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 steel air pollution related to the distance of the source of pollution.

REFERENCES

Babani F. and Lichtenthaler H.K. (1996). Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO2 assimilation rates and different kinds of chlorophyll fluorescence ratios. J Plant Physiol 148: 555-566

Buschmann C. and Lichtenthaler H.K. (1998). Principles and characteristics of multi-colour fluorescence imaging of plants. - J. Plant Physiol. 152, 297-314

Govindjee (1995): Sixty-three years since Kautsky: Chlorophyll a fluorescence. Australian Journal of Plant Physiology 22: 131-160

Govindjee (2004): Chlorophyll a fluorescence: A bit of basics and history. In: Papageorgiou G, Govindjee (eds) Chlorophyll fluorescence: A signature of Photosynthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 1-42

Krause G.H. and Weis E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. Ann Rev Plant Physiol Plant Mol. Biol 42: 313-349

Lichtenthaler H.K. (1987). Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce R, Packer L (eds) Methods Enzymol 148, pp. 350-382. Academic Press Inc, New York.

Lichtenthaler H.K. and Babani F. (2000). Detection of photosynthetic activity and water stress by imaging the red chlorophyll fluorescence. Plant Physiology Biochemistry 38: 889-895

Lichtenthaler H.K., Babani F. (2004). Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity during light adaptation, In George Papageorgiou G, Govindjee (eds): Chlorophyll Fluorescence: A Signature of Photosynthesis, Kluwer Academic Publishers. Dordrecht,The Netherlands, pp 713-736.

Lichtenthaler H.K. and Buschmann C. (2001). Chlorophylls and carotenoids–Measurement and characterisation by UV-VIS. Current Protocols in Food Analytical Chemistry (CPFA), (Supplement 1), pp. F4.3.1 - F 4.3.8. John Wiley, New York

Lichtenthaler H.K. and Miehe J.A. (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends Plant Sci 2: 316-320.

Lichtenthaler H.K., Babani F., Langsdorf G. (2007). Chlorophyll fluorescence imaging of photosynthetic activity in sun and shade leaves of trees. Photosynthesis Research, 93: 235-244.

Lichtenthaler H.K., Babani F., Langsdorf G., Buschmann C. (2000). Measurement of differences in red chlorophyll fluorescence and photosynthetic activity between sun and shade leaves by fluorescence imaging. Photosynthetica38: 521-529.

Krause, G.H., Weis, E. (1991): Chlorophyll fluorescence and photosynthesis: the basics. - Ann. Rev. Plant Physiol. Plant Mol. Biol. 42: 313-349.