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Chlorophyll fluorescence imaging of cadmium-treated white cabbage plants

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Abstract. The chlorophyll fluorescence imaging technique is a valuable tool to study the impact of heavy metal stress in plants. The aim of this paper was to investigate the influence of Cd on photosynthetic apparatus of white cabbage (*Brassica oleracea* subsp. *capitata* f. alba) plants. Two cabbage cultivars 'Ditmarska Najwcześniejsza' ('DN'; early) and 'Amager Polana' ('AP'; late) were used. Cd was applied before planting seedlings (10 mg Cd kg^{-1} DM of soil).. Measurements were performed at the 3rd leaf after 2 weeks of planting. The level of Cd-induced stress to plants was estimated by chlorophyll (Chl) content (photometrically) and analyses of images and numeric values of the major fluorescence parameters of Chl (Chl fluorescence imaging system FluorCam). Cd negatively affected the chlorophyll content and Fv/Fm, Fv'/Fm', ϕ_{PSII} and qP in leaves of early cultivar of white cabbage. However, in the case of late cv. we did not observe such distinct changes. It suggests that late cultivars. are more resistant to Cd than the early ones. Considering methodological aspect of the study, Chl fluorescence imaging can better reveal some alterations within the leaf, because numeric values of specific parameters, which are the averaged data collected from the whole leaf, cannot reflect the tissue specificity.

Abbreviations: HM – heavy metal, Cd – cadmium, Chl – chlorophyll, Fv/Fm – photochemical efficiency of PSII in the dark-adapted state, F'v'/F'm' – PSII maximum efficiency, ϕ_{PSII} – quantum efficiency of PSII electron transport, NPQ – nonphotochemical quenching of maximal Chl fluorescence, qP – photochemical quenching coefficient.

Key words: Chlorophyll fluorescence, imaging, photosynthetic apparatus, cadmium toxicity, phytoremediation, white cabbage.

Introduction

Cadmium is one of the most toxic heavy metals which occur in the environment as a result of industrial processes, intensive use of fertilizers in agriculture (Hsu and Kao, 2004), mining and also from the exhaust gases of automobiles (Das *et al,*. 1997). It can be easily uptaken and accumulated in plants leading to losses in agricultural yield and hazardous health effects as it enters the food chain (Hsu and Kao, 2004; Murakami *et al*., 2007). Phytoremediation of soils contaminated by HM is becoming more popular worldwide. There are several types of phytoremediation, which differ in the utilization of plants and a place where they are used. Phytoextraction is based on the use of plants that are able to accumulate large quantities of metals and produce high biomass (Wei *et al,*. 2008). The species belonging to botanical family *Brassicaceae* can fulfill these two main conditions.

Our earlier studies revealed the usefulness of white cabbage (*Brassica oleracea* subsp. *capitata* f. alba) for the purification of the soil from Cd by phytoextraction (Baczek-Kwinta *et al.,* 2011a).

In recent years Chl fluorescence techniques has become irreplaceable in detection and evaluation of stress in plants (Lichtenthaler and Miehė, 1997; Roháček *et al.,* 2008). They allow to perform highly accurate measurements without damaging the leaf. Moreover, they provide a lot of information about the actual state of photosynthetic apparatus of the leaf and also offer the possibility to screen gradients and irregularities of Chl fluorescence signatures over the whole leaf area (Lichtenthaler *et al.,* 2000).

The aim of this paper was to investigate the influence of Cd on the photosynthetic apparatus of white cabbage.

Materials and Methods

Plant material and growth conditions

Plants of white cabbage (*Brassica oleracea* subsp. *capitata* f. alba) cultivars: 'Ditmarska Najwcześniejsza' ('DN'; early) and 'Amager Polana' ('AP'; late) were produced from seeds in controlled conditions, in a phytotron of University of Agriculture in Cracow (day/night temperatures 16/12 ºC, 14-h photoperiod and relative humidity 60 ± 5 %).

Seedlings at their stage of 6-8 leaves were transferred into the pots filled with the local soil (clay silt, 35% silt and clay, pH 7,29). The concetration of Cd chosen for the present study was based on previous experiments (Antonkiewicz *et al.*, 2006, Baczek-Kwinta *et al.,* 2011a). Cd in the amount of 10 mg Cd kg^{-1} DM of soil (in the form of $CdSO_4 \cdot 8H_20$) was added to the soil before planting seedlings. Control pots were the ones with the same soil, but without adding of Cd.

Plants were grown for 2 weeks with temperature of 25-20 ºC day/ 20-17 ºC night and 30 % ralative humidity.

Measurements and analyses

Measurments were performed at 3rd leaf counting from the top of the plant. The level of Cd-induced stress to plants was estimated by the Chl content measured photometrically with SPAD chlorophyllmeter (Konica Minolta, Japan) and analyses of the major fluorescence parameters of Chl (Chlorophyll fluorescence imaging system FluorCam 701 MF, PSI, Brno, Czech Republic) revealing photosystem II (PSII) activity.

Statistical analysis:

The differences within the stage and the cultivar were estimated using the Student's t-test.

Results and Discussion

Effect of Cd on chlorophyll content

The amount of Chl was decreased in leaves of early cultivar ('DN') under the influence of Cd (Fig. 1.). However, in the case of late cv. ('AP'), there was no statistically significant differences in chlorophyll content. It suggests that early cultivars are more sensitive to Cd than the late ones.

The negative effect of Cd on pigment content in leaves has been described by several workers on various crops. Chen et al. (2011) reported that chlorophyll *a*, chlorophyll *b* and total chlorophyll content were limited by Cd toxicity in pakchoi and mustard leaves. Similar results were obtained by Haouari *et al.* (2012) on tomato and Shafi Tantrey and Agnihotri (2010) on gram. The toxic effect of Cd on pigment content in leaves was dependent on its concentration.

Effect of Cd on Chl fluorescence parameters

Images of Fv/Fm, Fv'/Fm', ϕ_{PSII}, NPQ and qP are shown in Fig. 2. These images illustrate the differences in photosynthetic characteristic between control and Cd-treated white cabbage plants.

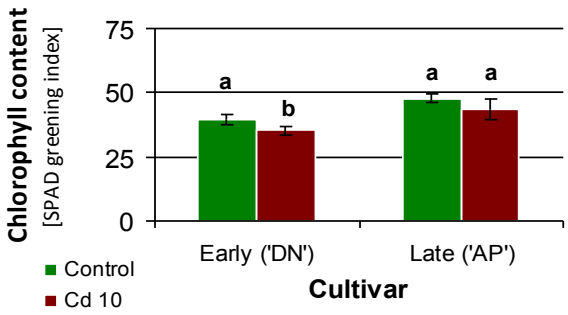

Fig. 1. The effect of Cd on chlorophyll content in leaves of white cabbage plants. Mean ± SD. The means labelled with the same letter are not significantly differentiated (n=4; P < 0.01; paired Student's t-test).

In case of early cv., they reveal more areas of lowered activity in Cd-treated plants comparing to control ones (Fig. 2A). Also the averaged values of Fv/Fm, Fv'/Fm', ϕ_{PSII} and qP decreased. However, NPQ seemed to be higher when analyzing the image (Fig. 2A), but averaged values remained unchanged (Tab. 1.). The decrease in Fv/Fm suggests diminished photochemical capacity of PSII while Fv'/Fm' gives an important information about PSII maximum efficiency (Bączek-Kwinta *et al*., 2011b). ϕ_{PSII} and qP can be interrelated with Fv/Fm (Maxwell and Johnson, 2000). The changes in values of these all parameters may be associated with alterations in chlorophyll content which were described in earlier paragraph (Fig. 1) and suggest that Cd caused PSII activity inhibition. NPQ alterations imply that the nonphotochemical quenching of maximal Chl fluorescence was increased by Cd treatment allowing to dissipate the excessive energy within the photosystems (Maxwell and Johnson 2000, Sofo et al., 2009).

In the case of late cultivar, the images of Chl fluorescence, did not show such discrepancies between control and Cd-treated plants (Fig. 2B), although control leaves revealed more sections of higher Fv'/Fm', ϕ_{PSII} and NPQ than control ones. .No changes in values of fluorescence parameters were noticed (Tab 1.). It suggests that late cultivar is more resistant to Cd treatment than the early one.

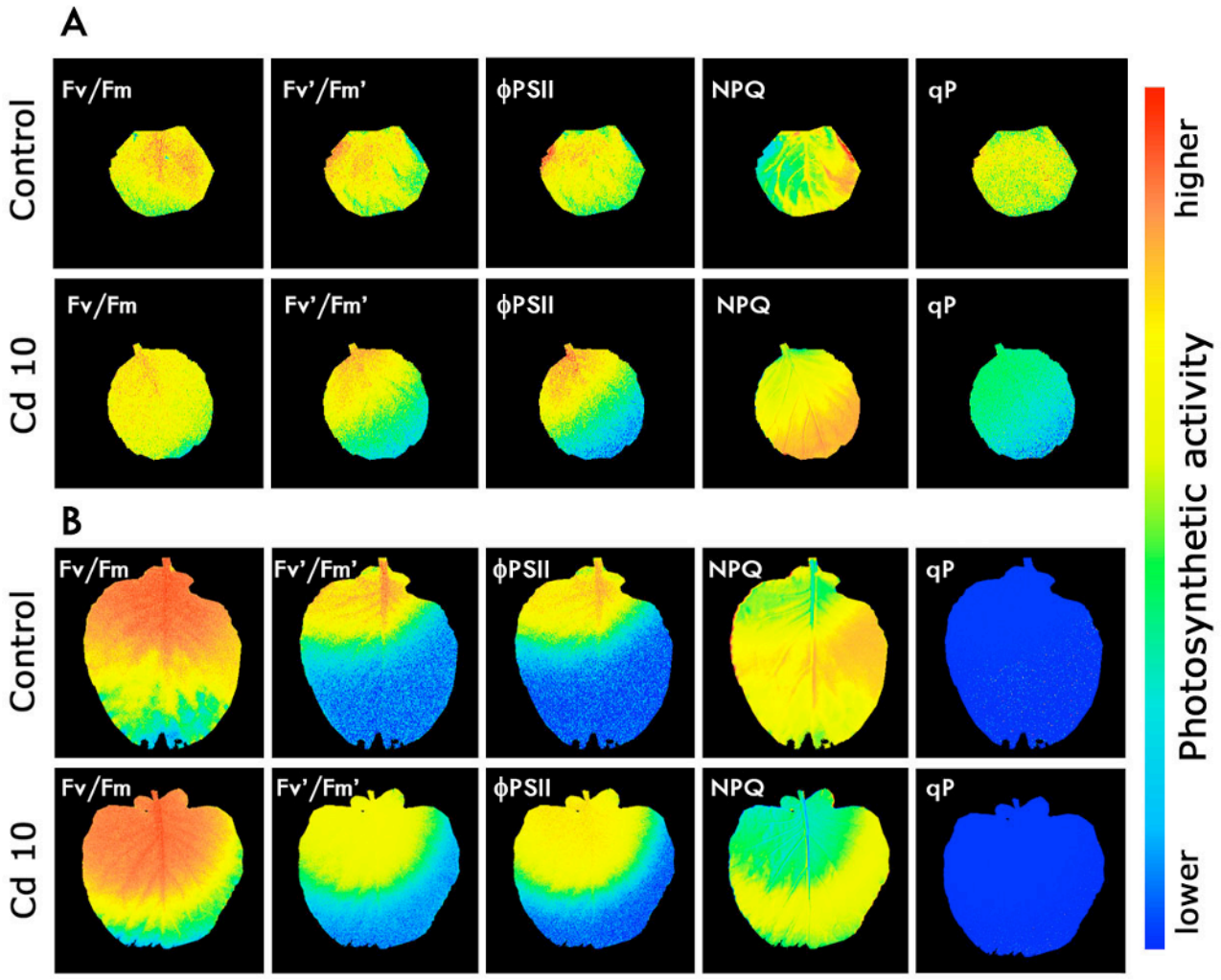

Fig. 2. Chl fluorescence images of Fv/Fm, Fv'/Fm', ϕPSII, NPQ and qP in leaves of early ('DN'; **A**) and late ('AP'; **B**) cultivar of white cabbage. All images are representative for both control and cadmium-treated plants (n=4).

Table 1. Summary of chlorophyll fluorescence parameters determined from fluorescence images of leaves of Cd- treated white cabbage plants similar to those shown in Fig. 2A, B. Mean ± SD, n=4. The means labelled with the same letter are not significantly differentiated ($P < 0.05$; paired Student's t-test).

Cultivar	Treatment	Fluorescence parameters [mean ± SD]				
		Fv/Fm	Fv'/Fm'	ϕ_{PSII}	NPQ	qP
Early 'DN'	Control	0.797 ± 0.007 a	0.504 ± 0.055 a	0.426 ± 0.043 a	1.537 ± 0.062 a	0.846 ± 0.016 a
	Cd 10	0.781 ± 0.008 b	0.431 ± 0.012 b	0.325 ± 0.011 b	1.612 ± 0.099 a	0.756 ± 0.034 b
Late 'AP'	Control	0.763 ± 0.028 a	0.425 ± 0.072 a	0.371 ± 0.061 a	1.494 ± 0.289 a	0.876 ± 0.061 a
	Cd 10	0.741 ± 0.016 a	0.446 ± 0.063 a	0.403 ± 0.069 a	1.204 ± 0.439 a	0.902 ± 0.030 a

Comparing the images of Chl fluorescence parameters, it was distinct that the distribution of photosynthetic activity was dependent on two factors: the cultivar and, among the cultivar, leaf section. Hence, numeric values, which are the averaged data collected from the whole leaf, cannot reveal the tissue specificity. The most photosynthetically active parts of the leaf were the areas located between the base (close to the petiole) and the center of the leaf blade. It can be important when measuring various processes on leaves and taking samples from them for further analyses.

Conclusions

The measurements of Chl content by greening index and assays of Chl fluorescence by imaging techniques can be applied in studies the efficiency of photosynthetic apparatus of HM-stressed plants. The most useful parameters of Chl fluorescence which can be helpful in assessing the level of cadmium stress in plants are Fv/Fm, Fv'/Fm', ϕ_{PSII} and qP, which allow to reveal the photosystem II (PSII) activity. Chl fluorescence imaging techniques also permit to observe the alterations in photosynthetic activity within the whole leaf.

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