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Changes of Photosynthetic Performance of *Brassica napus* Affecting Cadmium Phytoextraction Performance under Elevated Temperature, CO_2 and Drought

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Abstract. This study aimed to investigate the Cd-removal efficiency from the soil by rapes (*Brassica napus* L.) under changing climate conditions, i.e. elevated temperature and CO_2 with or without additional drought stress. As photosynthesis is the most important primary metabolic process, determining plant growth, Cd-phytoextraction performance was evaluated through the changes in chlorophyll *a* fluorescence (ChlF) parameters that are widely used to quantify abiotic stress responses. The results showed that, with the less affected photochemical quantum yields and better general physiological state of PSII, well-watered rapes grown under elevated temperature and CO_2 conditions produced significantly higher harvestable biomass and extracted significantly higher Cd content from the soil than those grown under ambient temperature and CO_2 conditions. Drought fully negated this gain; however, Cd-phytoextraction performance by rapes under the combined impact of elevated temperature, CO_2 and drought was not related to the changes of selected ChlF parameters, suggesting other restrictions to photosynthesis under additional drought stress than the reduced photochemical activity due to the decrease in electron transport flow.

Keywords: phytoextraction, *Brassica napus*, cadmium, photosynthetic performance, climate change

1. Introduction

Many pollutants have been, for decades, irresponsibly released to our environment, resulting in serious pollution problems in many areas throughout the world. It is estimated that in Europe there are 2.5 million potentially contaminated sites, of which 340 000 are actually polluted, and the main contaminant category in many of these sites is heavy metals (HMs) (EEA, 2014). Pollution by cadmium (Cd) as one of the most dangerous environmental contaminants is especially significant as sometimes contaminated soils are used for agriculture. The increasing presence of Cd in the environment has led to considerable concerns over the last decade (Cojocaru et al., 2016; Yang et al., 2017).

Unlike organic pollutants, HMs are non-degradable, and their detoxification in the environment mostly resides either in stabilization *in situ* or in their removal from the matrix, e.g., soil. Phytoextraction is a method of using plants with high shoot-accumulation ability to extract metals from soils/sediments/water, and it has been demonstrated to be an economically feasible method of treating polluted land (Fritioff and Greger, 2003).

Due to their rapid growth and high biomass productivity under different climatic and growing conditions, as well as their ability to accumulate and tolerate cadmium, *Brassica* species are more suitable for phytoremediation of Cd-contaminated soil than other cadmium-tolerant plants and their biomass after phytoextraction can be used for biofuel production (Romih et al., 2012). In addition, recent studies have shown that, under higher temperature and elevated CO_2 concentrations, the photosynthetic potential and biomass production of rapes increased significantly (Dikšaitytė et al., 2019, Juozapaitienė et al., 2019), which may improve the potential for Cd phytoextraction by rapes in the future.

Therefore, the aim of this study is to evaluate the Cd-removal efficiency from soil by rapes under changing climate conditions, i.e. elevated temperature and CO_2 with or without drought stress, using chlorophyll *a* fluorescence parameters as photosynthetic performance indicators affecting Cd phytoextraction performance.

2. Materials and methods

2.1. Growth conditions and experimental design

Field topsoil, taken from the VMU Academy of Agriculture Experimental Research Station, was crushed to particles up to 10 × 10 mm in size and mixed with agroperlite and sand, in a ratio of 5:3:2, by volume. The prepared soil was weighed 2.5 kg into 3-liter vegetation pots, adding 60 kg N ha^{-1} corresponding amount of complex NPK fertilizers (12-11-18 + microelements) to each pot. Soil in half of the pots was spiked with cadmium chloride ($CdCl_2 \times 2.5\ H_2O$) to get 100 mg Cd kg^{-1}. Then half of the pots were placed in a phytochamber, where ambient temperature and CO_2 conditions (aTC) were set: 21/14 °C day/night, 400 ppm CO_2 concentration in the air and 55-60/65-70% day/night relative air humidity (RH). The other half of the pots were placed in the phytochamber with simulated conditions of elevated temperature and CO_2 (eTC): 25/18 °C day/night air temperature, 800 ppm CO_2 concentration in air and 45-50/55-60% day/night RH. The other conditions were maintained identically in both chambers: a 14 h day period and ~ 300 mol $m^{-2}\ s^{-1}$ photon flux density photosynthetically active radiation. The seeds of rapes (*Brassica napus* L., var. 'Fenja') (15 units per pot) were sown in the prepared soil with incorporated cadmium after a 7-day incubation period under simulated aTC and eTC conditions in phytochambers. 15 days after germination, the plants were thinned, leaving 7 units in a pot, and 27 days after germination, all plants were fertilized again with 60 kg N ha^{-1} corresponding amount of the same NPK fertilizers.

Drought stress to half of the pots with plants growing under eTC conditions was applied by withholding watering for 7 days until the volumetric soil water content (SWC) dropped to an average of 5%. Then drought-stressed plants under eTC conditions (eTC+D) were maintained under 5% of SWC till the end of the experiment. Meanwhile, control plants were watered regularly with tap water throughout the experiment, maintaining 30% SWC. Duration of Cd treatment under aTC and eTC conditions from thinning to 7 plants – 43 days, duration of eTC+D treatment – 17 days. The experiment was performed in three replicates.

2.2. Chlorophyll a fluorescence measurement

Chlorophyll *a* fluorescence (ChlF) was measured with a plant efficiency analyzer (Handy PEA, Hansatech Instruments, King's Lynn, Norfolk, England) on the youngest fully expanded leaves of the plants. Leaves were adapted to darkness for 15 min and then illuminated for 1 s to a saturating ultra-bright red light pulse (650 nm, 1800 μmol photons $m^{-2}\ s^{-1}$) by an array of three light-emitting diodes providing uniform illumination over the area of leaf exposed by the leaf clip (4 mm dia). The measured data were used for the calculation according to the JIP-test equations (Stirbet et al., 2018). The biophysical parameters derived from the OJIP transients were calculated, and the following parameters, which refer to time zero (onset of fluorescence induction), were used. (1) Flux ratio of photosystem II (PSII): φPo, the maximum quantum yield of primary photochemistry; ψEo, the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φEo, the quantum yield of electron transport. (2) Flux ratios of PSI: δRo, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors; φRo, the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors. (3) Specific energy fluxes per active PSII reaction center (RC): absorption per active RC (ABS/RC); trapping per active RC (TR0/RC); dissipation per active RC (DI0/RC). (4) RC/ABS – the ratio of the total number of active PSII RCs per absorption flux (ABS). (5) Performance index (PI_{abs}) on an absorption basis.

2.3. Growth measurements

The shoots of all seven potted oilseed rape were cut at the base of growth on the last day of the experiment and weighed. To determine the dry mass, the shoots were dried in an electric oven at 70 °C to constant weight (for at least 72 hours) and then weighed again.

2.4. Determination of shoot Cd concentration

After being oven-dried at 70 °C to a constant weight, shoots were ground to a fine powder (Retsch MM400, Germany). Milled samples of shoots (~ 0.2 g) were microwave-digested (Milestone ETHOS One, Italy) with 65% HNO_3 and 30% H_2O_2 solutions (v/v=8/2). Then the shoot Cd concentration was determined by Optima 8000 ICP-OES (PerkinElmer, USA).

2.5. Evaluation of phytoextraction efficiency

Cd removal efficiency was determined by the harvestable plant biomass (shoot DW) multiplied by the concentration of Cd contained within this biomass, defined here as total uptake index (TU).

2.6. Statistical analysis

All analyses were performed in three replications using Fisher's least significant difference (LSD) criterion of variance post-hoc analysis with STASTICA 8 software package to evaluate the significance ($p < 0.05$) of differences among treatments. The Pearson linear correlation coefficient r and its *p* value (significance level $\alpha = 0.05$) were used to estimate the dependence between the variables.

3. Results and discussion

Figure 1 shows highly (up to 63%, $p < 0.05$) increased shoot dry weight (DW) of Cd-untreated (Cd-0) rapes grown under elevated temperature and CO_2 conditions (eTC) and to an even higher extent increased (2.4 times, $p < 0.05$) under Cd-100 treatment, compared to their respective controls, i.e. Cd-0 and Cd-100 rapes grown under ambient temperature and CO_2 (aTC) conditions. Drought stress almost negated this positive effect of eTC under both Cd-0 and Cd-100 treatments and the difference of shoot DW of drought-stressed rapes grown under eTC conditions (eTC+D) from their respective controls grown under aTC conditions was insignificant ($p > 0.05$) (Fig. 1A). Meanwhile, Cd shoot concentration in Cd-treated rapes significantly decreased under both eTC and eTC+D conditions, compared to those grown under aTC conditions (Fig. 1B). However, due to higher shoot biomass production, Cd-100 rapes grown under eTC conditions extracted significantly higher (up to 76%, $p < 0.05$) Cd content than under aTC conditions, while drought exacerbated Cd extraction efficiency and the difference from aTC was insignificant ($p > 0.05$) (Fig. 1C). Thus, the obtained results confirm the positive effect of elevated temperature and CO_2 on well-watered rape's biomass production as determined in previous studies (Dikšaitytė et al., 2019, Juozapaitienė et al., 2019). In addition, there was a statistically significant very strong positive correlation between well-watered rape's shoot DW and total uptake (TU) index under Cd-100 treatment (r = 0.99, $p < 0.05$). In this context, with a lower accumulation of Cd in their shoots but higher biomass production under elevated temperature and CO_2 conditions, well-watered rapes seem to be more efficient in the remediation of heavily Cd-contaminated soil in a future warmer climate with more CO_2 in the environment. However, drought stress fully negates this gain from a warmer climate.

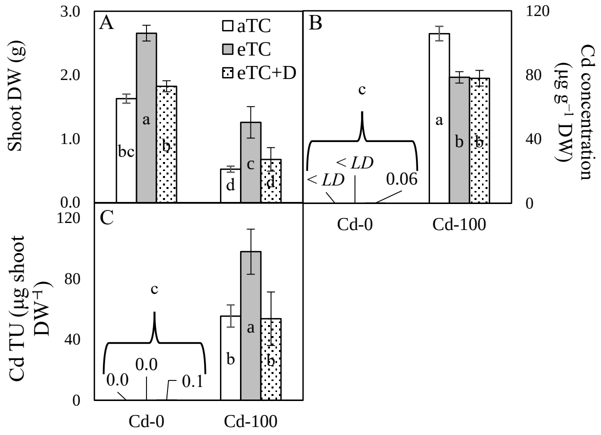

Figure 1. Shoot dry weight (DW), (B) Cd concentration and (C) total uptake index (TU) of rapes at 0 (Cd-0) and 100 mg kg^{-1} (Cd-100) cadmium concentration in soil. aTC – ambient temperature and CO_2 conditions (21/14 °C day/night, 400 ppm CO_2), eTC – elevated temperature and CO_2 conditions (25/18 °C day/night, 800 ppm CO_2), eTC+D – drought effects (5% SWC, compared to 30% SWC) under eTC conditions. The figure shows the means ± SE (n = 3). Different letters inside the columns indicate significant differences between the treatments at $p < 0.05$ (Fisher's LSD). *< LD* – below detection limit

JIP-test parameters were normalized to control, i.e. Cd-untreated (Cd-0) rapes grown under aTC conditions, and the deviations of Cd stress treatments (Cd-100) under aTC, eTC and eTC+D conditions from the control parameter values are shown on the radar plot (Figure 2). It can be seen from Figure 2 that rapes grown under eTC conditions appeared to be less affected by high Cd concentration in the soil, compared to those grown under aTC conditions. It is indicated by the smaller decreases in φPo and ψEo, δRo and φRo, and φEo, reflecting the quantum yields of the electron transport in PSII, PSI, and between them, correspondingly. In addition, there were a smaller decrease in RC/ABS and fewer increases in ABS/RC, TRo/RC and DIo/RC, under eTC conditions, compared to those grown under aTC conditions. Therefore, PI_{abs} that present combined measurements of φPo, ψEo, and RC/ABS and provides useful quantitative information about the state of plants and their vitality (Stirbet et al., 2018) also decreased less in eTC, compared to aTC conditions.

An increase in ABS/RC indicates inhibition of electron transport from quinone A (Q_A^-) to quinone B (Q_B^-), and transformation of active RCs to 'silent' RCs (Yusuf et al., 2010). This is in accordance with the decrease in RC/ABS, which reflects the ratio of the total number of active PSII RCs per absorption flux (ABS). An increase in DIo/RC also supports the change in RC functionality, as the increase in dissipation could indicate that some of the RCs have transformed to 'heat sinks' to dissipate excess energy (Strasser et al., 2000). Meanwhile, an increase in TRo/RC can indicate impairment of oxygen evolving complex (OEC) (Kalaji et al., 2014). Thus, these results indicate that average absorption (ABS/RC) and trapping (TRo/RC) per active RC increases owing to the inactivation of some RCs, and that the ratio of total dissipation to the number of active RCs (DIo/RC) increases because of the high dissipation of the inactive RCs. Similar results were previously reported for other species grown under different stress treatments (Fghire et al., 2015, Gupta, 2019).

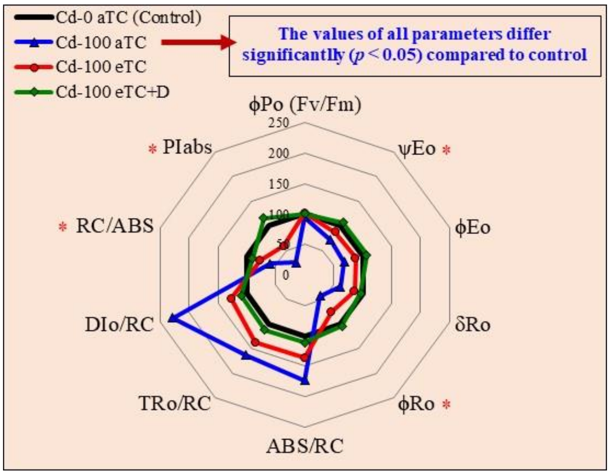

Figure 2. Radar plots depict changes in JIP test parameters in rapes treated with 100 mg kg^{-1} Cd concentration in soil (Cd-100), compared to Cd-untreated ones (Cd-0) under ambient temperature and CO_2 conditions (aTC), elevated temperature and CO_2 conditions (eTC) and drought stress under eTC conditions (eTC+D). Values are means (n = 3). The status of the stressed plants (Cd-100) under aTC, eTC and eTC+D conditions is shown relative to the status of control, i. e. Cd-0 under aTC (100% black line). * – indicates significant eTC differences from control (Cd-0 aTC) at $p < 0.05$ (Fisher's LSD)

Taken together, these changes in ChlF parameters, which were largely more pronounced under aTC than under eTC conditions, indicate a decrease in photochemical activity in rapes leaves due to the toxic effects of cadmium with reduced efficiency of absorbed light energy conversion to chemical energy (NADPH) for further metabolic reactions. A close linear relationship was found between all ChlF parameters used in this study and rape's shoot DW under aTC and eTC conditions. The significant positive correlation was determined between well-watered rape's shoot DW and PI_{abs} ($r = 0.59, p < 0.001$), RC/ABS ($r = 0.67, p < 0.001$), φPo ($r = 0.46, p < 0.01$), ψEo ($r = 0.52, p < 0.01$), φEo ($r = 0.54, p < 0.01$), φRo ($r = 0.61, p < 0.001$) and φRo ($r = 0.59, p < 0.001$). Whereas, between well-watered rape's shoot DW and ABS/RC, DIo/RC and TRo/RC was determined strong negative interaction ($r = -0.66$, $r = -0.59$, and $r = -0.67$, respectively, all $p < 0.001$). Meanwhile, absent significant changes in these ChlF parameters of Cd-100 rape under eTC+D conditions, compared to control ones, suggest that the decrease in their biomass production with Cd treatment was related to other restrictions than the reduced photochemical activity due to the decrease in electron transport flow. It could be the decrease in the activity of Calvin cycle enzymes such as Rubisco, decreased Ribulose 1,5-bisphosphate regeneration capacities and triose phosphate utilization, decrease in chlorophyll content (Song et al., 2019), or ultrastructural changes in chloroplasts (He et al., 2017) and others.

Conclusions

Although eTC conditions resulted in significantly lower Cd accumulation in rapes shoots, due to higher biomass production, they extracted significantly higher Cd content in the harvestable biomass than under aTC conditions. Drought diminished the positive eTC impact on Cd phytoextraction. Cd-phytoextraction performance by rapes under aTC and eTC conditions was strongly related to their photochemical activity and general physiological state of PSII. Meanwhile, drought-stressed rapes grown under eTC conditions demonstrated unchanged photochemical activity due to suppression of Cd, suggesting other restrictions to photosynthesis than the decrease in electron transport flow.

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