Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree

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Abstract: A serious problem in durum wheat cultivation is its genetic capacity to accumulate cadmium (Cd) in the grain. The aim of the study was to verify if the degree of durum wheat resistance to NaCl salinity is related to its tolerance to Cd contamination, and to search for physiological markers of Cd accumulation in the grain. The experiment involved a salt sensitive cv. Tamaroi and a salt resistant line BC_5Nax_2, as well as a moderately salt tolerant line SMH87. The plants grew in the soil supplemented with 3 mg or 5 mg Cd/kg dry weight. The plant response to Cd was evaluated based on chlorophyll fluorescence (ChlF) and Cd content in the grains. Toxic effects of both Cd levels on photosynthetic performance index were the strongest in salt sensitive cv. Tamaroi, which showed the highest Cd content in the seeds. We therefore assumed that tolerance to salinity and Cd has a common physiological background, and that ChlF parameters may be used as the markers of Cd tolerance.

Keywords: *Triticum durum* Desf.; heavy metal; salinity resistance; grain yield

Durum wheat is one of the cereals most commonly used by food industry and cultivated around the world (Oleson 1994). Durum wheat is grown due to its grain protein and fibre content, low glycemic index, and high level of vitamins (Olmos et al. 2003). Tolerance to salt stress is crucial in durum wheat cultivation. The mechanisms of plant tolerance to salinity entail mainly excretion of salt ions (Munns and Tester 2008). Two loci: *Nax1* and *Nax2* carrying genes that control excretion of sodium ions from xylem were transferred from *Triticum monococcum* L. to salt sensitive cultivar Tamaroi of *T. turgidum* L. ssp. *durum* (Desf.) to produce a new line resistant to high salinity (James et al. 2012). A serious problem in durum wheat cultivation is its genetic capacity to accumulate cadmium (Cd) ions in the grain (Cheli et al. 2010). The EU has proposed a limit of Cd concentration in food (0.2 mg/kg fresh weight of

a product) (CEC 2006). Heavy metal pollution of soil seriously limits crop yield and quality, and affects animals, humans and the natural environment (Paradiso et al. 2008). Crop plants growing at high levels of Cd show many disorders, such as inhibition of seed germination and plant growth (Rascio et al. 1993), leaf rolling, chlorosis and necrosis (Greger et al. 1991), nutrient distribution disorder (Moral et al. 1994), reduction of chlorophyll content and photosynthesis efficiency, and stomatal closure (Clemens 2006). Jalil et al. (1994) identified durum wheat plants as more effective accumulators of Cd in the grain than bread wheat. Zhang et al. (2012) found differences between species and cultivars in the amount of absorbed Cd and resistance to its toxic effects. Cadmium increases lipid peroxidation, and protein and nucleic acid oxidation. The element inactivates several enzymes by binding with their

sulfhydryl groups (Dorta et al. 2003). The measurement of chlorophyll *a* fluorescence (ChlF) is a widely used, non-invasive method of determining photosynthesis efficiency and can also be used to predict yields of crop plants under various environmental conditions (Kalaji and Pietkiewicz 2004). The aim of the presented study was to investigate whether: (1) the degree of durum wheat resistance to soil NaCl salinity is related to the species tolerance to soil Cd contamination and (2) to search for physiological markers of the ability to accumulate Cd in the grain. We compared plant response to Cd in three durum wheat genotypes: NaCl-resistant line BC_5Nax_2, NaCl-sensitive cv. Tamaroi, and SMH87 line moderately tolerant to salinity. The defence response of the studied durum genotypes included evaluation of chlorophyll *a* fluorescence, photosynthesis efficiency, as well as yield and Cd content in the grains.

MATERIAL AND METHODS

Plant material. The line SMH87, moderately tolerant to salinity, was obtained from the Plant Breeding Centre in Smolice (Poland). The salt resistant line BC_5Nax_2, involving Nax_2 locus, and the sensitive cv. Tamaroi were obtained from Dr. Richard A. James from Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry (Canberra, Australia).

Experimental treatments. Seeds sterilised with 70% ethanol for 1 min were germinated in the dark at 4 °C for 21 days on filter paper wetted with distilled water. Next, they were sown into pots (4 L) filled with soil classified as degraded chernozem. Cadmium was added as $3 CdSO_4 \cdot 8 H_2O$ salt. Cd dose of 3 mg/kg DW (dry weight) was used in the experiment as average soil pollution with Cd ions, while 5 mg/kg DW corresponded to the maximum Cd content (Tóth et al. 2016). After adding Cd at both concentrations, soil salinity did not exceed 0.2 mS. Each pot harboured five plants, while each treatment (genotype/Cd dose) involved 10 pots. The plants were grown until full seed ripening in an air-conditioned glasshouse at 22 °C/18 °C day/night, in daylight (March–May), supplemented with light intensity by AGRO Philips sodium lamps (Philips, Aache, Germany) of 400 μmol/m²/s PPFD (photosynthetic photon flux density), up to a 16 h photoperiod. Relative humidity was 65%. The plants were fertilised with Hoagland medium (Hoagland and Arnon 1938) once a week.

Chlorophyll *a* fluorescence. Measurements involved the middle part of the flag leaf and the plant efficiency analyser (PEA) (Hansatech Ltd., King's Lynn, UK) was used. Excitation irradiance was 3 000 μmol/m²/s (peak at 650 nm). The measurements were taken after 30 min of the leaf adaptation to darkness. The ChlF parameters were calculated based on the theory of energy flow in photosystem II (PSII) and JIP test as described by Strasser et al. (2000).

Gas exchange. The gas exchange was measured in the flag leaf. Net photosynthesis rate (P_n), transpiration rate (E), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were analysed with an infrared gas analyser (CIRAS-1, Hansatech Ltd., King's Lynn, UK), with a Parkinson leaf chamber (PLC6). The irradiation system consisted of halogen lamps. The flow rate of air with constant CO_2 concentration (400 μmol CO_2/mol air) through the assimilation chamber was 300 cm³/min. The temperature of the leaves was 25 °C, the air humidity was 40%, and the irradiance 800 μmol photon/m²/s. The measurements involved 10 plants for each genotype/treatment.

Grain yield. Mature seeds were harvested and their number and dry weight per spike were calculated. Analyses were done in 25 replicates for each genotype/treatment.

Cd content in grains. The Cd content was determined as described by Ostrowska et al. (1991). The grains were dried in an air flow dryer at 65 °C for 48 h, weighed, and powdered. The milled samples were dried at 105 °C for determination of hygroscopic water and then they were mineralised at 450 °C for 12 h. The residue was dissolved in diluted nitric acid (acid:water ratio of 1:2; *v/v*). Cd content was determined using the ICP-OES method in Optima 7300DV apparatus (Perkin Elmer, Norwalk, USA). Reference material NCS DC73348 (China National Analysis Center for Iron & Steel, Beijing, China) was applied to each analytical series (Fuentes et al. 2004). Analyses were done in five replicates for each genotype/treatment.

Statistical analysis. The experiments were arranged in a fully randomised design. Two-way analysis of variance (ANOVA) and Duncan's multiple range test (at $P < 0.05$) were performed using the statistical package Statistica 13.0 (Stat-Soft, Inc., Tulsa, USA). Data were presented as means ± SE (standard error). Pearson's correlation coefficients were assumed as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Cadmium affected all investigated ChlF parameters. The most evident changes in ChlF were noted in cv. Tamaroi the most sensitive to salinity. Both Cd levels significantly declined energy absorption (ABS/CS) in cv. Tamaroi and BC_5Nax_2, while in SMH87 this only occurred for 3 mg/kg variant (Figure 1A).
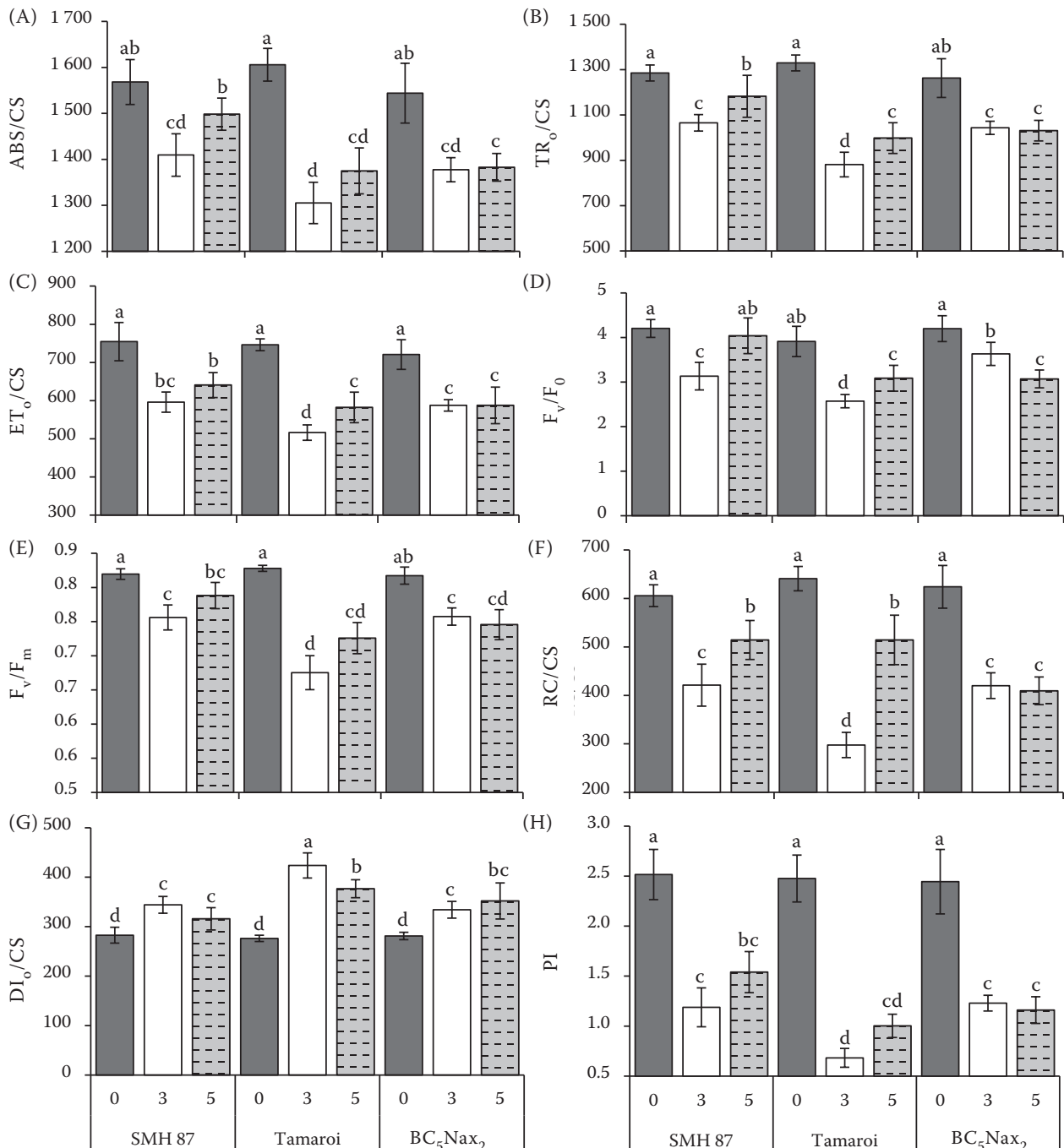

Figure 1. Changes in chlorophyll *a* fluorescence in three durum wheat genotypes grown in the soil with cadmium (Cd) at 0 mg (control), 3 mg/kg DW (dry weight) and 5 mg/kg DW. Values represent means (*n* =10) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test; $P < 0.05$). (A) light energy absorbed by leaf cross-section (ABS/CS); (B) trapped energy flux (TR_o/CS); (C) quantum yield of photosynthetic electron transport chain (ET_o/CS); (D) maximum efficiency of water photodissociation (F_v/F_0); (E) potential photochemical PSII efficiency (F_v/F_m); (F) reaction centres (RC/CS); (G) dissipation energy flux (DI_o/CS), and (H) performance index (PI)

A stream of energy resulting from the reduction of plastoquinone (TR_o/CS) thinned after Cd treatment in all plants (Figure 1B). This decrease may indicate partial inactivation of the active reaction centres (RC). Energy flux declined in all plants (Figure 1C), which is typical under stress and may be related to inactivation of RC and oxygen evolving complex. This effect was confirmed by changes in F_v/F_o, describing maximum efficiency of water photodissociation (Figure 1D). Potential photochemical efficiency (F_v/F_m) dropped in all plants at 3 Cd mg/kg DW, whereas in cv. Tamaroi and BC_5Nax_2 this effect occurred also at higher Cd dose (Figure 1E). The density of RC decreased under stressful conditions (Figure 1F). The strongest Cd-induced decline in RC/CS was observed in cv. Tamaroi. An increase in energy dissipation (DI_o/CS) noticed in all plants indicated a decrease in the energy necessary for photochemical transformations (Figure 1G). This effect was particularly pronounced in cv. Tamaroi plants. The strongest reduction of the photosynthetic performance index (PI) at both Cd doses was visible in cv. Tamaroi (Figure 1H). Negative effects of Cd observed by Paunov et al. (2018) in durum wheat also involved five-fold suppression of the efficiency of energy transformation in PSII, and a disruption in oxygen-evolving complex. This effect on ET_o/CS and F_v/F_0 was manifested mainly in cv. Tamaroi.

A decline in P_n occurred in SMH87 line at 5 mg Cd/kg DW and in BC_5Nax_2 line exposed to 3 mg Cd/kg DW (Figure 2A). In cv. Tamaroi no Cd effect on P_n was observed, although all ChlF parameters were clearly reduced in this cultivar. Transpiration rate decreased in all plants only at higher cadmium concentration (Figure 2B). Stomatal conductance dropped gradually along with increasing Cd dose in cv. Tamaroi and BC_5Nax_2, while in SMH87 the stomata were more closed in the plants exposed to 5 mg Cd/kg DW (Figure 2C). This caused a dramatic reduction of internal CO_2 concentration (C_i) (Figure 2D). Hart et al. (1998) stated that stomata closure is one of the mechanisms preventing Cd influx into the transpiration stream. Significant correlation was found between C_i and g_s and E and it amounted to $r = 0.824$ ($P < 0.05$) and $r = 0.794$ ($P < 0.05$), respectively, but C_i did not correlate with P_n. Harley and Sharkey (1991) reported that P_n may decline at high CO_2 concentration.
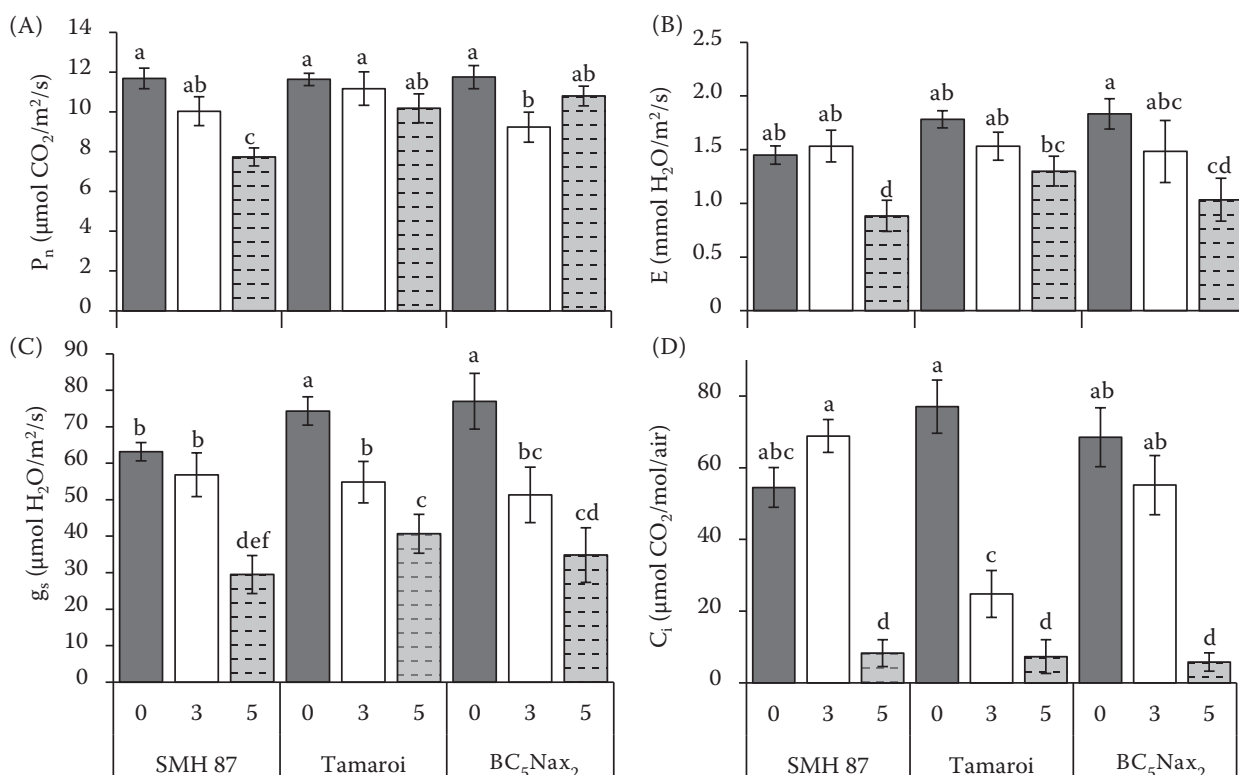

Figure 2. (A) Net photosynthesis (P_n); (B) transpiration rate (E); (C) stomatal conductance (g_s) and (D) internal CO_2 concentration (C_i) in the leaves of durum wheat genotypes grown in the soil with cadmium (Cd) at 0 mg (control), 3 mg/kg DW (dry weight) and 5 mg/kg DW. Values represent means ($n = 10$) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test, $P < 0.05$)
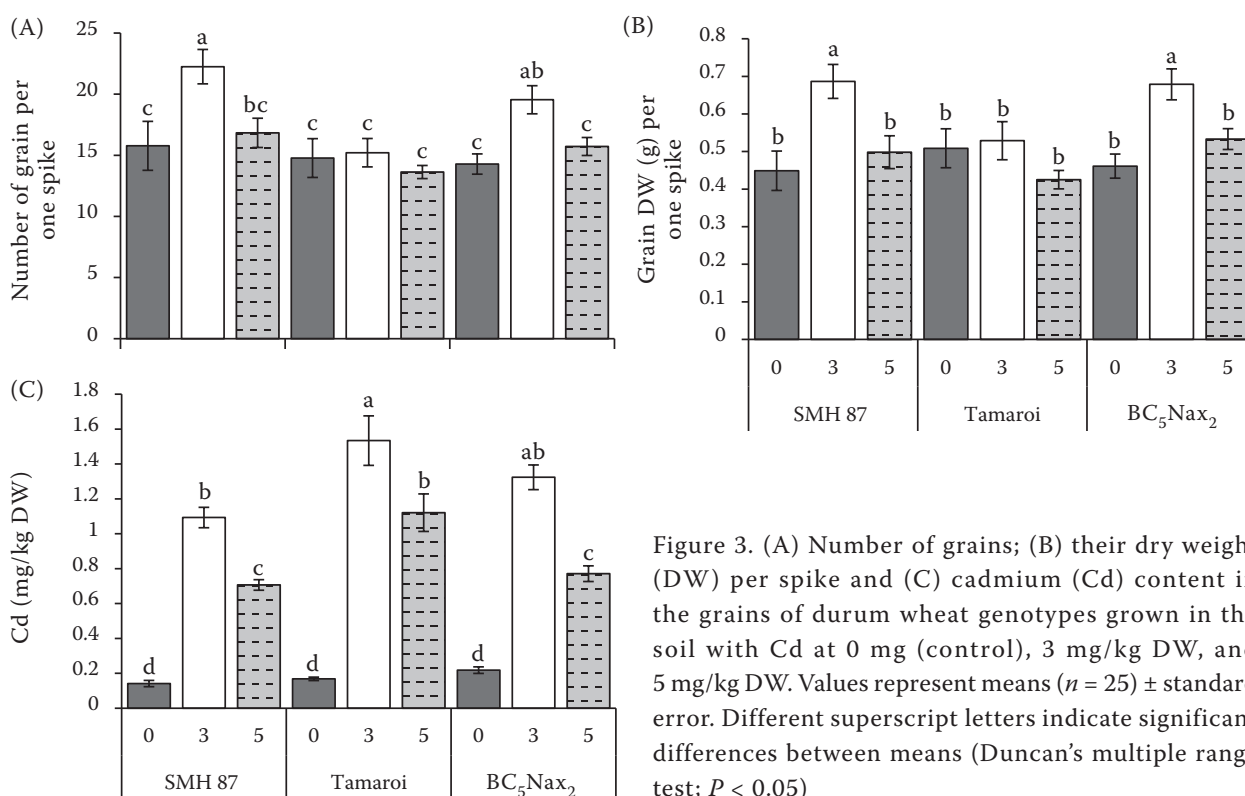
Figure 3. (A) Number of grains; (B) their dry weight (DW) per spike and (C) cadmium (Cd) content in the grains of durum wheat genotypes grown in the soil with Cd at 0 mg (control), 3 mg/kg DW, and 5 mg/kg DW. Values represent means ($n = 25$) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test; $P < 0.05$)

Cadmium did not affect the yield of cv. Tamaroi (Figures 3A,B). The number of grains and their dry weight were the highest in SMH87 and BC_5Nax_2 lines grown at 3 mg Cd/kg DW, as compared with the control and the plants exposed to 5 mg Cd/kg DW. Moral et al. (1994) reported that Cd may provoke phytotoxic effects even at relatively low concentrations. In the studied plants, Cd content in the grains increased at both applied Cd doses *vs*. control (Figure 3C). The grains of the plants grown at 5 mg Cd/kg DW accumulated less Cd than those of the plants grown at 3 mg Cd/kg DW. Cv. Tamaroi grains accumulated greater Cd amounts at 3 mg Cd/kg DW as compared with SMH87, and greater Cd amounts at 5 mg Cd/kg DW than SMH87 and BC_5Nax_2 lines. Cadmium accumulation in the seeds correlated negatively with most of the studied ChlF parameters (Table 1). Moral et al. (1994) demonstrated lowered tomato yield in Cd presence but no changes in fresh weight of the fruit. Dai et al. (2017) revealed that the introduction of nutrients, namely nitrogen and sulphur, could be an important factor of wheat response to Cd. Coordination between N and S assimilation can strengthen plant defence mechanisms and effectively alleviate Cd negative effects (Khan et al. 2016). Gill et al. (2012) reported that high Cd concentrations

in the soil disturb photosynthesis and coordination between carbon, nitrogen, and sulphur metabolism. On the other hand, *Fagopyrum tararicum* plants exogenously treated with sulphur increased Cd uptake in the root vacuoles (Lu et al. 2019). Decreased cadmium translocation to the leaves can result from its chelation and vacuolar sequestration by non-

Table 1. Correlation coefficients for cadmium (Cd) amount in the grains (mg/kg dry weight) and chlorophyll fluorescence (ChlF) parameters of all studied durum wheat plants grown in the presence of Cd

ChlF	Cd amount in grains
PI	0.588***
ABS/CS	0.446**
TR_o/CS	0.530***
ET_o/CS	0.448**
DI_o/CS	0.465***
RC/CS	0.577***

**$P < 0.01$; ***$P < 0.001$; PI – performance index; ABS/CS – energy absorption; TR_o/CS – reduction of plastoquinone; ET_o/CS – quantum yield of photosynthetic electron transport chain; DI_o/CS – energy dissipation; RC/CS – reaction centres

protein thiols, and its inhibited transport from roots to shoots. We can assume also that higher Cd amount was toxic enough to block the transport of assimilates and ions to the seeds. Hart et al. (1998) showed that Cd uptake rates in xylem translocation to the shoots of durum wheat were not responsible for increased Cd accumulation in mature grains. Lachman et al. (2015) observed the effect of cadmium on essential elements transport into the plant. Especially, cadmium had an antagonistic influence of calcium and manganese. The adverse effects of heavy metal ions, including cadmium, are similar to the influence of the salinity caused by sodium and chlorine ions, on damage to cytoplasmic membranes and induction of osmotic stress. According to Munns and Tester (2008) osmotic stress evokes stronger effects than ionic stress. Both salinity and heavy metal ions cause cell dehydration, an increase in plasma membrane permeability, a decrease in photosynthesis rate and generation of oxidative stress, which evoke a decrease in yield (Munns and Tester 2008, Li et al. 2010, Kalaji et al. 2011). We showed a negative effect of salinity on the growth and the photosynthetic efficiency of *Miscanthus × giganteus* in previous work (Płażek et al. 2014). Moreover, our experience gained in research on the effects of high and low temperature indicates that the parameters of chlorophyll fluorescence strongly correlate with the yield obtained from plants grown under these stress conditions (Płażek et al. 2018, Hornyák et al. 2020). The photosynthetic apparatus is very sensitive to various environmental stresses, and therefore the plant's ability to maintain normal photosystems function correlates with the plant tolerance to unfavourable environmental conditions. Summing up, Cd accumulation was the highest in the grains of the salt sensitive cultivar. The decrease in the efficiency of the photosynthetic apparatus could play an important role in Cd deposition in the seeds. It may be supposed that the tolerance to Cd and NaCl stresses has a common physiological background.

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