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Mario Franić, mag. educ. biol. et chem.

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Učinci kadmija na parametre fotosinteze u različitim genotipovima kukuruza

Mario Franić

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Kratki sažetak doktorske disertacije:
Kadmij uzrokuje mnoge negativne posljedice u funkcionalnosti biljaka, posebno u fotosintetskoj aktivnosti. U ovom istraživanju korištena su četiri genotipa kukuruza kako bi se procijenio utjecaj akumuliranog kadmija u listu ispod klipa na fotosintetsku učinkovitost. Izdvojila su se dva genotipa sa visokom akumulacijom kadmija (Mo17, Os6-2) i dva genotipa sa niskom akumulacijom kadmija (B73, B84). Inbred linija B73 se može smatrati tolerantnom na kadmij s obzirom na minimalna smanjenja in PI_{ABS} i PI_{total} vrijednosti, dok se linija B84 može smatrati osjetljivom na kadmij. Ovi rezultati mogu se koristiti u oplemenjivanju kukuruza za razvoj genotipova kukuruza sa visokom akumulacijom kadmija za fitoekstrakciju, te za razvoj genotipova sa niskom akumulacijom kadmija za proizvodnju na tlu kontaminiranom kadmijem.

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1. prof. dr. sc. Zdenko Lončarić, redoviti profesor Poljoprivrednog fakulteta u Osijeku; predsjednik
2. prof. dr. sc. Hrvoje Lepeduš, redoviti profesor Filozofskog fakulteta u Osijeku; član
3. prof. dr. sc. Vera Cesar, redovita profesorica Sveučilišta Josipa Jurja Strossmayera u Osijeku, Odjela za biologiju; član
4. doc. dr. sc. Ivna Štolfa Čamagajevac, docentica Sveučilišta Josipa Jurja Strossmayera u Osijeku, Odjela za biologiju; zamjena

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Effects of cadmium on photosynthetic parameters in different maize genotypes

Mario Franić

Thesis performed at: Agricultural Institute Osijek; Faculty of Agriculture in Osijek, Department for Agroecology

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Short abstract:
Cadmium causes many adverse effects in plant functionality, especially in photosynthetic activity. In this research four maize genotypes were used to assess the impact of cadmium uptake in maize ear-leaves on their photosynthetic performance. Two high cadmium accumulating (Mo17, Os6-2) and two low cadmium accumulating (B73, B84) genotypes were identified. The inbred line B73 can be considered cadmium tolerant according to minor decreases in PI_{ABS} and PI_{total}, while B84 could be considered cadmium sensitive. These results could be used in maize breeding for development of high cadmium accumulation genotypes for phytoextraction and for production of low cadmium accumulation maize on cadmium contaminated soil.

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Reviewers:
1. Zdenko Lončarić, PhD, full professor at Faculty of agriculture in Osijek
2. Hrvoje Lepeduš, PhD, full professor at Faculty of humanities and social sciences in Osijek
3. Vera Cesar, PhD, full professor at University of Josip Juraj Strossmayer in Osijek, Department of biology
4. Ivna Štolfa Čamagajevac, PhD, assistant professor at University of Josip Juraj Strossmayer in Osijek, Department of biology

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1. Introduction

Maize (*Zea Mays* L.) is one of the most important cereal crops in the world, in 2015 it accounted for 18.6% of the cereals produced in the EU-28 (source: Eurostat). It is known as one of the few major cultivated species in the Western Hemisphere about 7 000 – 10 000 years ago (Wilkes 2004). Botanically, maize is an annual grass that belongs to *Poaceae* family. Apex of the maize stem ends in tassel, an inflorescence of male flowers. Female inflorescences are wrapped in several layers of ear leaves and develop around the midsection of the plant. Female inflorescences mature to become edible ears with usually 300 – 1000 kernels. Maize has a high economic value as it is a major staple food and a source for many industrial applications. Besides the economic importance, maize has been widely used as a model organism for basic, translational and applied research.

Agricultural production worldwide is affected by different abiotic stress conditions which cause extensive agronomic and economic losses (Boyer 1982). Abiotic stress conditions such as heat, drought, salinity, heavy metal stress and low temperatures have been subjects of intense research (Santa di Toppi and Gabrielli 1999, Bray et al. 2000, Cushman and Bohnert 2000). Majority of abiotic stress experiments are conducted in controlled conditions and therefore do not reflect actual conditions that plants are subjected to in the field which are always a specific combination of different stresses.

Heavy metals are elements with atomic number higher than 20. They are conventional elements with various properties (conductivity, ligand properties, stability as cations, etc.). Some of them, like copper, zinc, magnesium, iron, nickel and cobalt, are essential micronutrients crucial for normal functioning of plant metabolism acting as enzyme cofactors and participating in redox reactions (Santa di Toppi and Gabrielli 1999). Other heavy metals, such as cadmium, mercury and lead, generally have no role in the metabolism and, like their essential counterparts, when present in excess can become extremely toxic.

Cadmium (Cd), as a non-essential metal, causes many adverse effects in plant functionality. Once accumulated by the plant, it causes damage of various molecular mechanisms and cell compartments (Das et al. 1998). Exposure of plants to cadmium leads to alterations in many cellular processes and functions such as photosynthetic activity, antioxidant activity, ion channels and plant water status and redox imbalance (Perfus-Barbeoch et al. 2002, Ortega-Villasante et al. 2005, Ekmekçi et al. 2008, Sharma and Dietz 2009), reduction of cell proliferation and growth (Schützendübel et al. 2001). Key sources of excess cadmium concentrations in soils are antropogenic activities such as traffic, industry, and application of phosphate fertilizers (Di Toppi and Gabrielli 1999). Its uptake by roots is mostly a transpiration driven passive process; although it has no physiological role, plants have no cadmium exclusion mechanism (Gallego et al. 2012). Mechanisms for cadmium to enter plant cell include the transport systems involved in micronutrient uptake, specifically through transmembrane divalent metal carriers.

Contamination of soil by single metal is rare and usually where one metal is concentrated there are others present in higher concentrations as well. Metals also tend to interact, and the

interactions are hard to predict. It is generally assumed that heavy metals enter plant cells through transporters of essential metals and heavy metal uptake is in competition with essential metals uptake, such as potassium, calcium, magnesium, iron, manganese (Santa di Toppi 1999, Perfus-Barbeoch et al. 2002). The cause of Cd toxicity could originate from its similarity to zinc (Zn) which is an essential metal to biological systems and an important factor in the protection of biological membranes against oxidative stress (Aravind and Prasad 2003); by competing with divalent cations for protein binding sites it causes imbalances in essential metals (Cakmak et al. 2000). There is a significant variation in affinity for heavy metals accumulation among as well as within plant species (Grant et al. 1998). Maize inbreds have been shown to differ in uptake of heavy metals (Florijn and van Beusichem 1993, Brkić et al. 2003) and temperate inbred lines B84 and Os6-2 have been designated as different according to their respective ionomic profiles (Sorić et al. 2011, Šimić et al. 2012) and leaf cadmium accumulation (Sorić et al. 2009).

Large number of studies has been done on the effect of cadmium on photosynthetic machinery – from isolated thylakoid membranes to hydroponically grown plants but relatively few studies have been done on the influence of cadmium on the photosynthetic apparatus in plants grown from seeds on soil polluted with cadmium (Baryla et al. 2001). It was previously shown that soil affects uptake of heavy metals and influences their toxicity (Clijsters and Van Assche 1985). One of the oldest approaches to photosynthesis research is chlorophyll *a* fluorescence, with first such experiments dating back to more than 80 years ago (Kautsky and Hirsch 1931). In the 1960s Kautsky and coworkers observed an increase in the yield of chlorophyll fluorescence when photosynthetic material was transferred from dark into the light. Plotting chlorophyll fluorescence values, on a logarithmic scale, from minimum F0 (O) to maximum Fm (P) reveals a polyphasic rise, with two intermediate steps J and I (Strasser and Govindjee 1992). A procedure for quantification of this fluorescence transient (O-J-I-P), known as JIP test, was developed by Strasser and Strasser (1995). These fluorescence transients, along with phenomenological and biophysical parameters, have shown to be reliable indicators of stress (Krause and Weiss 1991; Schreiber et al., 1994; Tsimilli-Michael et al., 1999). The JIP-test is being used in investigating stress physiology in a number of plant species under controlled and field conditions (Reddy and Strasser 2000). Analysis of the chlorophyll fluorescence increase, known as JIP test (Strasser and Strasser 1995) gives information about changes in photochemistry efficiency and heat dissipation and is widely used for assessment of plant reaction to various types of stress conditions (Appenroth et al. 2001, Hermans et al. 2003). Negative effects of cadmium on photosynthesis, especially on PSII, are easily detectable using methods which measure chlorophyll *a* fluorescence (Drazkiewicz et al. 2003, Mallick and Mohn 2003, Cherif et al. 2012).

1.1. Literature review

1.1.1. Photosynthesis

Photosynthesis is a process that involves a chain of oxidation and reduction reactions by which light energy is transformed into chemical energy stored in organic molecules. Plants, algae and cyanobacteria release oxygen as a byproduct of photosynthesis. Two separated groups of reaction are involved in the process of photosynthesis: light or primary reactions

and secondary reactions. Primary reactions include absorption of light that is used to synthesize ATP and NADPH. In these reactions water is oxidized and oxygen is released. In secondary reactions (Calvin cycle) energy stored in ATP and NADPH is used to reduce CO_2 to sugar. These reactions can take place in the light or in the dark. Light reactions take place in the thylakoid membranes of the chloroplast and the processes of the Calvin cycle take place in the chloroplast stroma. Energy that enters light reactions is linearly proportionate to light intensity, but it is temperature independent. Energy consumed in secondary reactions is temperature dependent but independent on the light intensity Disturbances in energy supply and demand can cause thylakoid membrane damage. Besides the photochemical processes, part of absorbed energy can be lost as heat dissipation which can be measured as chlorophyll *a* fluorescence. Through chlorophyll *a* fluorescence energy distribution between photochemical and non-photochemical processes can be quantified (Pevalek-Kozlina 2003).

Two main types of fluorimeters are used to measure chlorophyll *a* fluorescence: PAM (pulse amplitude modulation) fluorimeters measure fluorescence induced by modulated light (Schreiber 2004) while devices like PEA (plant efficiency analyzer) induce fluorescence with continuous light.

1.1.2. Biophysics of light absorption and energy distribution

Light is electromagnetic radiation, radiation range between 400 and 700 nm is photosynthetically active meaning that plant pigments can use this radiation range to transform it to chemical energy through photosynthesis. Pigments absorb light in the visible spectra. Chlorophyll *a* best absorbs light at 430 and 662 nm wavelengths while chlorophyll *b* best absorbs light at 453 and 642 nm wavelengths which correspond to blue and red part of the spectra. Middle part of the spectra (520-570) is not used for photosynthesis and it corresponds to green part of the spectra (Pevalek-Kozlina 2003).

Photosynthetic pigments that absorb light are connected to two protein complexes: photosystem II (PSII) and photosystem I (PSI). Photosynthesis in higher plants takes place in chloroplasts which are organelles 5-10 μm in size located in mesophyll cells of the leaf (Pevalek-Kozlina 2003). Chloroplasts have two membranes (inner and outer) and an intermembrane space between them. Interior of chloroplasts is filled with liquid matrix called stroma with a continuous system of thylakoid membranes. Reactions of energy transformation, electron transport and formation of proton gradient take place on thylakoid membranes. Two types of thylakoid membranes are in the chloroplast: granum and stromal thylakoids. Granum thylakoids are arranged in parallel stacked one on top of the other and stromal thylakoids go through the stroma connecting granum thylakoids (Figure 1) (Alerts et al. 2002).

Main protein complexes of the photosynthetic electron transport chain are PSII, cytochrome b_6f complex (cyt b_6f), PSI and ATP synthase.

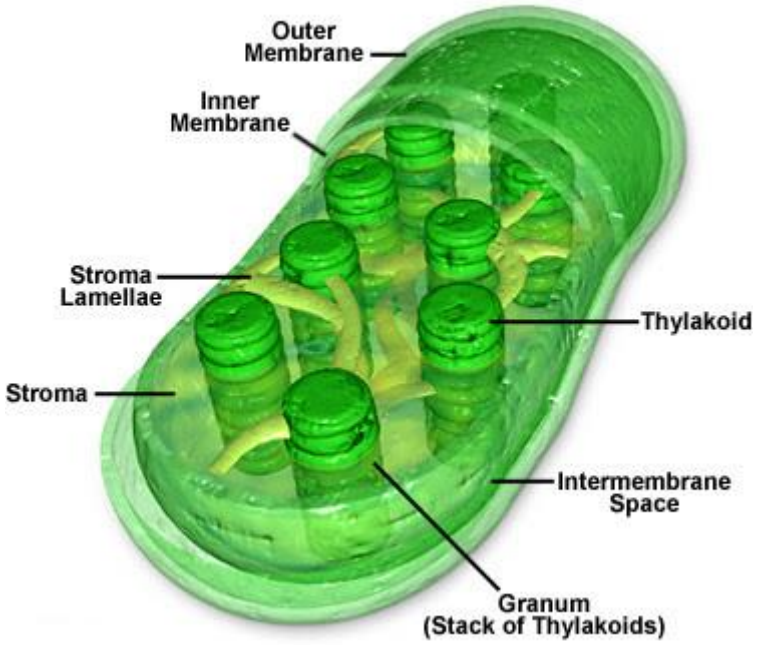


Figure 1. Schematic model of a chloroplast (from http://www.biologyjunction.com/chloroplast_diagram.htm)

1.1.3. Structure of main proteins of the photosynthetic electron transport chain

1.1.3.1.Photosystem II (PSII)

Photosystem II is built from light harvesting complexes (LHCII) and reaction centers. LHCII complexes include hundreds of pigment molecules, mainly chlorophylls *a, b*, and carotenoids. Antenna systems are built from an internal antenna that s located near the reaction center and external antennas. PSII is usually organized as a dimer where every monomer of the PSII represents one functional unit (Shen et al. 2008). Internal antennas of PSII have two subunits CP43and CP47 while external part of the LHCII consists of several subunits.

External antennas of LHCII absorb more than half of the photons in PSII, while internal antennas function as absorption units and a link between external antennas and reaction centers. External antennas are usually trimers in which every unit includes chlorophylls *a* and *b* and four binding sides where one of them (L2) can bind carotenoids of the xanthophyll cycle.

Core of PSII is the reaction center built from 17 transmembrane protein subunits and 3 external proteins. Transmembrane proteins are D1 and D2, CP47, CP43, twelve subunits (E, F, H, I, J, L, M, Tc, X) and Ycf12. Three external proteins (with sizes of 33, 23 and 17 kDa) are connected to the lumen side. These external proteins, D1, D2, CP43 and CP47 subunits with manganese cluster (Mn_4Ca) form the oxygen-evolving complex (OEC) (Shen et al. 2008).

Four chlorophyll and two pheophytin (Pheo) molecules are located in the center of D1 and D2 subunits. Two chlorophyll molecules are located between D1 and D1 proteins while other two are symmetrically connected to D1 and D2 proteins. Two pheophytin molecules are linked to D1 and D2 subunits. Primary electron acceptor molecule, which is plastoqinone (Q_A), is located on the D2 protein and the secondary electron acceptor is on the D1 protein.

1.1.3.2.Photosystem I (PSI)

Components of photosystem I are light harvesting complexes and reaction centers. This membrane complex transfers electrons from plastocyanin (PC) to feredoxin (Fd) (He and Malkin 1998). Photosystem I antennas are built from four different LHCI proteins: Lhca1, Lhca2, Lhca3 and Lhca4 which form dimers Lhca1-Lhca4 and Lhca2-Lhca3 and they form a belt around the reaction center. Reaction center of photosystem I is a heterodimer built from 12 Psa protein subunits (PsaA to PsaL) and about 100 chlorophyll molecules (Amuntus et al. 2008). Primary electron acceptor (P700) consists of two chlorophyll *a* molecules and is located between PsaA and PsaB subunits (He and Malkin 1998).

1.1.3.3.Cytochrome b_6f complex (Cyt b_6f)

Cytochrome b_6f is a membrane protein complex acting as a plastokinon-plastocyanin oxidoreductase and is involved in the formation of proton gradient that is used in ATP synthesis (Clark and Hind 1983). In the reactions of photosynthesis cytochrome b_6f is a link between PSII and PSI (Cramer et al. 1996). This complex is a dimer formed from four large and four small subunits. Large subunits are cytochrome *b6*, cytochrome *f*, Rieske-iron sulphur protein and subunit VI. Small subunits are PetG, PetL, PetM and PetN (Baniulis et al. 2011).

1.1.3.4.ATP-synthase

ATP-synthase is a membrane protein complex consisting of two large subunits: CF_0 and CF_1. Hydrophobic CF_0 subunit is formed from four subunits (I, II, III and IV). Hydrophilic CF_1 subunit consists of five subunits (α, β, γ, δ, ε) and it is located above CF_0 on the stoma side of the membrane. Two units are connected where CF_0 unit is a proton channel and the CF_1 unit controls the proton flow. The difference in proton concentration between the membranes creates electrochemical gradient which runs ATP-synthase which creates ATP from ADP and Pi. Plastoquinon (PQ) binds protons on the stroma side of thylakoid membrane and transports them into the lumen (stroma pH under light is 7.95 and lumen pH is 5.6). Protons can move through thylakoid membrane only through ATP-synthase.

PSII, cytochrome b_6f, PSI and Fd-$NADP^+$-oxidoreductase are connected through mobile components of the electron transport chain and they are plastoquinon (PQ), plastocyanin (PC) and ferredoxin (Fd).

1.1.4. Photosynthetic electron transport

Light is absorbed by antenna complexes and transfer the excitation energy to PSII and a chlorophyll molecule on the D1 protein where charge separation occurs. During the charge separation an electron is transferred from primary electron donor (P680) to pheophytin (Pheo) and P680 becomes reduced ($P680^+$). Final electron source is water that gets oxidized in the oxygen-evolving-center (OEC) and the released electrons replace electrons from PSII reaction

center. Oxidation of water releases oxygen (O_2) and four protons which are released in the lumen. Four electrons are donated to $P680^+$. Pheo transfers electrons to primary electron acceptor (Q_A) which is immobile. Electrons are further transferred to mobile secondary electron acceptor (Q_B). After double reduction and protonation (two protons from stoma) Q_B is released (as plastohydroquinon PQH2) from the binding side and is diffused into the thylakoid membrane (Barber 1997). Mobile PQH2 can freely pass through thylakoid membrane and transfer electrons to FeS protein on the acceptor side of cytochrome *b_6f* and then to cytochrome *f*. Two protons are released into the lumen by plastoquinon (PQ). One proton is transferred to $NADP^+$ through cyt *b_6f* and PC and the other one is transferred back to PQ through cyt *b_6f* (Q cycle). Proton gradient is formed during the transfer of protons from stroma to lumen. This gradient runs ATP-synthase. Through the electron transport chain electros are transferred to PSI reaction center (P700) and ATP is formed in the process of non-cyclic photophosphorylation (Figure 2). Last step in this electron transfer is transfer of electrons to ferredoxin which reduces $NADP^+$ to NADPH and the reaction is catalyzed by ferredoxin-$NADP^+$-oxidoreductase. NADPH is used in Calvin-Benson cycle (Berg et al. 2013).

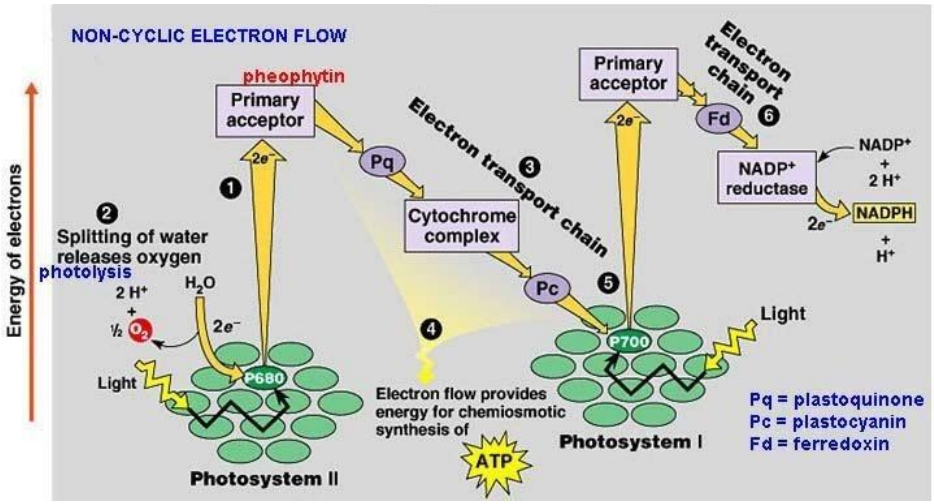


Figure 2. Schematic representation of non-cyclic photophosphorylation (from https://www.quora.com/What-is-the-final-electron-accepter-in-noncyclic-photophosphorylation)

PSI can function independently of PSII when the ratio of NADPH to $NADP^+$ is high (there is not enough of $NADP^+$ to accept electrons from reduced ferredoxin) in the process of cyclic electron transport (cyclic phosphorylation). In this way electrons are transferred from reaction center of PSI to Fd or NADPH through NADP(H) dehydrogenase and ferredoxin-quinon-oxidoreductase (FQR) (Cleland and Bendall 1992) to PQ. PQ is oxidized through cyt *b_6f* and the reduction of PC. PC oxidizes with the transfer of electrons to reaction center of PSI (P700) (Figure 3.). This electron transport generates proton gradient for the function of ATP-synthase. Through the reactions of photosynthetic electron transport transformed light energy is stored in the form of NADPH and ATP that are used as an energy source for the reactions of CO_2 assimilation.

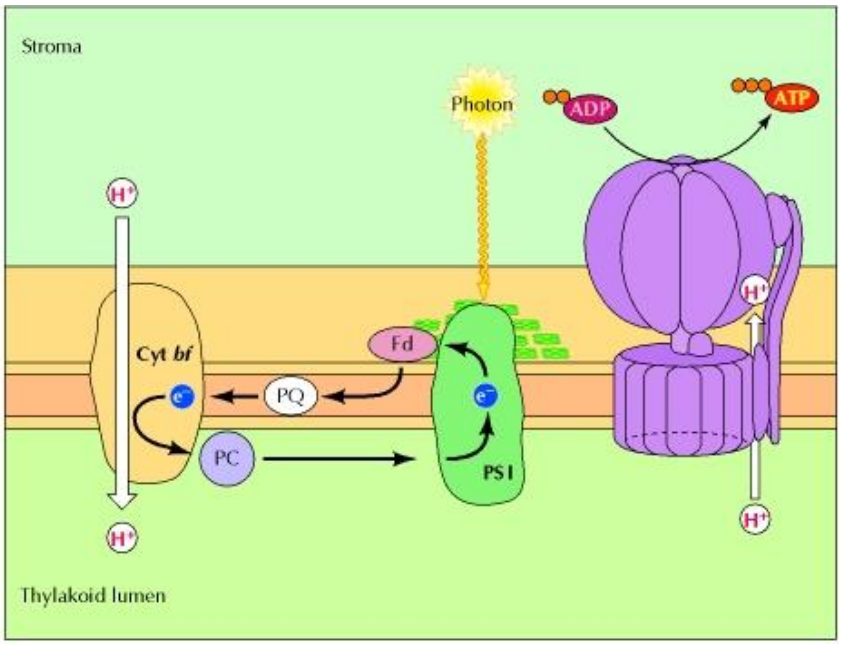


Figure 3. Schematic representation of cyclic phosphorylation (from http://biology-themiracleoflife.blogspot.com.tr/2011/03/photosynthesis-cyclic-electron-flow.html)

One of the approaches of photosynthetic efficiency research is chlorophyll *a* fluorescence measurement. It is a non-destructive method for *in vivo* measurement of energy distribution in PSII.

1.1.5. Chlorophyll *a* fluorescence

Approximately 1-2 % of total absorbed light is emitted as chlorophyll *a* fluorescence, hence this fluorescence represents only a small amount of dissipation energy and it carries the information on the structure and functioning of electron transport (Strasser et al. 2004). Light energy that is absorbed can be used in three ways: for running photosynthetic processes, it can be thermally dissipated and it can be dissipated in the form of photons as excitation energy. These processes are connected and changes in one cause changes in the other two, hence chlorophyll *a* fluorescence can be used to gain information on photosynthetic efficiency and thermal dissipation (Maxwell and Johnson 2000). Recording the fluorescence emitted from chlorophyll molecules is a widely used non-destructive tool in the research of photosynthesis and it has allowed an increased understanding of both photochemical and non-photochemical processes. The availability of commercial devices enabled accurate and easy measurements of chlorophyll fluorescence even in the field conditions.

1.1.6. Polyphasic chlorophyll *a* fluorescence rise

Chlorophyll fluorescence represents a small deactivation process for excited chlorophyll molecules, but the time-course provides useful insight into utilization of excitation energy by PSII and other complexes within the thylakoid membrane, although indirectly (Waker 1987). For chlorophyll *a* fluorescence, leaves (usually) need to be dark adapted and the measurement itself last for 1 second. Dark adaptation of 30 minutes is usually enough to open all reaction centers. Open PSII reaction centers (Q_A oxidized) emit 2 % of absorbed light as fluorescence

and closed reaction centers (Q_A reduced) emit 8-10 % of absorbed light as fluorescence. The sample is illuminated with continuous red light (~650 nm) and high light intensity (about 3000 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$). Changes that occur in the chlorophyll fluorescence intensity during illumination of dark adapted sample are known as "Kautsky effect" (Strauss et al. 2006). On the onset of illumination fluorescence yield is at minimum (F_0 or O). Illumination causes reaction centers to close (due to charge separations in PSII) and the intensity of fluorescence rises accordingly, reaching maximum value (F_m, F_P or P) at 1 second (Strasser et al 2004).

During the 1 second measurement the fluorescence curve quickly rises (polyphasically) after which a gradual decline to steady state occurs (Strauss et al. 2006). It is assumed that the polyphasic rise in fluorescence is an indicator of primary reactions (Krause and Weis 1991). When dark adapted leaves (usually 30 minutes) are illuminated with saturating light (usually 3000 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$) maximum fluorescence is reached (F_m) and two intermediate steps can be detected on the chlorophyll fluorescence transient curve: J step (at ~ 2 ms) and I step (at 30 ms). Since the steps are called O, J, I and P this polyphasic chlorophyll *a* transient curve is called the OJIP curve (Figure 4). A new peak can sometimes occur at 300 µs under high temperature or specific heavy metal stress and is called K step (Kalaji and Loboda 2007, Lazar 1999). Appearance of this step suggests that the donor side of PSII is damaged (or more specifically, impairment Mn cluster in oxygen evolving center in a considerable percentage of PSII reaction centers).

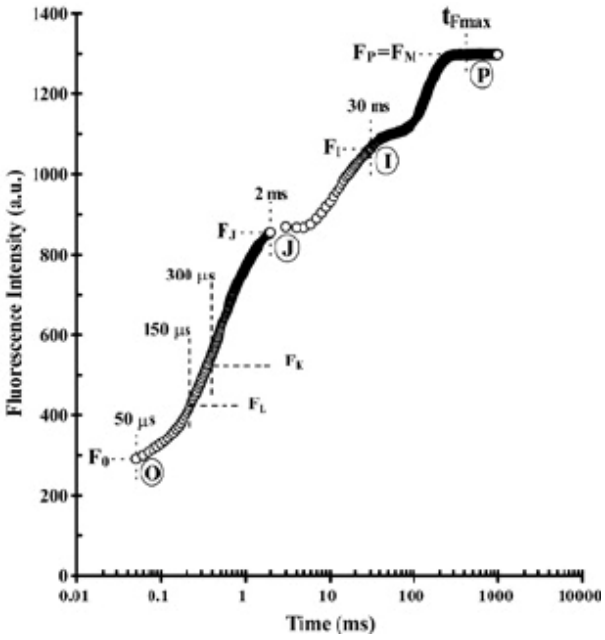


Figure 4. Typical chlorophyll *a* polyphasic fluorescence rise (OJIP). Plotted on a logarithmic time scale as presented by Strasser et al. (2004).

Data extracted from the recorded fluorescence transients (OJIP) can be directly used as parameters of chlorophyll fluorescence, like minimal fluorescence intensity (F_0), maximal fluorescence intensity (F_M), time (in ms) to reach maximum fluorescence (t_{fm}) among others.

From this extracted parameters others can be derived, like maximum variable fluorescence (F_v), initial slope of the fluorescence transient (M_0) and so on.

Based on the theory of energy fluxes through thylakoid membranes the JIP test (Strasser and Strasser 1995) indirectly provides information on the structure and functioning of the photosynthetic apparatus and its variables explain energy flow through PSII. JIP test provides information on the specific energy fluxes (per Q_A reducing PSII reaction center), quantum yields (for primary photochemistry and electron transport), and performance indexes which are products of terms expressing partial potentials at steps of energy bifurcations.

Photosynthesis is a process which is susceptible to stressful conditions and in field conditions it often functions in unfavorable conditions. Chlorophyll fluorescence measurements are used to detect changes in organization and functioning of PSII under stressful conditions and it has been used in physiological and ecophysiological investigations of various plant species. It has been used to study various types of stresses, like drought (Goltsev et al. 2012), high temperature (Toth et al. 2005), and heavy metal (Perreault et al. 2010).

1.1.7. Effect of environmental and abiotic factors on plant growth and development

During vegetation period plants are often exposed to various adverse environmental effects that influence their growth and development and in the end resulting in reduction of yield and its quality. Conditions or substances that negatively affect plant growth, development and metabolism are considered as stressful factors (Lichtenthaler 1996) and they can be classified as biotic or abiotic (Boyer, 1982). Abiotic stress factors occur due to extreme physical, chemical and environmental conditions such as salinity, UV radiation, drought, heavy, metals, temperature extremes, nutrient deficiency, air pollution, herbicides, etc. (Singh Gill and Tuteja 2010). Biotic stress factors are biological and are caused by other organisms like pathogenic microorganisms, insects, herbivores etc. (Gaspar et al. 2002). If the plants´ tolerance limit and adaptability to stress is exceeded it can cause permanent damage or even plant death (Pahlich 1993). Plant response to a certain stress factor can depend on the intensity and the duration of the stress factor (Kranner et al. 2010).

Exposure of plants to stress factors, either biotic or abiotic, causes disturbances in plant metabolism and physiological processes which ultimately leads to yield decreases. The effects of stress factors on plant are mostly conducted in controlled laboratory conditions which do not reflect environmental conditions to which plants are exposed during growth and usually include more than one abiotic or biotic stress factors (Moffat et al. 2002).

Environmental conditions that plants grow in affect plant growth and development and if these conditions are not optimal they will become limiting factors of plant productivity. In order to overcome these restrictions and increase plant production it is necessary to develop and produce cereal cultivars with higher yield potential and greater yield stability (Khush 1999).

Since plants are rooted in the ground they cannot escape from stress factors and these unfavorable environmental conditions tend to express themselves in plants as oxidation and the increase in generation of reactive oxygen species (ROS) which cause various damages in

the plant including lipid peroxidation, protein and amino acid oxidation, DNA damage and cell death (Mittler 2002, Wang et al. 2003) (Figure 5). In general, ROS are normal by products in different metabolic reactions and are localized in different cell compartments (chloroplasts, mitochondria, peroxisomes).

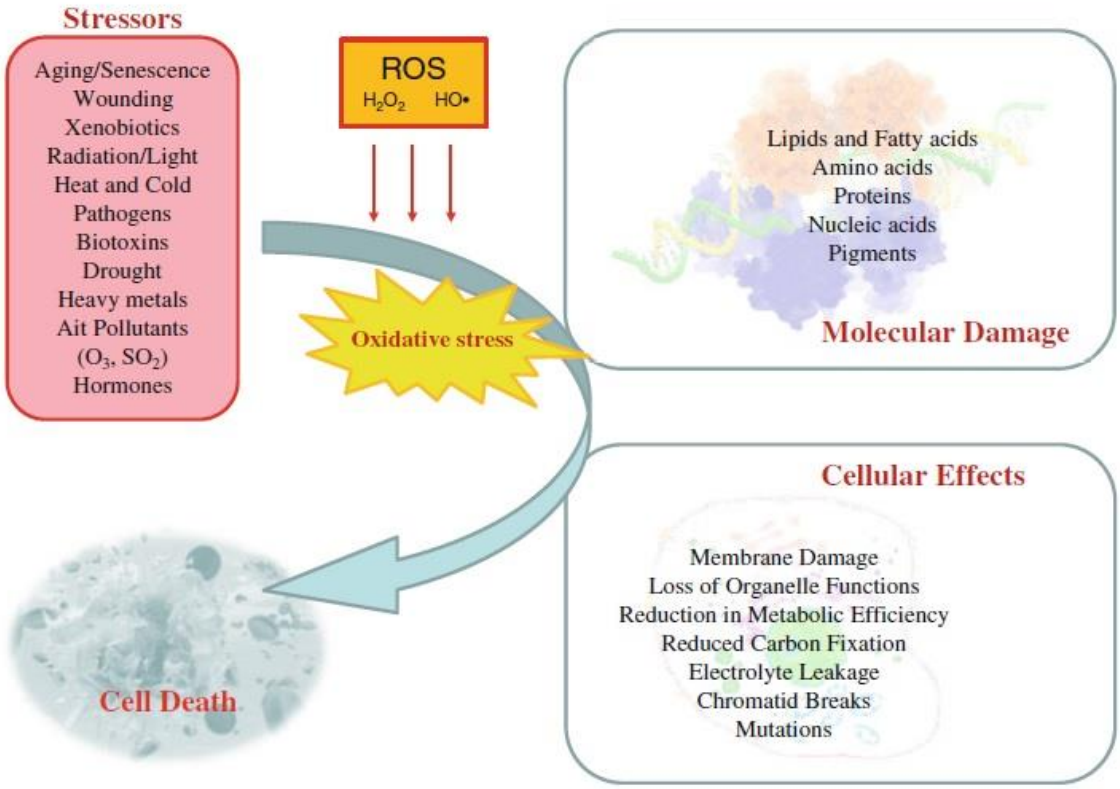


Figure 5. Initiators of reactive oxygen species (ROS) and different physiological dysfunctions caused by ROS (Zitka et al. 2013)

During their evolution plants have developed different mechanisms to cope with various stresses they are exposed to that secure their adaptation and survival. In contrast to this, plants can also display different levels of sensitivity depending on the environmental conditions, plant species, developmental phase, intensity and duration of stress (Mittler and Blumwald 2010). Plant response to stressful conditions is very complex and includes multiple genes and different biochemical and molecular mechanisms.

Photosynthesis is the basis of plant growth and it is also affected by stressful environmental. Internal factors that influence photosynthetic performance and productivity are pigment concentration, leaf surface and orientation while external factors are light, temperature, carbon-dioxide concentration, water, humidity, nutrient availability and pollution. When conditions are optimal, photosynthesis efficiency is high while unfavorable environmental factors can reduce CO_2 fixation, disturb photochemical and non-photochemical processes and in that way reduce photosynthetic efficiency and hence plant biomass and yield.

1.1.8. Heavy metals in soil

Plants represent an important link between the atmosphere and soil and between consumers in the food chain, directly influencing the flow of matter and energy in the food chain. Pollutants are often introduced into the food chain through plants that uptake them from soil. Once in the food chain these pollutants cause irreversible damage to different organisms as a result of accumulation processes. The uptake of a certain nutrient by the plant is specified by its amount in the medium and its availability. Plants can´t change the amount of nutrients present but can change their availability by modifying the pH of the soil solution, releasing organic acids with chelating properties from the roots, or by mycorrhiza, etc. Most readily available elements in soil are present as ions or soluble soil complexes. On the other hand, the least readily available elements in soil are tightly bound to the soil structure. Between these two opposite states are small particles loaded with metals and have large surface areas (clay, sludge, organic material).

Metals are often characterized by their physical properties which distinguish them from nonmetals. However, their physical properties are lost after the metal has been chemically transformed into a compound that a plant can uptake (Shaw, 2004). The term "heavy metal" is a loose category, specifying metals which density is ranging from 3.5 to 7 g cm^{-3}(Duffus 2002). Adriano (2001) determined heavy metals as metal or metalloid with density exceeding 5 g cm^{-3}.

Heavy metals in soil occur naturally, but are rarely present in toxic concentrations. Various anthropogenic activities such as mining, smelting, use of synthetic products (pesticides, batteries, paints, industrial or municipal sludge) can result in heavy metal contamination of soil. Most common problems from cationic metals come from mercury, cadmium, lead, nickel, copper, zinc, chromium, and manganese. Several metal ions, called micronutrients, are toxic at high concentrations but are crucial to metabolism functioning at low concentrations (Marschner 1995). Essentially all of the micronutrient cations are classified as heavy metals – Fe, Mn, Cu, Zn, and Ni. Based on the research of Naumann et al. (2007) the toxicity of ten different heavy metals on *Lemna minor* based on fresh and dry weight, chlorophyll (*a*, *b*) and carotenoid content decreased as follows:

$$Ag^{+} > Cd_2^{+} > Hg_2^{+} > T1^{+} > Cu_2^{+} > Ni_2^{+} > Zn_2^{+} > Co_6^{+} > Cr_6^{+} > As_3^{+} > As_5^{+}.$$

Excessive heavy metal accumulation in soil can lead to decreased microbial activity, soil fertility, and ultimately reduction in yield (McGrath et al. 1995). In arable soils main sources of metal pollution are fertilizers, agricultural chemicals, and liquid and solid wastes. Estimation is that average cadmium input to agricultural lands in Europe is approximately 8 g ha^{-1} yr^{-1} from the atmosphere and 5 g ha^{-1} yr^{-1} from the usage of phosphatic fertilizers (Hutton 1982). In Belgium, metal contaminations from fertilizers and atmospheric deposit is estimated to be on average 16, 20, 260, and 3800 g ha^{-1} yr^{-1} for arsenic, cadmium, lead, and zinc (Navarre et al. 1980).

1.1.9. Cadmium in soil

The total concentration of cadmium in soil comprises from the geological parent material with inputs from other mostly anthropogenic sources. Soil is the ultimate sink for heavy metals in continental areas. Due to their high affinity for the soil matrix, metals are immobile in soil and tend to accumulate there especially in the surface soil layers. Average concentrations of cadmium (reviewed from world literature) in soil that is not exposed to (obvious) sources of pollution is in the range 0.06 – 1.1 mg kg^{-1} with a minimum of 0.01 and maximum of 2.7 mg kg^{-1} (Kabata-Pendias and Pendias 1992). Concentrations of cadmium in topsoil in Europe, which include natural background and human sources, are presented in Figure 6. Most important sources of cadmium which contaminate soils can be divided into three categories (Alloway and Steinnes 1999):

1. Atmospheric emissions (Metalliferous mining and smelting, metal-using industries, manufacture of phosphatic fertilizers, industrial emissions, coal combustion, etc.)
2. Direct application (Phosphatic fertilizers, phosphogypsum and by-products of gypsum, sewage sludge, composted municipal waste, etc.)
3. Accidental contamination (industrially contaminated land, mine waste dumps, corrosion of galvanized metal structures).

For agriculture, most significant source of cadmium is direct application in with mineral fertilizers. They are the most ubiquitous source of cadmium contamination in the world. All soils fertilized with these types of fertilizers will have an input of cadmium, and the amount will depend on the type of fertilizer, source of rock phosphate, and the amount applied. Phosphatic fertilizers can contain up to 300 mg Cd kg^{-1}, while N and K fertilizers contain significantly less cadmium of about 9 mg Cd kg^{-1} (Fergusson 1990). Phosphate rocks have high concentrations of several heavy metals but cadmium is probably agriculturally most important. This elevated cadmium content in phosphate rocks is thought to be due to substitution of Ca^{2+} with Cd^{2+} in apatite. Kongshaug et al. (1992) summarized that the average composition of 91 % of the phosphate reserves in the world contain (in mg kg^{-1}): As (11), Cd (25), Cr (188), Pb (10), Hg (0.05), Ni (29), and V (88).

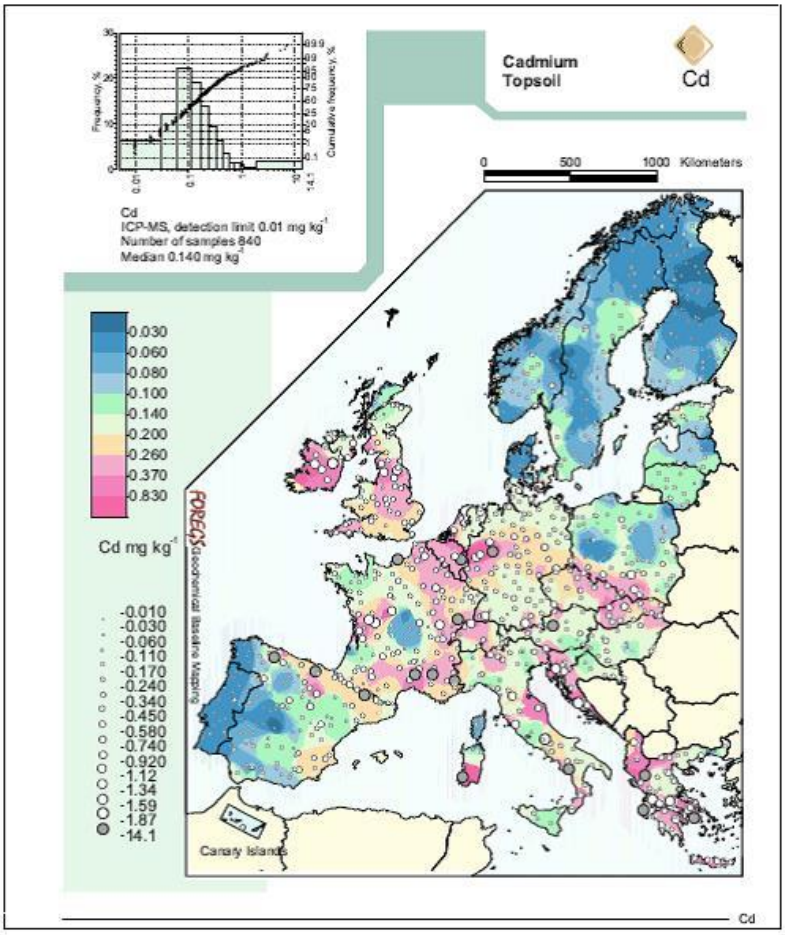


Figure 6. Cadmium concentrations (mg kg^{-1}) in European topsoil. Concentrations include natural background and human sources. Source: Geochemical Atlas of Europe – Soil data and information system – FOREGS and JRC Ispra

Cadmium solubility and bioavailability is affected by pH where acidic soils favor the solubility of cadmium, but they are also controlled by organic matter content, sand, clay or micro-nutrients like zinc, iron or manganese. Bioavailability is also dependent on crop variety, rainfall, and farming practices.

1.1.10. Heavy metal stress in plants

Agricultural soil in many parts of the world are by some degree contaminated by heavy metals such as cadmium, copper, zinc, nickel, cobalt, arsenic, lead and chromium. Causes of this contamination are various but the most frequent are long-term use of phosphatic fertilizers, sewage sludge application, dust from smelting, industrial waste and bad irrigation practices (Bell et al. 2001, Passariello et al. 2002). Under normal physiological conditions there is a balance between ROS production and scavenging in all cell compartments. Main response of plants upon exposure to elevated levels of heavy metals is the generation of ROS. ROS can be directly generated through Haber–Weiss reactions which create hydroxyl radical (HO) referred to as the most reactive oxygen species (Kehrer 2000) or the overproduction of ROS

and the generation of oxidative stress can be an indirect consequence of heavy metal presence (Mithofer et al. 2004, Wojtaszek 1997). This indirect mode of action includes heavy metal interaction with the antioxidant system (Srivastava et al. 2004), disturbing the metabolism of essential elements (Dong et al. 2006) and disrupting the electron transport chain (Qadir et al. 2004). ROS usually damages cellular components are membranes, chloroplast pigments, nucleic acids and enzymatic and non-enzymatic antioxidants (Figure 5).

Plants have evolved mechanisms to cope with heavy metals. Plant tolerance to heavy metals can be defined as the ability to survive in a soil that is toxic to other plants with an interaction between genotype and the environment (McNair et al. 2000). Plants that are resistant to high metal concentrations have developed two basic mechanisms – avoidance and tolerance. Avoidance involves exclusion of metals outside the roots and tolerance includes complexation of metals to avoid cellular damage. Heavy metals have a strong effect on oxidative processes in plants and it is the base for the connections with signaling response. Plant tolerance to heavy metals largely depends on the efficiency of uptake, translocation, and sequestration in specialized tissues and cell organelles (Gupta and Sandalio 2012). Complexed and sequestered metals in cellular structures are not available for translocation to the shoot (Lasat et al. 1998). Metal transport across plasma membranes is essential for plant growth, development, signal transduction and toxic metal phytoremediation (Cherian and Oliveira 2005). Plants have several classes of metal transporters that are involved in metal uptake and homeostasis and that also have a key role in tolerance (Yang et al. 2005). Several protein classes have been implicated in plant heavy metal transport – heavy metal ATP-ases, natural resistance macrophage protein (Nramp) family of proteins, cation diffusion facilitator (CDF) family proteins and the zinc-ion permease (Williams et al. 2000, Yang et al 2005).

1.1.11. Cadmium stress in plants

Higher plants uptake cadmium from soil or water, depending on the availability and concentration (Clemens 2006). Cadmium is toxic to plants even at low concentrations and leaf concentration that exceed 5–10 μg Cd g^{-1} DM are toxic to most plants (White and Brown 2010). Plants tolerant to cadmium are often excluders that limit the entry and root-to-shoot translocation. Nontolerant plants mainly accumulate metals in roots, just like tolerant excluders, but they differ in the capacity to translocate the metal to shoots which is a fact involved in tolerance (Verbruggen et al. 2009). Some of the mechanisms plants cope with excess cadmium are metal exclusion, active excretion, metal binding to cell walls, chelation, compartmentalization in vacuoles and restricted distribution in sensitive tissues (Benavides et al. 2005).

Rate of metal accumulation in plants is driven by physiological requirements and the first barrier for heavy metals to enter cells is the cell wall. It is defined as the key site of heavy metal storage in plants and deposition of heavy metals in cell walls is considered as one of the crucial mechanisms of tolerance (Vasquez et al. 2006). Due to negative charge of the cell wall it has a large capacity for heavy metal binding and immobilization. Toxic heavy metals enter plant cells via transport systems that are involved in micronutrient uptake. Cadmium (Cd^{2+}) uptake occurs through transmembrane carriers that have a role in the uptake of Ca^{2+},

Fe^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} (Clemens 2006). Low levels of these metals can lead to increased uptake of cadmium because cadmium competes with them for the same transport channels which can result in cadmium toxicity (Wojas et al. 2007). It has been shown that cadmium inhibits root Fe(III) reductase and thereby leading to Fe(II) deficiency which seriously affects photosynthesis (Alcantara et al. 1994).

Cadmium is highly mobile in the phloem and it can accumulate in any part of the plant. Biochemical changes it can induce in the roots and leaves are lignification of the cell walls in roots and leaf main vein. Visual symptoms include stunted growth, leaf epinasty and chlorosis (Chaffei et al. 2004, Zhao et al. 2006). It modifies chloroplast ultrastructure, reduces net photosynthetic rate, stomatal conductance and leaf transpiration (Souza et al. 2011). Associated with reduction in photosynthesis is the decrease in transcription of genes related to photosynthesis (psbA, psaB, rbcL), inactivation of CO_2 fixation involved enzymes and chlorophyll biosynthesis, induction of lipid peroxidation, disturbances in N and S metabolism and antioxidant machinery of the plant (Qian et. al 2010, Perfus-Barbeoch et al. 2002, Laspina et al. 2005, Garcia et al. 2006). One of the reasons for cadmium toxicity is its chemical similarity with ions that are part of enzyme active sites and signaling components, mostly zinc but also iron and calcium (Roth et al. 2006). Main targets for cadmium and plant responses to cadmium are presented in Figure 7.

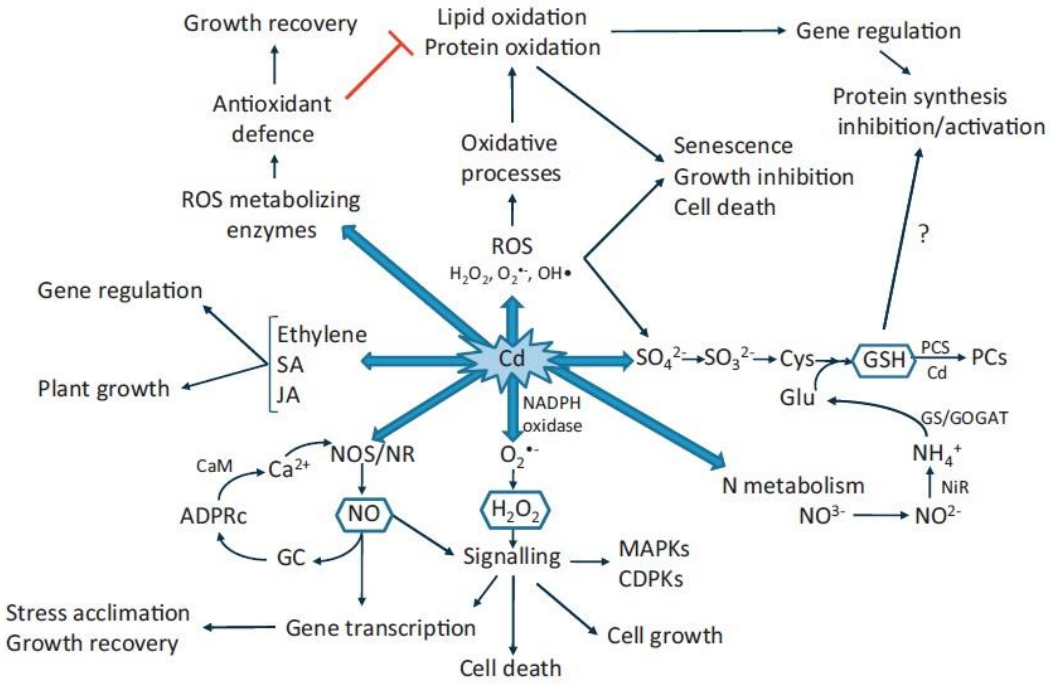


Figure 7. Schematic representation of main cadmium targets and responses to cope with cadmium stress (Gallego et al. 2012)

1.1.12. The effect of excess cadmium on photosystem functioning

In photosynthetic organisms, cadmium is easily taken up and affects different metabolic activities in different cell compartments which include inhibition of photosynthesis, chlorosis, growth inhibition, decrease in water and nutrient uptake etc. One has to distinguish between short-term and long-term effects of cadmium exposure, because organisms can adapt to higher concentrations by employing different mechanisms of resistance (as expression of sequestering compounds or exporters) (Faller et al. 2005). The photosynthetic apparatus is especially sensitive to cadmium and it can be directly or indirectly affected by the metal ion. Toxic action *in vivo* can be the result of interactions at different metabolic levels yielding complex responses. Lipid peroxidation in photosynthetic membranes and disturbances in photosynthetic pigment synthesis and degradation are one of the ways photosynthetic processes are challenged by cadmium.

A major mechanism of toxicity is the inhibition of RubisCo which, for cadmium, occurs due to SH-interactions (Stiborova 1988) or impaired protein biosynthesis (Kremer and Markham 1982). It may also affect RubisCo activity by damaging its protein structure (disconnecting the subunits) and replacing cofactors (Mg^{2+}) necessary for carboxylation (Krupa 1998). Cadmium has been shown to affect both reduction and re-oxidation of Q_A as seen through the OJIP curve kinetics. Slower net reduction of the Q_A pool after the J-step suggests back-pressure from the electron transport, suggesting inhibition of the Calvin cycle (Ciscato et al. 1999).

Several steps of the electron transport chain are affected by cadmium, either directly or indirectly. It has been shown that light-harvesting protein complex II (LHCII), which is the major light-harvesting antenna in photosynthesis, is affected by cadmium. Cadmium causes disturbances in its oligomeric structure by decreasing the level of *trans*-Δ^3- hexadecenoic fatty acid (Krupa 1988) which leads to inefficient light energy utilization. Besides the effect on LHCII, cadmium disturbs the integration of chlorophyll into stable chlorophyll-protein complexes in the thylakoid membranes (Horvath et al 1996).

Oxygen evolving complex (OEC) has been proposed as a primary target of cadmium toxicity. Destruction of OEC and interaction with metal ions (Mn^{2+}, Ca^{2+}, Cl^-) have been proposed as mechanisms of cadmium toxicity (Skorzynska and Baszynski 1993). Also the total plastoquinone pool is reduced by cadmium leading to a decrease in electron transport to PSI. In this way, PSI is also affected by diminished activity of Fd due to inhibition of electron flow (Siedlecka and Baszynski 1993). Reduction in the energy transfer from PSII antennae to the reaction center and change in the ultrastructure of thylakoid membrane have been reported in maize (Ekmekçi et al. 2008).

Toxic effects of cadmium on photosystem functioning in plants are diverse and complex, which makes the distinction between direct and indirect mechanisms almost impossible. Its accumulation affects ultrastructural organization of cell walls dramatically which could play a role in the decrease in photosynthetic activity in cadmium stressed plants.

1.1.13. Maize

Maize (*Zea mays* ssp. *Mays* L.) (Figure 8 is a cereal plant of the grass family (*Poaceae*) and genus *Zea*. It is cultivated around the world but its origin was the subject of controversy until the middle of 20th century and the development of molecular techniques.

Figure 8. Illustration depicting maize plant – male and female inflorescence and fertilized ear with kernels. From: http://philschatz.com/biology-book/contents/m44722.html

Now it is clear that teosintes are the closest relatives of maize and one form of teosintes (*Zea mays* ssp. *parviglumis*) is the direct progenitor of maize (Hufford et al. 2012) and it can still be found in southwest of Mexico. Maize and teosintes paradox has fascinated botanists and geneticists for a long time due to striking differences in morphology (tassels, ears, seeds) between maize and teosintes (Figure 9). Now it is known that this large transformation is mainly due to human mediated selection and large diversity in the teosinte genome (Tian et al. 2009). From Mexico, maize was diffusely spread through Central America towards the Caribbean islands and then to Chile, Peru and Argentina. Maize was introduced in Northern America around 700 A.D. in the valley of Rio Grande, toward Northern and Western parts of the Rocky Mountains and along the rivers of Mississippi, Arkansas and Ohio. It has reached New England and Canada by 1200 (Galinat 1992). Maize was introduced in Europe in 1493 in Southern Spain when Columbus brought it from the Caribbean islands. Since then, a large number of local maize populations have been created through natural selection and breeding, which resulted in adaptation to different ecological conditions (Camus-Kulandiavelu et al. 2006).

As a tropical plant, maize is cold and drought intolerant: minimal temperature for initial growth and development is 8 °C, and in vegetation 12 °C. Optimal temperature during

vegetation is 24 – 28 °C. Maize is very susceptible to frost and above ground parts freeze at – 1 °C. For optimal yields 500 – 600 mm of water is needed during vegetation period. Water regime of maize changes during vegetation and most critical periods are silking, fertilization and grain filling. A common feature of cereal responses to abiotic stresses, such as drought, heat, cold etc., in the stages near flowering and early grain filling is reduction in reproductive fertility (which affects grain formation) and can be attributed to different metabolic causes (insufficient supply of photosynthates, ABA accumulation, etc.) (Collins et al. 2008). One of the most important leaves in a miaze plant is the ear-leaf since it affects plant morphology and grain yield (Zheng and Liu 2013). Relationship between chlorophyll content in leaves, which directly affects photosynthetic processes, and total canopy chlorophyll has been established in maize. Chlorophyll content in maize ear-leaves explains more than 87% of variation in total chlorophyll in a maize canopy (Ciganda et al. 2009), and hence could represent chlorophyll related traits (Lepeduš et al. 2012). Maize has a very developed root system, and leaf anatomy adapted to collect even the smallest amounts of water and in the case of drought leaf rolling is one of the ways it

Figure 9. Visual differences between teosinte plant (left) and maize plant (right). From: http://evolution-textbook.org/content/free/figures/ch11.html

reduces transpiration. In our climate excess of water can arise as a problem in maize cultivation, which leads to anaerobic conditions, growth retardation, chlorosis and decrease in phosphorous uptake. Photosynthetically maize differs from other cereals (wheat, barley, rice) by the way it partitions CO_2 fixation; maize is a C4 plant opposed to e.g. wheat which is a C3 plant. CO_2 fixation in maize includes two spatially separated steps which eliminate

photorespiration. Since maize is most sensitive to various types of stresses in stages near flowering and grain filling and a relationship between maize ear-leaf chlorophyll content and total canopy chlorophyll content has been established, chlorophyll related traits which directly affect photosynthesis could be assessed through ear-leaf measurements.

2. Objectives of the study and hypotheses

2.1. Objectives

The objectives of the study were:

(i) to investigate the variation of cadmium uptake in maize ear-leaves of four different maize genotypes by means of ICP-OES analysis,

(ii) to detect the effects of cadmium uptake on photosystem II of the selected genotypes through chlorophyll *a* fluorescence,

(iii) to identify any possible tolerance mechanisms or sensitivity of the selected genotypes to cadmium.

2.2. Hypotheses

Main hypotheses of the study were:

(i) There is variation of cadmium uptake in leaves of four selected maize genotypes

(ii) There are high and low cadmium accumulating genotypes

(iii) Cadmium induces changes in photosystem II that are detectable through chlorophyll *a* fluorescence and can be identified through the use of JIP-test

(iv) Highest cadmium soil concentration will have the highest effect on decrease of performance index (PI_{ABS})

3. Materials and methods

3.1.Soil preparation

Soil was collected from the field of the Agricultural Institute Osijek, sieved through a 5 mm sieve and separated into four equal parts, 350 kg of soil per each part. Soil chemical properties are presented in Table 1. According to WRB classification (FAO/ISRIC/ISSS, 1998) soil type was determined as eutric gleysol with 1.5% sand, 71.2% silt, and 27.2% clay. Soil was neutral to weak alkaline reaction, slightly calcareous, moderate fertility with medium humus content (Table 1). Soil potassium was in range moderate availability (class C) and phosphorus in range high availability (class D) determined by AL-acetic acid method (Egner et al. 1960).

Table 1. Soil properties (mean ± SE, n = 2 for organic matter (%), P_2O_5-AL, K_2O-AL, CaCO3 (%); n = 8 for all other parameters).

Parameter	Concentration mg kg^{-1}	Parameter	Concentration mg kg^{-1}
pHKCl	6.99 ± 0.03	Cu (mg kg^{-1})	24,00 ± 0.19
pHH_2O	8.05 ± 0.02	Fe (mg kg^{-1})	29358.75 ± 208.80
Organic matter (%)	2.57 ± 0.08	Mn (mg kg^{-1})	676.05 ± 5.53
P_2O_5-AL	29.58 ± 0.64	Zn (mg kg^{-1})	69.40 ± 0.64
K_2O-AL	25.60 ± 3.43	Ni (mg kg^{-1})	31.32 ± 0.22
CaCO3 (%)	1.26 ± 0.02	Co (mg kg^{-1})	13.83 ± 0.11
Pb (mg/kg)	20.50 ± 0.20	Cd (mg kg^{-1})	0.110 ± 0.01

The total concentrations of all heavy metals determined after extraction by *aqua regia* were very low, mainly lower than 50% of maximum allowed concentrations (MAC) in agricultural soils, and less than 25% of MAC for Pb (20,5 mg kg^{-1}) and Cd (0,1 mg kg^{-1}).

Soil was contaminated to three different Cd levels: 0.5, 1 and 5 mg of Cd per kilogram of soil (mg Cd kg^{-1} soil). Control soil was left uncontaminated. Cd was applied as $CdCl_2$ solution and solutions were made as follows: Cd0.5 – 0.408 g of $CdCl_2$ was dissolved in 5 L of deionized water, Cd1 – 0.816 g of $CdCl_2$ was dissolved in 5 liters of deionized water and Cd5 – 4.08 g of $CdCl_2$ was dissolved in 5 liters of deionized water. Soil was contaminated by applying 10 milliliters of prepared $CdCl_2$ solution per every kilogram of soil. Soil was spread in a few centimeters thick layer and sprayed (using a spray bottle) with 2,240 liters of prepared $CdCl_2$ solution per treatment. Plastic 12 liter pots (r =275 mm, h = 250 mm) were filled with prepared soil, 14 kg of soil per pot, 4 pots per genotype, 16 pots per each treatment giving total of 64 pots for the experiment. Cd concentrations in soil per treatment were determined in 2013 by ICP-OES. Control treatment, Cd0.5, Cd1 and Cd5 treatments had (mean ± SE): 0.11 ± 0.01, 0.62 ± 0.04, 1.07 ± 0.46 and 4.89 ± 0.06 mg Cd kg^{-1} soil, respectively.

3.2.Plant material and growth conditions

Seeds of four maize genotypes (B73, Mo17, B84, Os6-2) were planted in pots in the beginning of May (May 9, 2012; May 7, 2013). Seeds were planted by hand 5 cm deep in four replications with eight seeds per pot. Pots were watered with 200 mL of water daily and the amount of water was increased up to 2 liters as the plants grew. Fertilization was made according to the recommendations based on the soil analysis. Plants were grown to physiological maturity (R6 stage). Plants were grown in a greenhouse until V3 phase and later pots were transferred outside to the field environmental conditions. There were differences in two growing years mostly in temperature and precipitation. 2012 had a little less rainfall than 2013 but both years were in the normal rainfall range in July when measurements were made. 2012 was warmer than 2013; deviations from the normal average temperature were 0.4, 3.0 and 3.6°C for May, June and July 2012, respectively. Deviations from the normal average temperature in 2013 were 0.2, 0.5 and 1.8°C for May, June and July, respectively. Cumulative insolation duration was higher in 2012 and it was above the average normal cumulative insolation duration.

3.3.Chlorophyll *a* fluorescence measurements

Chlorophyll fluorescence was measured in the first half of July (2012 and 2013) on attached leaves, during flowering (tasseling), using Plant Efficiency Analyser (Model HANDY PEA, HANSATECH). Measurements for both years were done early in the morning (7 – 9 AM) due to midday depression of photosynthesis in maize (Shen and Xu 2001). Temperatures during measurements ranged between 19 and 21°C. Chlorophyll fluorescence was measured on the middle section of the upper side of ear-leaves on 4 plants per pot, making 16 measurements per genotype for each treatment. After dark adaptation time of 30 minutes chlorophyll fluorescence transient was induced by applying a pulse of saturating red light (peak at 650 nm, 3200 mmol m^{-2} s^{-1}) provided by 3 ultra-bright LED´s. LED´s are focused via lenses on the leaf surface that is exposed by the leaf clip (4 mm in diameter). Saturating light pulse induces chlorophyll *a* fluorescence increase from minimal fluorescence (F_0), when all reaction centers open, to maximal fluorescence (F_m), when all reaction centers closed. 120 data points are collected during the 1 second measurement. Chlorophyll *a* fluorescence data was processed with PEA plus software provided with the Plant Efficiency Analyser. Data points and parameters obtained by chlorophyll *a* fluorescence were analyzed according to the OJIP-test that outputs multiple parameters quantifying the photochemistry of PSII. OJIP-test was described by Strasser et al. (1995, 2004, 2010). Parameters are given in Table 2.

Fluorescence induction OJIP curves were created using data points measured by the Handy PEA device. Double normalization between O (F_0) and P (F_m) and logarithmic time scale was used for plotting.

Table 2. Definition of terms and formulae of OJIP-test parameters and expressions (Source: Strasser et al. 2004)

Parameter	Description
	Data extracted from the recorded fluorescence transient
t_{max}	Time Needed To Reach F_m
V_J	Relative Variable Fluorescence At J Step; $V_J=(F_J–F_0)/(F_m–F_0)$
V_I	Relative Variable Fluorescence At I Step; $V_I=(F_I–F_0)/(F_m–F_0)$
M_0	Initial Slope Of Relative Variable Fluorescence; $M_0=4(F_{300\mu s}–F_0)/(F_m–F_0)$
N	Number of reduction turnovers, oxidation, and re-reduction of QA in the time between turning on the light and reaching the F_M; $N = S_m M_0 (1/V_J)$
S_m	Normalized total complementary area above the O-J-I-P transient (reflecting multipleturnoverQA reduction events); $S_m \equiv (Area)/(F_M – F_0)$
	Density Of Reaction Centres
RC/CS_0	Density Of Reaction Centres Per Excited Cross Section; $RC/CS_0=F_v/F_m\cdot(V_J/M_0)\cdot F_0$
	Yields or ratios of fluxes
TR_0/ABS	Maximum quantum yield of primary photochemistry; $TR_0/ABS = [1-(F_0/F_m)]$
ET_0/ABS	Maximum yield of electron transport; $ET_0/ABS = [1-(F_0/F_m)]\cdot(1-V_J)$
ET_0/TR_0	Efficiency of a trapped exciton to move an electron into the electron transport chain further than Q_A^-; $ET_0/TR_0 = (1-V_J)$
φ_{Ro}	Quantum yield for reduction of end electron acceptors at the PSI acceptor side; $[1-(F_0/F_m)]\psi_{Eo}\,\delta_{Ro}$
φ_{Do}	Quantum yield (at t = 0) of energy dissipation; $\varphi_{Do} = F_0/F_m$
	Specific Fluxes Per Active Reaction Center
ABS/RC	Absorption Per Active Reaction Centre; $ABS/RC=M_0\cdot(1/V_J)\cdot[1/(F_v/F_m)]$
TR_0/RC	Trapping Per Active Reaction Centre; $TR_0/RC=M_0\cdot(1/V_J)$
ET_0/RC	Electron Transport Per Active Reaction Centre: $ET_0/RC=M_0\cdot(1/V_J)\cdot(1–V_J)$
DI_0/RC	Dissipation Per Active Reaction Centre; $DI_0/RC=(ABS/RC)–(TR_0/RC)$
	Performance Index
PI_{ABS}	Performance Index On Absorption Basis; $PI=(RC/ABS)\cdot(TR_0/DI_0)\cdot[ET_0/(TR_0-ET_0)]$
PI_{total}	Performance index (potential) for energy conservation from exciton to the reduction of PSI end-electron acceptors e.g. NADP; $PI_{total} = (PI_{ABS})(\delta_{Ro}/(1-\delta_{Ro}))$
RC/ABS	Density Of Reaction Centres On Chlorophyll *a* Basis;$RC/ABS=(RC/TR_0)\cdot(TR_0/ABS)=[(F_J–F_0)/4(F_{300\,\mu s}–F_0)]\cdot(F_v/F_m)$
TR_0/DI_0	Flux Ratio Trapping Per Dissipation; $TR_0/DI_0=F_v/F_0$
$ET_0/(TR_0-ET_0)$	Electron Transport Beyond QA^-; $ET_0/(TR_0–ET_0)=(F_m–F_J)/(F_J–F_0)$

3.4.ICP-OES analysis – plant material and soil

Ear-leaves were collected after chlorophyll *a* fluorescence measurements. Samples from each pot were put in separate paper bags and dried. After drying samples were milled with a heavy metal free mill (ZM 200, RETSCH). The leaf samples were digested with 10 ml of a 5:1 mixture of HNO3 and H2O2 at 180°C for 60 min in microwave oven (Model MARS 6, CEM). After cooling, total Cd concentrations were measured using ICP-OES (Model OPTIMA 2100 DV, PERKINELMER). Leaf samples were analyzed with an internal pooled plasma control and with the reference material (Rice flour, IRMM - 804, Sample No. 0533,

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium) prepared in the same way as the other leaf samples.

The total heavy metal concentrations in soil samples were analyzed after grinding using heavy metal free grinder (Retsch RM 200), sieving through the sieves of 2 mm, and digesting with 10 ml of a 3:1 mixture of HCl and HNO3 (ISO 11466) at 210°C for 60 min in microwave oven (Model MARS 6, CEM). The total Fe, Mn, Zn, Cu, Ni, Co, Cr, Pb and Cd concentrations in digested soil samples were measured by ICP-OES (Model OPTIMA 2100 DV, PERKINELMER). Soil samples were analyzed with an internal pooled plasma control and with the reference material (Loam soil, ERM CC141, Sample No. 0037, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium) prepared in the same way as were the soil samples extracted by *aqua regia*.

3.5.Statistical analysis

Mean and standard error of the mean was calculated for every parameter of chlorophyll *a* fluorescence (n = 16). Mean and standard errors (SE) were calculated for cadmium and zinc content in ear-leaves with n = 4, as well as for cadmium and zinc content in soil for every treatment (n = 4). Initially, analysis of variance (ANOVA) was used for data of two years of experiment analyzed separately with factors: treatment, genotype, and replication. Combined analysis of variance across both years was also conducted where sources of variation were treatment, genotype and year and their respective two- and three-factor interactions.

Repeatability estimates were calculated in order to show how much is to be gained by the repetition of measurements and to set upper limits to the ratio of variance components, throwing light on the nature of the environmental variance. Repeatability was estimated in the combined analysis based on entry means (Hallauer et al. 2010) which corresponds to the heritability estimate. Heritability takes into account genotypic variance (σ^2_g), the pooled error variance (σ^2_e) and variance due to genotype by environment interaction, (σ^2_{ge}):

$$\hat{h}^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_e/re + \hat{\sigma}^2_{ge}/e + \hat{\sigma}^2_g}$$

where r and e are the number of replications and environments, respectively.
As post-hoc test, Fischer´s least significant difference (LSD) at the $p < 0.05$ level was determined for analyzing differences between factor-level means. Mean and SE (bar plot) for OJIP test parameters and metal contents are displayed graphically as where different letters represent statistically significant differences at the $p < 0.05$ level. Pearson´s correlation coefficient was calculated between OJIP test parameters and cadmium content in ear-leaves and soil. Statistical analysis was performed using R (R core team 2013).

4. Results

4.1.Cadmium content in ear-leaves

Mean values with standard errors of measured cadmium content determined by ICP-OES analysis in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter revealed highly significant effects of cadmium treatment and genotype and significant effect of their interaction in 2012 (Table 3. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance for Treatment and Genotype but for their interaction there was higher level of significance than in 2012 ($p < 0.001$) (Table 3. B).

Table 3. Analysis of variance for **cadmium content** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variarion	Degrees of freedom	F value
Treatment	3	12.501 ***
Genotype	3	24.415 ***
Treatment×Genotype	9	3.082*
Replication	3	0.445

B

Source of variation	Degrees of freedom	F value
Treatment	3	23.116 ***
Genotype	3	21.220 ***
Treatment×Genotype	9	20.135***
Replication	3	0.562

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Figure 10 A, B shows mean values, standard errors and results of LSD test for cadmium ear-leaf content in four maize genotypes determined by ICP-OES analysis in 2012 and 2013. With highest cadmium level in soil (Cd5) genotypes Mo17 and Os6-2 had the highest content of cadmium in ear leaves in 2012 24.46 and 29.95 mg Cd kg^{-1}, respectively and 2013 17.08 and 32.65 mg Cd kg^{-1}, respectively. Other two genotypes accumulated significantly less cadmium practically in all treatments. In Cd5 treatment in 2012 B73 and B84 had accumulated 1.01 and 3.83 mg Cd kg^{-1}, respectively. In 2013 these two genotypes accumulated less cadmium than in 2012 (0.53 and 0.88 mg Cd kg^{-1}, respectively).

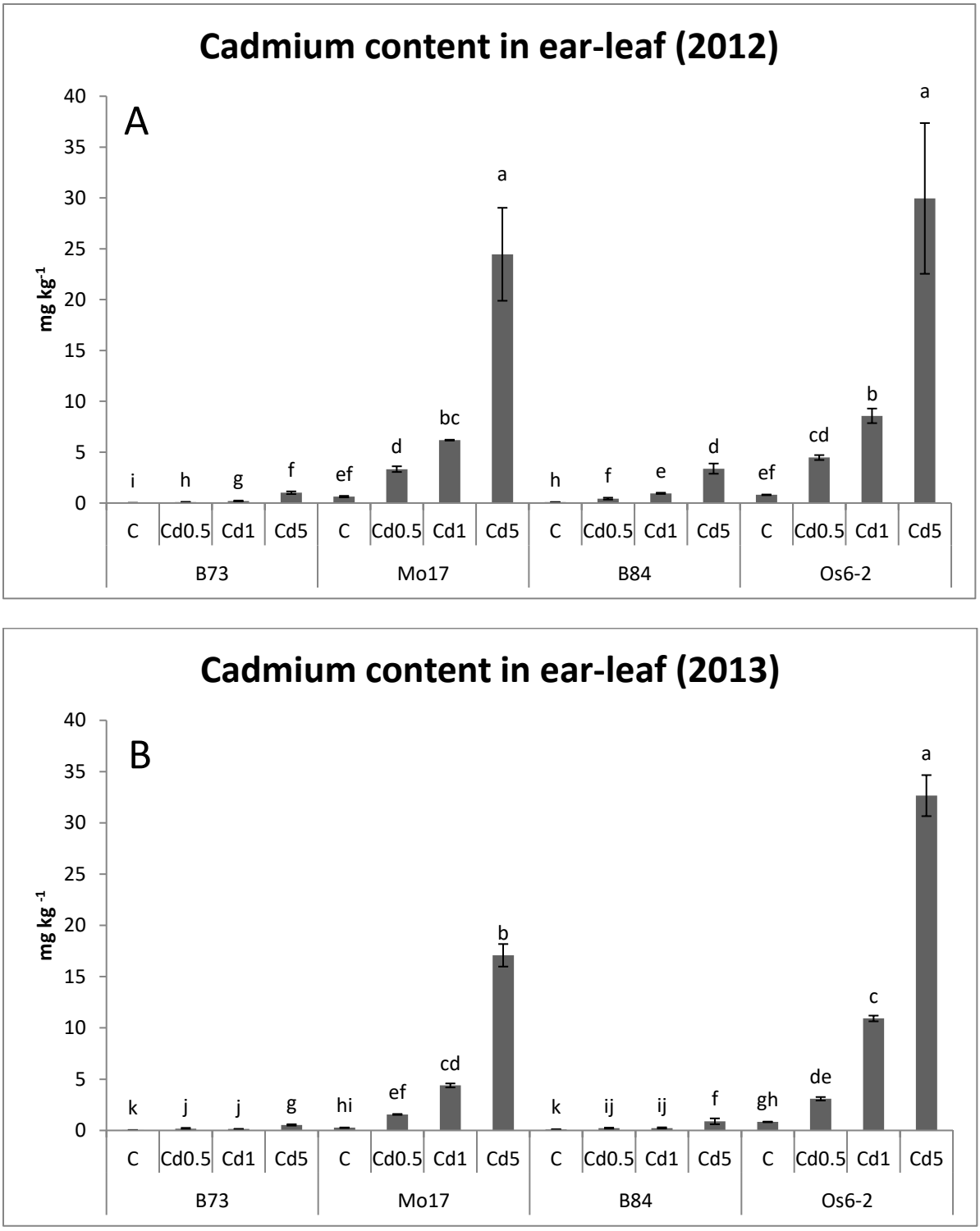


Figure 10. Mean values with corresponding standard errors for **cadmium ear-leaf content** (mg kg^{-1}) in four maize genotypes Bars with different letters denote least significant differences (LSD) at the 0.05 probability level.

4.2. Zinc content in ear-leaves

Mean values with standard errors of measured zinc content determined by ICP-OES analysis in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown highly significant effects of cadmium treatment and genotype on zinc

accumulation and significant effect of their interaction in 2012 (Table 4. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance for Genotype but lower level of significance of Treatment ($p<0.05$) and higher for Treatment×Genotype interaction ($p < 0.001$) (Table 4. B).

Table 4. Analysis of variance for **zinc content** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

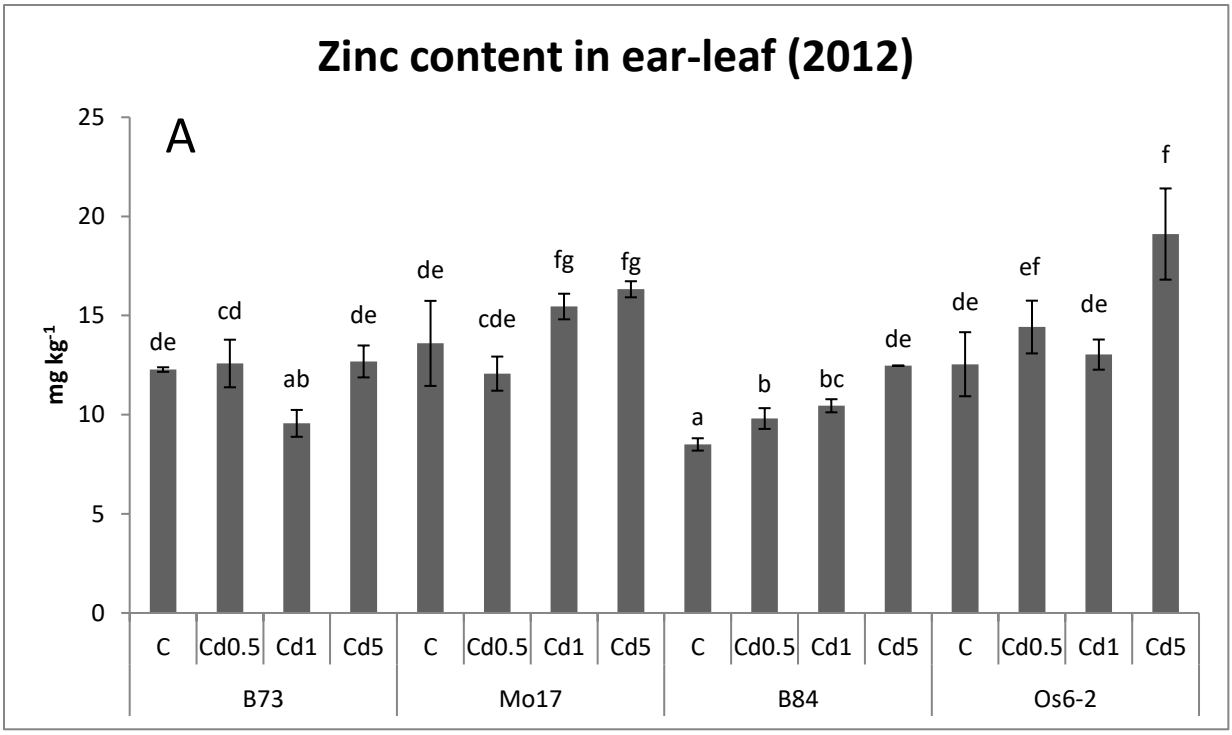
Source of variarion	Degrees of freedom	F value
Treatment	3	13.797***
Genotype	3	30.062***
Treatment×Genotype	9	3.940**
Replication	3	0.625

B

Source of variation	Degrees of freedom	F value
Treatment	3	2.310*
Genotpye	3	29.831***
Treatment×Genotype	9	6.389***
Replication	3	1.125

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Highest zinc content in 2012 and 2013 was measured in Os6-2 genotype in Cd5 treatment (19.11 and 71.74 mg Zn kg^{-1}, respectively) (Figure 11 A, B). B84 genotype accumulated slightly less zinc in ear-leaves than other genotypes, which is especially visible in 2012 (Figure 11 A). Generally lines that accumulate more cadmium (Mo17, Os6-2) accumulated more zinc with increasing cadmium concentrations in soil.



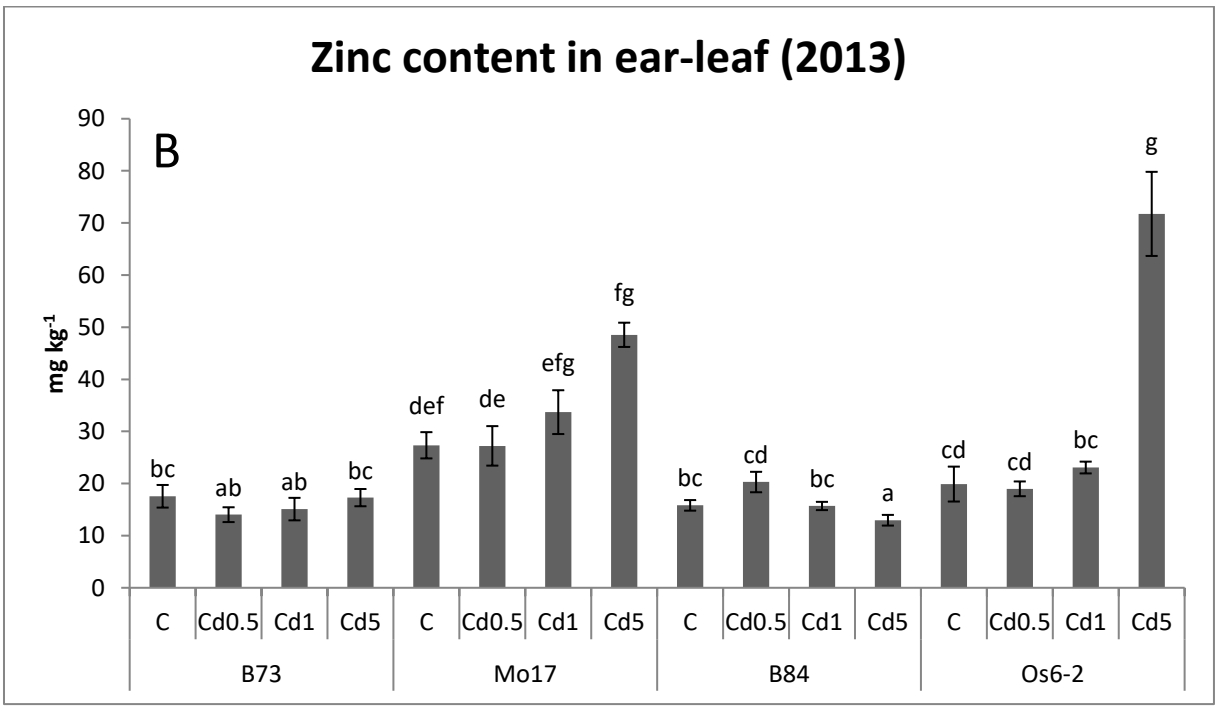


Figure 11. Mean values with corresponding standard errors for **zinc ear-leaf content** (mg kg^{-1}) in four maize genotypes Bars with different letters denote least significant differences (LSD) at the 0.05 probability level.

4.3.ICP-OES analysis of plant material

Combined analysis of variance for cadmium and zinc across both years showed significant effects of main sources of variation (Treatment, Genotype and Year) for the accumulation of cadmium and zinc in maize ear-leaves at the $p < 0.001$ level, except for the effect of Year ($p < 0.01$) (Table 5). All interactions were significant for zinc ($p < 0.001$), but not for cadmium.

Only significant interaction for cadmium was Treatment×Genotype ($p < 0.001$). Heritabilities of these two traits were high: 0.894 for cadmium and slightly less for zinc (0.755).

Table 5. . F values and significance levels from the combined analysis of variance across both years of experiment with estimated heritabilities for **cadmium and zinc content**.

Source of variation	Degrees of freedom	Cadmium (mg kg^{-1})	Zinc (mg kg^{-1})
Treatment	3	258.514***	26.847***
Genotype	3	172.873***	54.885***
Year	1	8.93**	197.503***
Treatment×Genotype	9	76.993***	12.701***
Treatment×Year	3	0.945	10.807***
Genotype×Year	3	1.918	26.918***
Treatment×Genotype×Year	9	2.007	8.922***
Heritability		0.894	0.755

*, **, *** significance at the 0.05, 0.01, 0.001 probability levels, respectively

4.4.Polyphasic chlorophyll *a* fluorescence rise (OJIP)

OJIP chlorphyll *a* fluorescence transients (double normalized between O and P) measured in July 2012 and 2013 on ear-leaves of four maize genotypes challenged by four different levels of cadmium in soil are shown in Figures 12. A, B, C, D and 13. A, B, C, D, respectively. In both years, OJIP transients of all four genotypes exhibited a typical OJIP shape. In both years, B73 inbred line showed the smallest deviation from the control; with only slight decrease in I step under cadmium treatments (Figure 12. A, Figure 13. A). Shapes of OJIP curves in all treatments for this genotype were almost identical to control. B84 inbred line showed increases in J and I step in 2012 and similarly in 2013 except increase in I step was smaller. Only slight increases in J and I steps are visible (Figure 12. B, Figure 13. B). Mo17 inbred line exhibited larger increases in J and I steps in both years and these increases are consistent with increasing cadmium in treatments (Figure 12. C, Figure 13. C). Inbred line Os6-2 also exhibited large increases in J and I steps in 2012, and especially in 2013 (Figure 12. D, Figure 13. D). According to OJIP curves, Os6-2 inbred line seems to be the most sensitive to cadmium, while B73 inbred line does not show any sensitivity to cadmium.

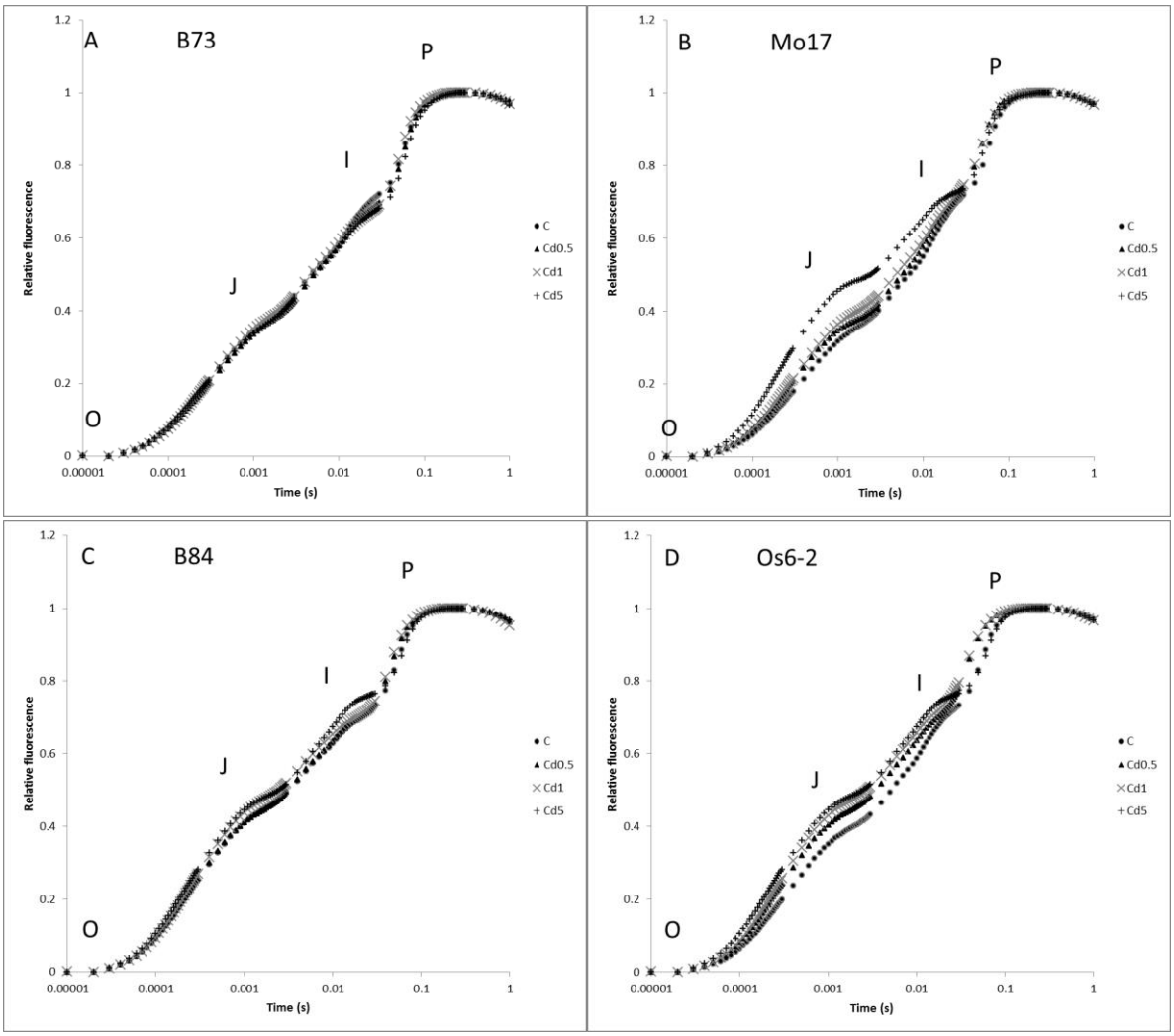


Figure 12. Normalized OJIP chlorophyll *a* fluorescence rise measured in **July 2012** on ear-leaves of four maize genotypes (B73 (A), Mo17 (B), B84 (C), Os6-2 (D)) challenged by four different cadmium levels in soil (Control (C), 0.5 mg Cd kg^{-1} (Cd0.5), 1 mg Cd kg^{-1} (Cd1), 5 mg Cd kg^{-1} (Cd5)).

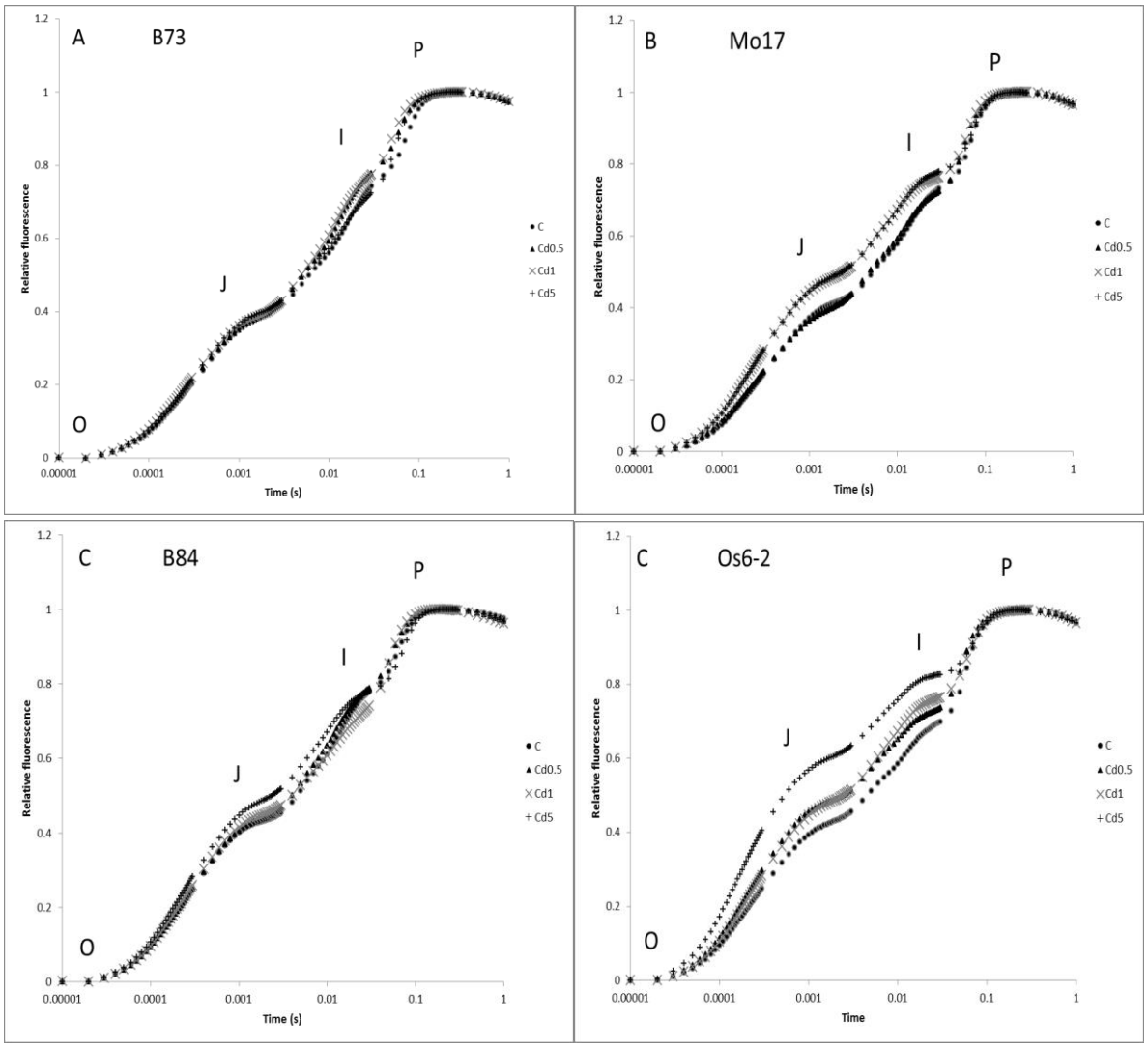


Figure 13. A, B, C, D Normalized OJIP chlorophyll *a* fluorescence rise measured in **July 2013** on ear-leaves of four maize genotypes (B73 (A), Mo17 (B), B84 (C), Os6-2 (D)) challenged by four different cadmium levels in soil (Control (C), 0.5 mg Cd kg^{-1} (Cd0.5), 1 mg Cd kg^{-1} (Cd1), 5 mg Cd kg^{-1} (Cd5)).

4.5.Chlorophyll *a* fluorescence

Combined analysis of variance across both years of experiment for all measured chlorophyll *a* fluorescence revealed significant effects of main sources of variation, except for Year in ET_0/RC, t_{max}, TR_0/ABS, ET_0/ABS, ET_0/TR_0, $ET_0/(TR_0-ET_0)$ and PI_{total} (Table 6). Treatment×Genotype interaction was significant for all parameters ($p < 0.001$) except for PI_{total}. Treatment×Year interaction was significant in four parameters: ET_0/RC, RC/CS_0, TR_0/ABS and TR_0/DI_0. Genotype×Year interaction was significant in seven parameters: F_0, F_m F_{300}, DI_0/RC, RC/SC_0, TR_0/DI_0 and PI_{ABS}. The three-fold Treatment×Genotype×Year interaction was mostly not significant. Exceptions were F_{300}, ABS/RC, DI_0/RC and TR_0/ABS.

Heritability estimates for all measured parameters were similarly high as for cadmium and zinc accumulation and it ranged from the lowest for F_0 to the highest for PI_{total} (Table 6). indicating high repeatability of all traits and parameters examined in the experiment.

Table 6. F values and significance levels from the combined analysis of variance across both years of experiment with estimated heritability for all measured chlorophyll *a* fluorescence traits are shown.

Source of variation	Degrees of freedom	F_0	F_m	F_{300}	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC	RC/ABS	RC/CS_0
Treatment	3	137.84***	47.03***	88.86***	110.67***	185.55***	64.45***	94.76***	101.94***	36.11***
Genotype	3	11.08***	40.10***	25.18***	33.53***	64.69***	29.41***	79.01***	37.48***	33.26***
Year	1	837.15***	929.90***	732.59***	6.97**	3.99*	0.07	13.89***	5.60*	390.51***
Treatment×Genotype	9	8.08***	6.89***	8.61***	18.42***	20.47***	19.18***	10.88***	14.87***	5.57***
Treatment×Year	3	0.93	2.21	2.51	2.12	0.36	4.49**	2.20	0.91	4.59**
Genotype×Year	3	3.41*	4.02**	5.01**	2.50	0.12	0.53	3.52*	1.41	5.47**
Treatment×Genotype×Year	9	0.64	0.44	4.55***	2.02*	0.68	0.82	2.04*	0.93	1.76
Heritability		0.74	0.91	0.828	0.85	0.93	0.85	0.94	0.89	0.88

Source of variation	Degrees of freedom	t_{max}	TR_0/ABS	ET_0/ABS	ET_0/TR_0	TR_0/DI_0	ET_0/(TR_0-ET_0)	PI_{ABS}	PI_{total}	V_J	V_I
Treatment	3	28.57***	99.96***	83.42***	63.92***	65.12***	63.97***	38.59***	29.71***	31.77***	45.59***
Genotype	3	6.86***	55.81***	24.14***	17.88***	41.64***	37.02***	100.24***	25.16***	52.18***	22.19***
Year	1	0.86	2.18	0.71	0.92	53.64***	0.67	50.065***	4.31**	1.34	1.42
Treatment×Genotype	9	3.01**	7.98**	6.90***	6.30***	12.63***	5.72***	3.96***	1.42	8.06***	5.76***
Treatment×Year	3	0.39	5.21**	1.11	0.31	5.59**	1.01	0.70	1.02	0.63	1.58
Genotype×Year	3	1.17	1.84	1.27	0.15	4.71**	1.76	24.53***	1.06	2.11	4.49**
Treatment×Genotype×Year	9	0.55	2.99**	0.35	0.37	1.44	0.49	0.77	0.40	0.53	0.95
Heritability		0.83	0.94	0.91	0.91	0.88	0.94	0.88	0.96	0.89	0.90

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

4.5.1. Minimal fluorescence (F_0)

Mean values with standard errors for measured minimal fluorescence intensity (F_0) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 7. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment ($p<0.01$), Genotype and their interaction ($p < 0.001$) (Table 7. B).

Table 7. Analysis of variance for **minimal fluorescence intensity (F_0)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	49.052***
Genotype	3	5.443**
Treatment×Genotype	9	3.379**
Replication	15	0.231

B

Source of variation	Degrees of freedom	F value
Treatment	3	98.153***
Genotype	3	9.681**
Treatment×Genotype	9	5.648**
Replication	15	0.231

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of F_0 between different genotypes and that there are significant differences in mean values between different treatments. In Figure 14 A, B mean values and results of LSD test are shown for minimal fluorescence intensity parameter (F_0) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of F_0 parameter were higher in 2012 than in 2013. In general, in both years values of F_0 were highest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in F_0 and in 2012 there were no significant differences in F_0 between treatments for that genotype (Figure 14 A), while in 2013 changes were very small (Figure 14 B). Other three genotypes responded to increasing cadmium content in soil with increasing F_0 values.

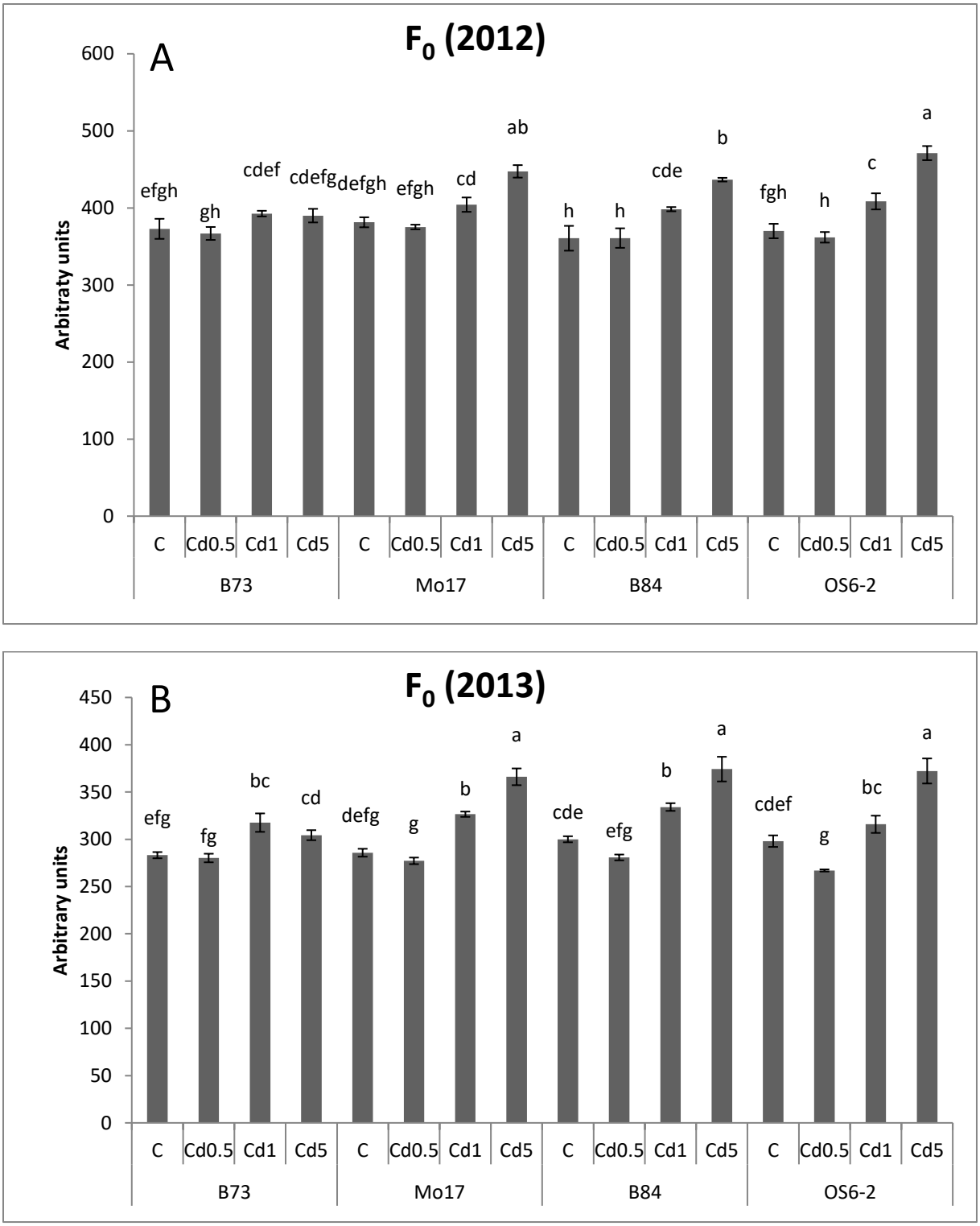


Figure 14. A, B Mean values, standard errors and results of LSD test for **minimal fluorescence intensity (Fo**) in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.2. Maximum fluorescence intensity (F_m)

Mean values with standard errors for measured maximum fluorescence intensity (F_m) in July 2012 and 2013 are shown in Table 27 in the Appendix. As for F_0, analysis of variance for F_m parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 8. A). Analysis of variance for the same parameter in 2013 has shown the same level of significance for Treatment as in 2012, but higher for Genotype and their interaction ($p < 0.001$) (Table 8. B).

Table 8. Analysis of variance for **maximum fluorescence intensity (F_m)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	16.471***
Genotype	3	18.267**
Treatment×Genotype	9	3.337**
Replication	15	1.037

B

Source of variation	Degrees of freedom	F value
Treatment	3	46.241***
Genotype	3	31.792***
Treatment×Genotype	9	4.445***
Replication	15	0.362

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of F_m between different genotypes and that there are significant differences in mean values between different treatments. In Figure 15. A, B mean values and results of LSD test are shown for maximum fluorescence intensity parameter (F_m) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of F_m parameter were higher in 2012 than in 2013. In general, in both years values of F_m were lowest at lowest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in F_m and showed no significant differences in F_m values between treatments (except in 2012 Cd0.5 treatment is significantly different from control) (Figure 15. A, B). Other three genotypes responded to increasing cadmium content in soil with decreasing F_m values.

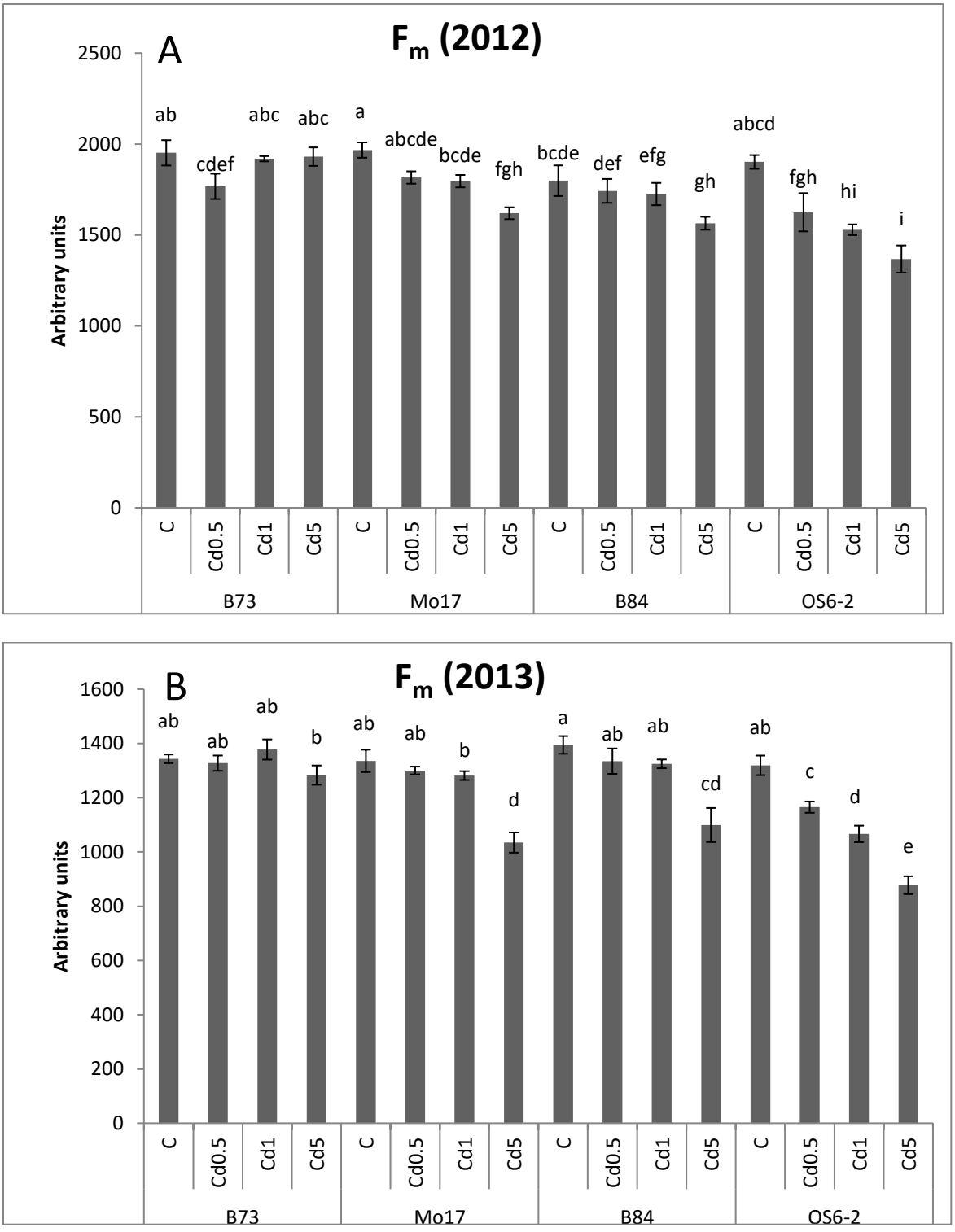


Figure 15. A, B Mean values, standard errors and results of LSD test for **maximum fluorescence intensity (F_m)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.3. Fluorescence intensity at 300 μs (F_{300})

Mean values with standard errors for measured fluorescence intensity at 300 μs (F_{300}) in July 2012 and 2013 are shown in Table 27 in the Appendix. Similarly to F_0 and F_m, analysis of variance for F_{300} parameter has shown significant effects of cadmium treatment, genotype and

their interaction in 2012 (Table 9. A). In 2013, analysis of variance for the same parameter has shown the same levels of significance for Treatment and Genotype ($p<0.001$) but higher level of significance their interaction than in 2012 ($p < 0.001$) (Table 9. B).

Table 9. Analysis of variance for **fluorescence intensity at 300 μs (F_{300})** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

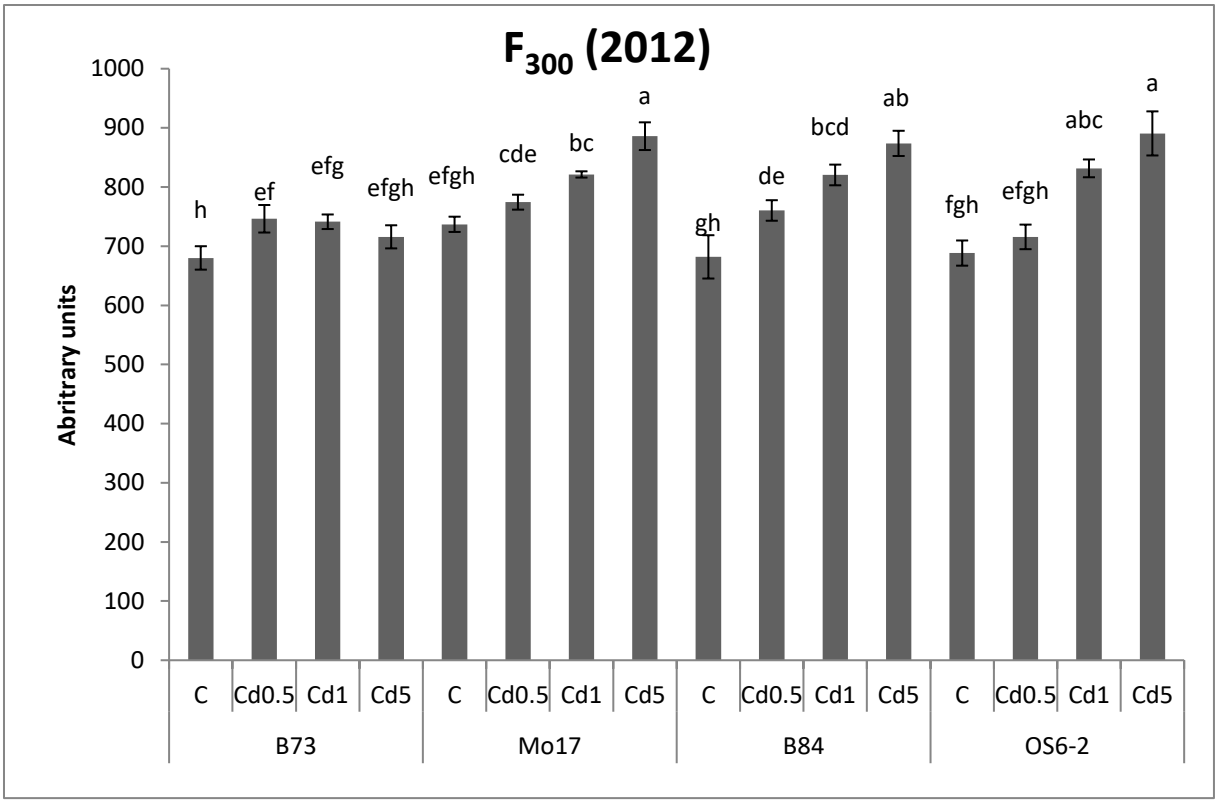
Source of variation	Degrees of freedom	F value
Treatment	3	33.771***
Genotype	3	11.011***
Treatment×Genotype	9	3.495**
Replication	15	0.346

B

Source of variation	Degrees of freedom	F value
Treatment	3	58.83***
Genotype	3	19.66***
Treatment×Genotype	9	10.640***
Replication	15	0.050

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of F_{300} between different genotypes and that there are significant differences in mean values between different treatments. In Figure 16 A, B mean values and results of LSD test are shown for fluorescence intensity at 300 μs (F_{300}) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of F_{300} parameter were higher in 2012 than in 2013. In general, in both years values of F_{300} were highest at highest cadmium concentration in soil (Cd5 treatment). According to LSD test significantly highest values were in Mo17. Inbred line B73 showed the smallest changes in F_{300} and showed no significant differences in F_{300} values between treatments in 2013 (Figure 16 B). Other three genotypes responded to increasing cadmium content in soil with increasing F_{300} values in a gradual manner.



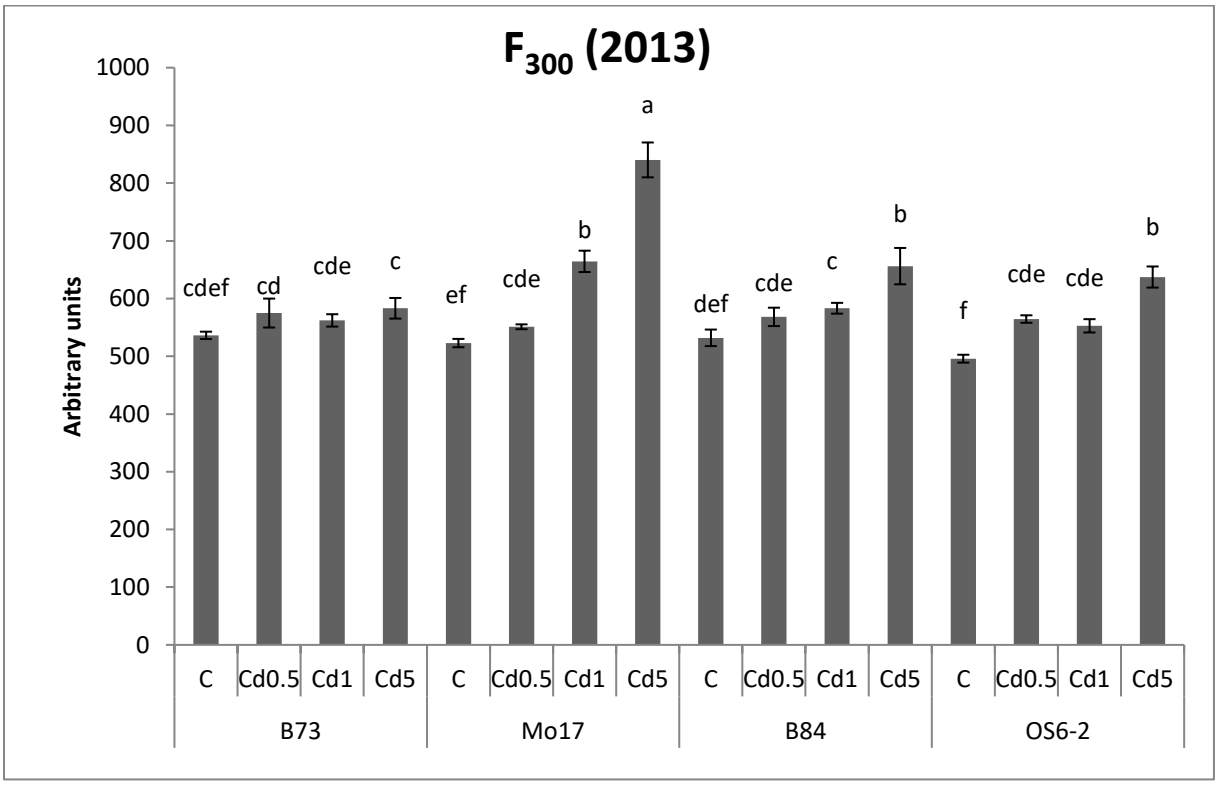


Figure 16. A, B Mean values, standard errors and results of LSD test for **fluorescence intensity at 300 µs (F_{300})** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.4. Variable fluorescence at J step (V_J)

Mean values with standard errors for measured variable fluorescence at J step (V_J) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 10. A), and similarly to F_{300} there were significant effect of Treatment×Genotype interaction. Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment ($p < 0.001$) Genotype ($p < 0.01$). Interaction Treatment×Genotype was also significant in 2013 ($p< 0.05$) (Table 10. B).

Table 10. Analysis of variance for **variable fluorescence at J step (V_J)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	10.059***
Genotype	3	25.247***
Treatment×Genotype	9	3.553**
Replication	15	0.735

B

Source of variation	Degrees of freedom	F value
Treatment	3	23.740***
Genotype	3	27.948***
Treatment×Genotype	9	5.085***
Replication	15	0.265

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of V_J between different genotypes and also between treatments. In Figure 17 A, B mean values and results of LSD test are shown for variable fluorescence at J step (V_J) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of V_J parameter had almost the same intensity in both years of the experiment. Generally, in both years values of V_J were highest at highest cadmium concentration in soil (Cd5 treatment). According to $LSD_{0.05}$ test highest values were in Os6-2 (in both years) followed by Mo17 and B84 in 2012 and by B84 and Mo17 in 2013 (Figure 17 A, B). Inbred line B73 showed the smallest changes in V_J; there were no significant differences from the control in any of the treatments with no increasing or decreasing pattern. Other three genotypes responded to increasing cadmium content in soil with increasing V_J values (Figure 17 A, B).

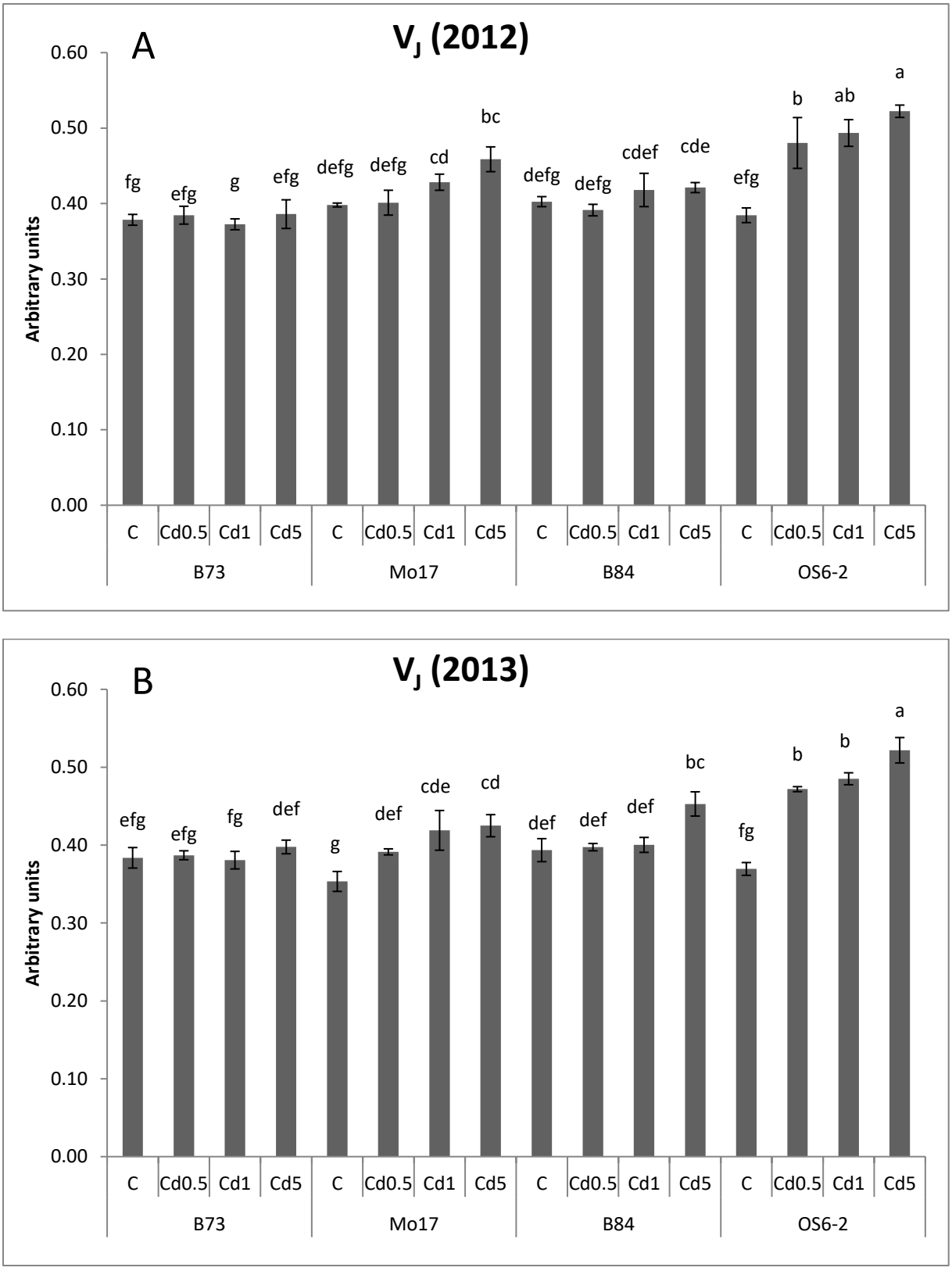


Figure 17. A, B Mean values, standard errors and results of LSD test for **variable fluorescence at J step (V_J)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.5. Variable fluorescence at I step (V_I)

Mean values with standard errors for measured variable fluorescence at I step (V_I) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of all sources of variation, except for Replicaiton, in both years (Table 11 A, B). Unlinke for V_J parameter, interaction effects were significant in both years (for 2012 $p < 0.05$, and for 2013 $p < 0.001$) (Table 11. B).

Table 11. Analysis of variance for **variable fluorescence at I step (V_I)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

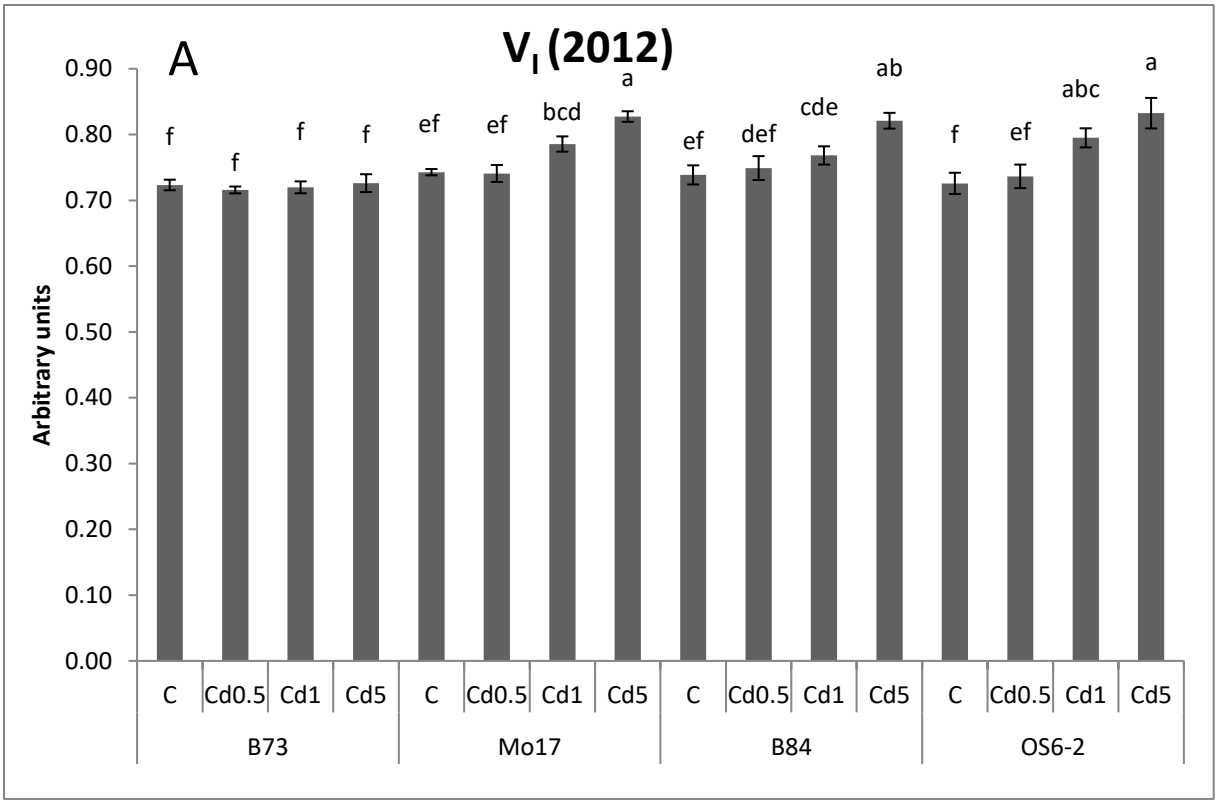
Source of variation	Degrees of freedom	F value
Treatment	3	23.446***
Genotype	3	14.446***
Treatment×Genotype	9	2.579*
Replication	15	1.490

B

Source of variation	Degrees of freedom	F value
Treatment	3	24.449***
Genotype	3	11.598***
Treatment×Genotype	9	4.928***
Replication	15	0.507

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of V_I between different genotypes and also between treatments. In Figure 18 A, B mean values and results of LSD test are shown for variable fluorescence at I step (V_I) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of V_I parameter had almost the same intensity in both years of the experiment. Generally, in both years values of V_J were highest at highest cadmium concentration in soil (Cd5 treatment). According to $LSD_{0.05}$ test highest values in 2012 were in Cd5 treatment in OS6-2, B84 and Mo17 (Figure 18 A). In 2013 highest values according to $LSD_{0.05}$ test were Cd5 treatment in OS6-2 and Mo17, while B84 had somewhat lower values in that treatment (Figure 18 B). Inbred line B73 showed the smallest changes in V_I; there were no significant differences from the control or between any of the treatments, and there was no decreasing or increasing pattern. Other three genotypes responded to increasing cadmium content in soil with increasing V_J values (Figure 18 A, B).



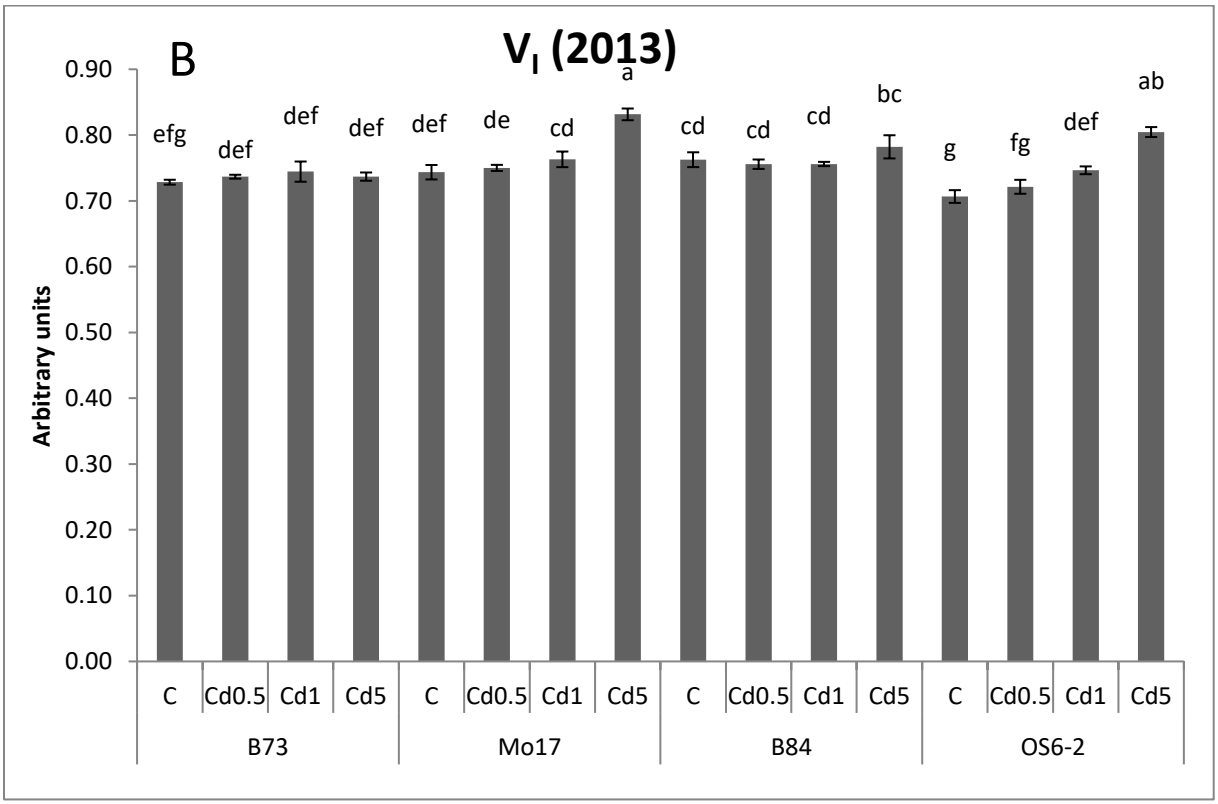


Figure 18. A, B Mean values, standard errors and results of LSD test for **variable fluorescence at I step (V_I)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.6. Absorption per active reaction center (ABS/RC)

Mean values with standard errors for measured absorption per active reaction center (ABS/RC) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 12. A), and unlike in F_{300} there was no significant effect of Treatment×Genotype interaction. Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment ($p < 0.001$) Genotype ($p < 0.01$). Interaction Treatment×Genotype was also significant in 2013 ($p< 0.05$) (Table 12. B).

Table 12. Analysis of variance for **absorption per active reaction center (ABS/RC)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	7.927***
Genotype	3	4.514**
Treatment×Genotype	9	1.010
Replication	15	1.192

B

Source of variation	Degrees of freedom	F value
Treatment	3	19.715***
Genotype	3	6.415**
Treatment×Genotype	9	2.746*
Replication	15	2.024

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of ABS/RC between different genotypes and that there are significant differences in mean values between different treatments. In Figure 19 A, B mean values and results of LSD test are shown for absorption per active reaction center (ABS/RC) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of ABS/RC parameter were slightly higher in 2013 than in 2012. In general, in both years values of ABS/RC were highest at highest cadmium concentration in soil (Cd5 treatment). According to $LSD_{0.05}$ test highest values were in Os6-2 followed by B84 and Mo17. Inbred line B73 showed the smallest changes in ABS/RC; treatment Cd0.5 seems to have a greater effect on ABS/RC value in this genotype than Cd1 or Cd5 where differences were significant only in 2013 between Cd5 and control (Figure 19 B). Other three genotypes responded to increasing cadmium content in soil with increasing ABS/RC values (Figure 19 A, B). Genotype Os6-2 had highest values of ABS/RC while B73 had the lowest.

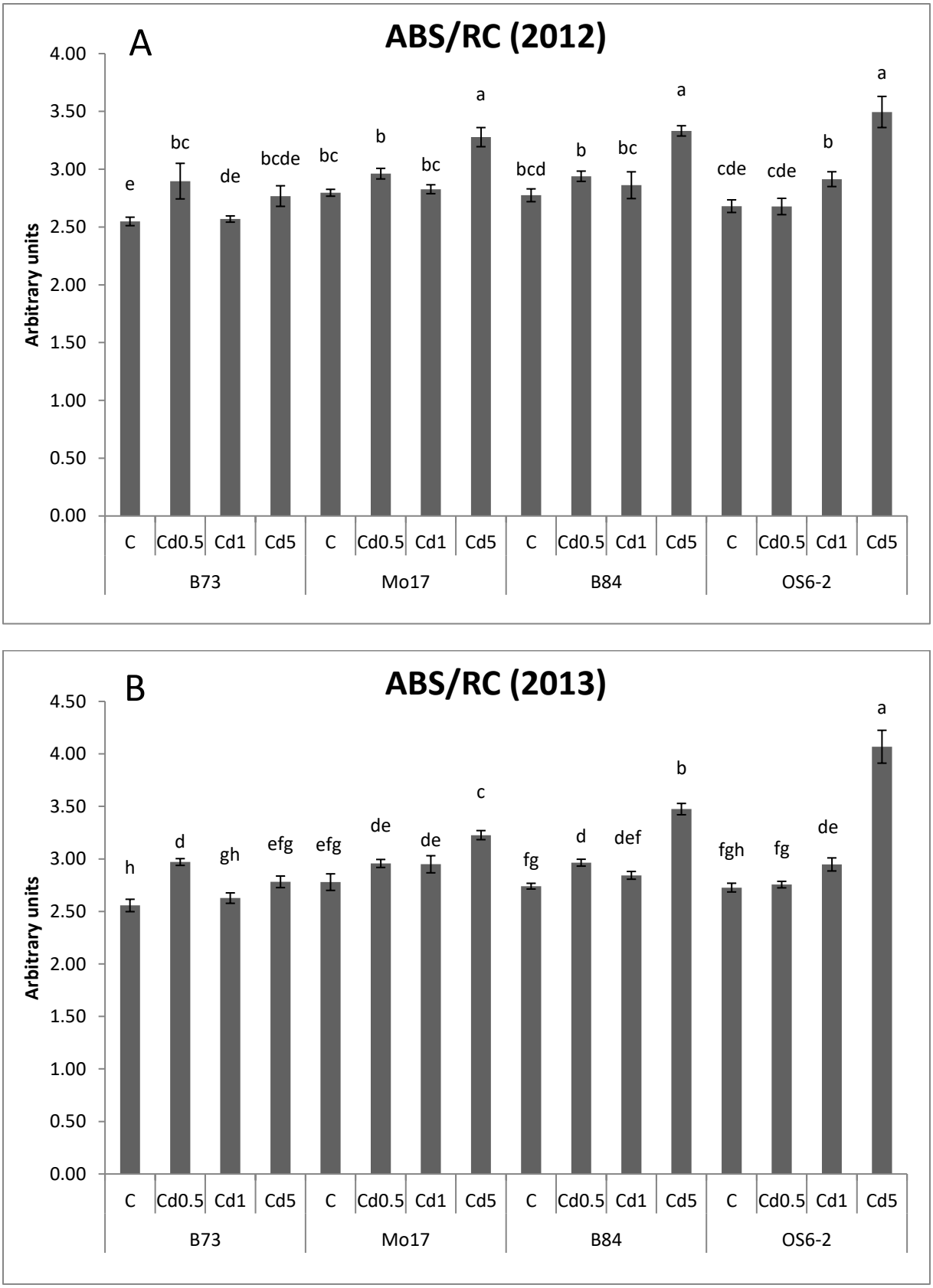


Figure 19. A, B Mean values, standard errors and results of LSD test for **absorption per active reaction center (ABS/RC)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.7. Trapped energy flux per active reaction center (TR_0/ABS)

Mean values with standard errors for measured trapped energy flux per active reaction center (TR_0/ABS) in July 2012 and 2013 are shown in Table 27 in the Appendix. Similarly as for ABS/RC, analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 13. A). Analysis of variance for the same parameter in 2013 has shown significant effects of cadmium treatment ($p<0.001$), genotype ($p < 0.001$) and their interaction ($p < 0.05$) (Table 13. B).

Table 13. Analysis of variance for **trapped energy flux per active reaction center (TR_0/ABS)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	8.692***
Genotype	3	4.455**
Treatment×Genotype	9	0.992
Replication	15	0.854

B

Source of variation	Degrees of freedom	F value
Treatment	3	24.120***
Genotype	3	13.024***
Treatment×Genotype	9	2.668*
Replication	15	0.803

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD have shown that there are differences in mean values of TR_0/RC between different genotypes and that there are significant differences in mean values between different treatments. In Figure 19 A, B mean values and results of LSD test are shown for trapped energy flux per active reaction center (TR_0/ABS) measured on ear-leaves of four maize genotypes in 2012 and 2013. Generally, in both years values of TR_0/ABS were highest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in TR_0/ABS; treatment Cd0.5 seems to have a greater effect on TR_0/ABS value in this genotype than Cd1 or Cd5. Differences for B73between Cd5 and control were not significant in 2012 or 2013 (Figure 19 B). Other three genotypes responded to increasing cadmium content in soil with increasing TR_0/ABS values (Figure 19 A, B). Os6-2 had highest values of TR_0/ABS while B73 had the lowest.

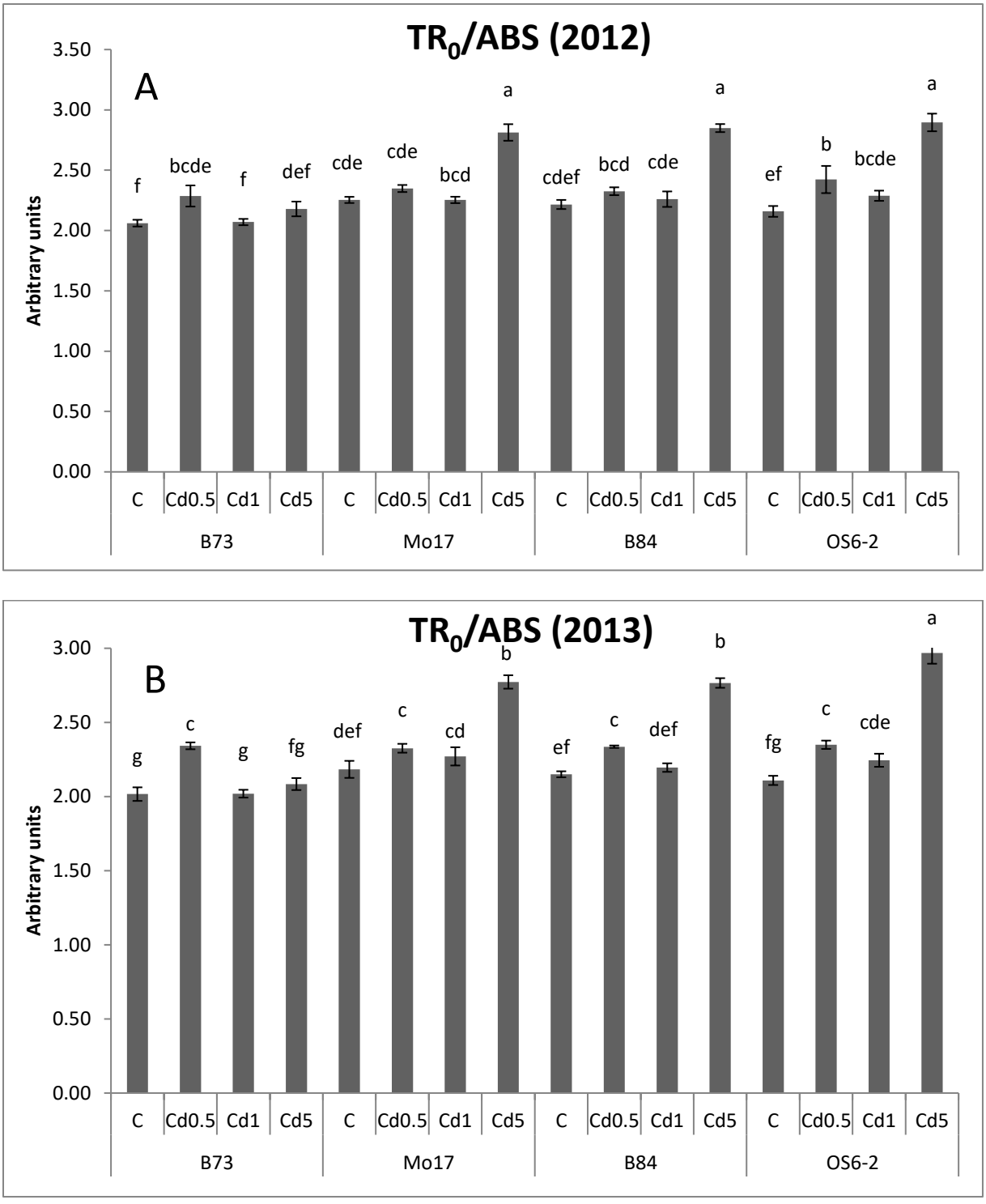


Figure 19. A, B Mean values, standard errors and results of LSD test for **trapped energy flux per active reaction center (TR_0/ABS)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.8. Electron transport flux per active reaction center (ET_0/RC)

Mean values with standard errors for measured electron transport flux per active reaction center (ET_0/RC) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 14. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for all sources of variation (Table 14. B).

Table 14. Analysis of variance for **electron transport flux per active reaction center (ET_0/RC)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

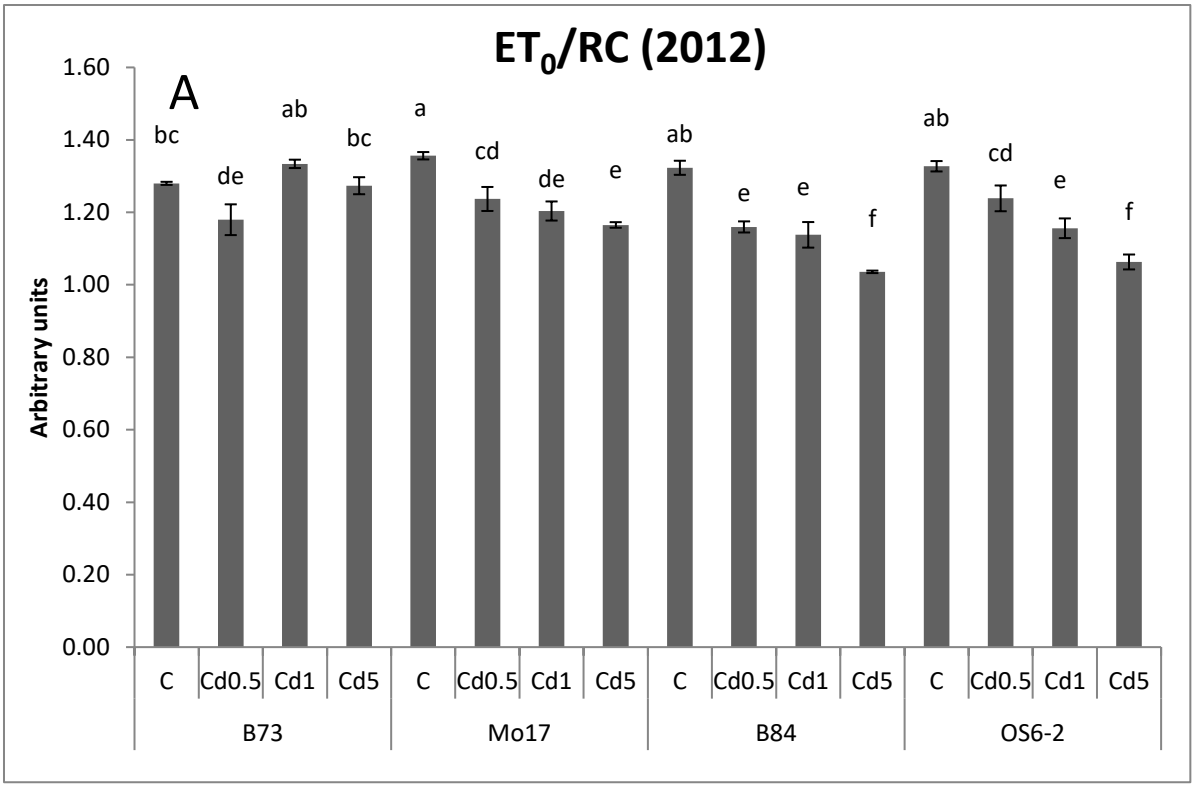
Source of variation	Degrees of freedom	F value
Treatment	3	41.773***
Genotype	3	14.496***
Treatment×Genotype	9	7.982***
Replication	15	0.585

B

Source of variation	Degrees of freedom	F value
Treatment	3	16.660***
Genotype	3	27.770***
Treatment×Genotype	9	20.490***
Replication	15	0.660

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of ET_0/RC between different genotypes and that there are significant differences in mean values between different treatments. In Figure 21 A, B mean values and results of LSD test are shown for electron transport flux per active reaction center (ET_0/RC) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of ET_0/RC were lowest at highest cadmium concentration in soil (Cd5 treatment). According to$LSD_{0.05}$ test significantly lowest values were in B84 and Os6-2 in Cd5 treatment, followed by Mo17 and B73. Inbred line B73 showed the smallest changes in ET_0/RC and in 2012 there were no significant differences in ET_0/RC between treatments for that genotype (except in Cd0.5 treatment) (Figure 21 A), while in 2013 values slightly increased in Cd1 and Cd5 treatments (Figure 21 B). Other three genotypes responded to increasing cadmium content in soil with decreasing ET_0/RC values.



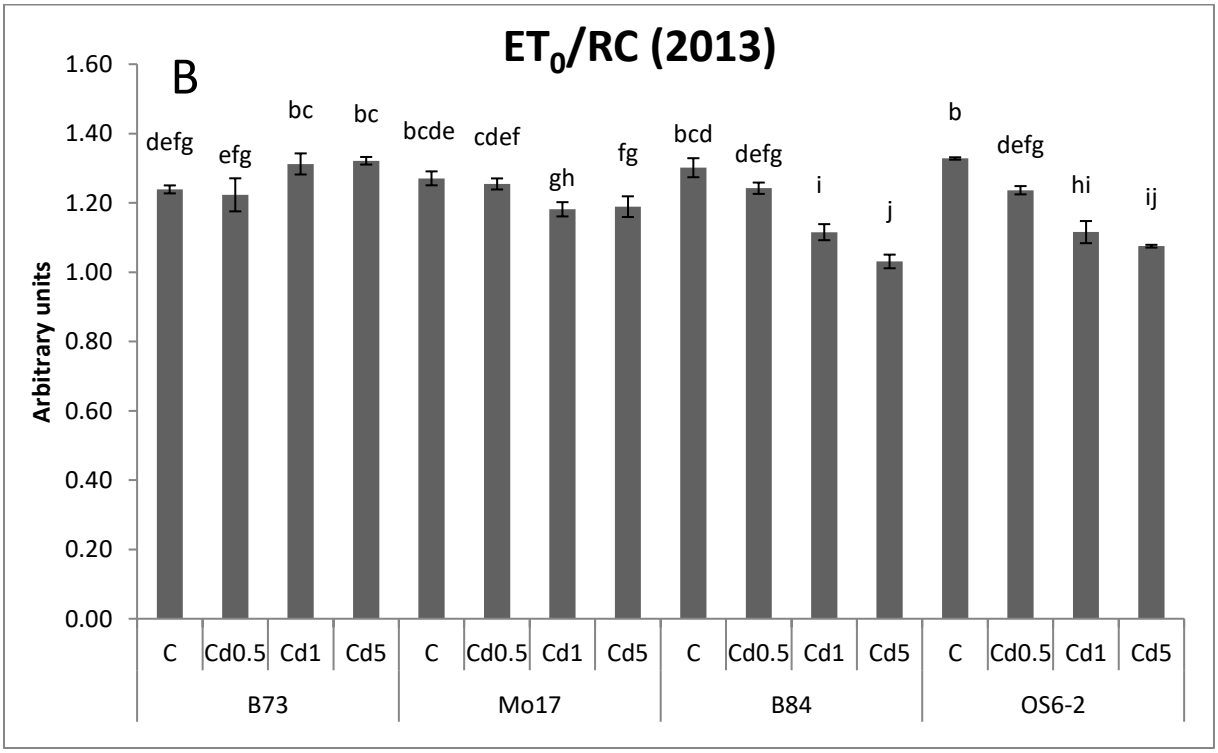


Figure 21. A, B Mean values, standard errors and results of LSD test for **electron transport flux per active reaction center (ET_0/RC)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.9. Dissipation energy per active reaction center (DI_0/RC)

Mean values with standard errors for measured dissipation energy per active reaction center (DI_0/RC) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 15. A), and unlike ET0/RC there was no significant effect Treatment×Genotype of interaction. Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$). Interaction Treatment×Genotype was also significant in 2013 ($p < 0.01$) (Table 15. B).

Table 15. Analysis of variance for **dissipation energy per active reaction center (DI_0/RC)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	14.538***
Genotype	3	9.300***
Treatment×Genotype	9	1.859
Replication	15	1.795

B

Source of variation	Degrees of freedom	F value
Treatment	3	43.012***
Genotype	3	18.382***
Treatment×Genotype	9	3.781**
Replication	15	2.234

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of DI_0/RC between different genotypes and that there are significant differences in mean values between different treatments. In Figure 22 A, B mean values and results of LSD test are shown for dissipation energy per active reaction center (DI_0/RC) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of DI_0/RC were highest at highest cadmium concentration in soil (Cd5 treatment). Highest values in both years were in Os6-2 and B84 genotypes, while lowest values were in B73 genotype. In B73 genotype increase in dissipation energy is not as clear as in other three genotypes and there are no significant differences between treatments in both years (Figure 22 A, B). Other three genotypes responded to increasing cadmium content in soil with increasing DI_0/RC in a gradual manner. Increasing cadmium content in soil had largest effect on dissipation energy in lines Os6-2 and B84 (Figure 22. A, B).

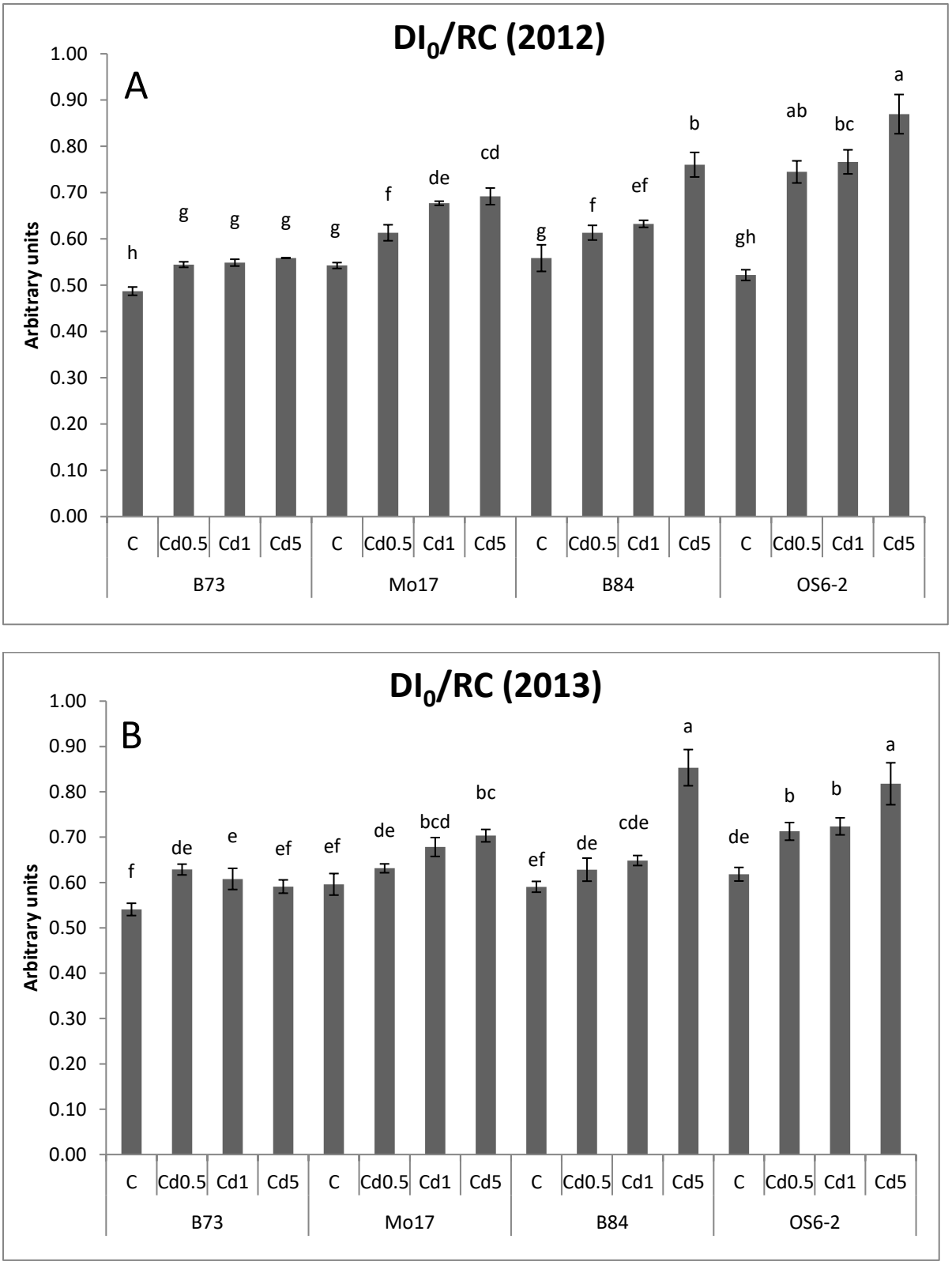


Figure 22. A, B Mean values, standard errors and results of LSD test for **dissipation energy per active reaction center (DI_0/RC)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.10. Q_A-reducing reaction centers per PSII antenna chlorophyll (RC/ABS)

Mean values with standard errors for measured RC/ABS parameter in July 2012 and 2013 are shown in Table 27 in the Appendix. Same as for DI_0/RC, analysis of variance for RC/ABS parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 16. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$). In 2013 interaction Treatment×Genotype was also significant ($p < 0.05$) (Table 16. B).

Table 16. Analysis of variance for measured **Q_A-reducing reaction centers per PSII antenna chlorophyll (RC/ABS)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	13.419***
Genotype	3	9.891***
Treatment×Genotype	9	0.791
Replication	15	1.776

B

Source of variation	Degrees of freedom	F value
Treatment	3	41.452***
Genotype	3	13.610***
Treatment×Genotype	9	2.821*
Replication	15	1.217

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of ET_0/ABS between different genotypes and that there are significant differences in mean values between different treatments. In Figure 23 A, B mean values and results of LSD test are shown for Q_A-reducing reaction centers per PSII antenna chlorophyll (RC/ABS) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of RC/ABS were lowest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in both years and Os6-2 line showed the largest changes in RC/ABS values (Figure 23 A, B). All genotypes, except B73, showed a gradual decrease in RC/ABS values with the increase of cadmium content in soil. In B73 genotype largest decrease was in Cd0.5 treatment, Cd1 treatment did not have any significant effect on the decrease of RC/ABS, while Cd5 treatment caused a slight decrease in RC/ABS values.

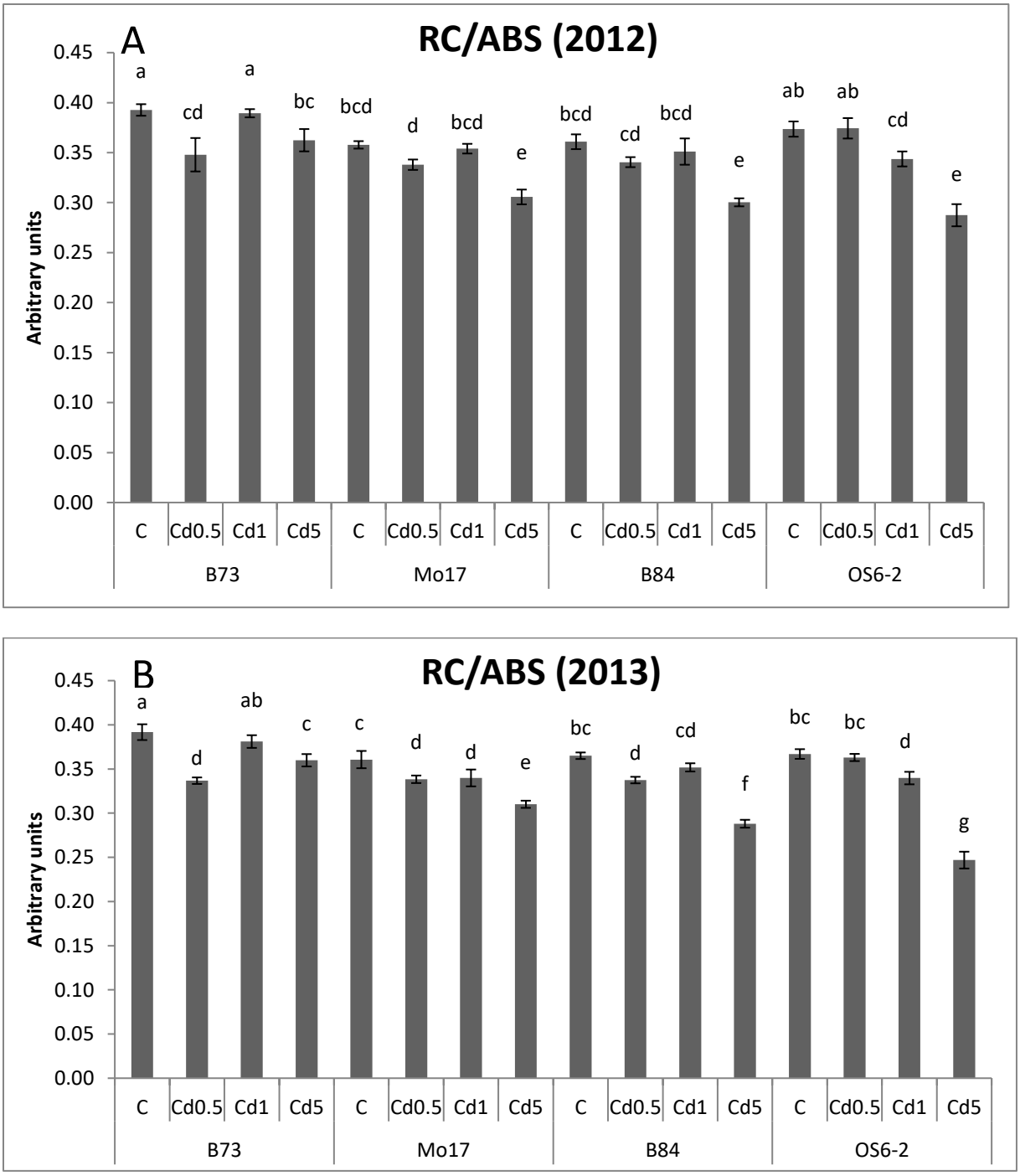


Figure 23. A, B Mean values, standard errors and results of LSD test for **Q_A-reducing reaction centers per PSII antenna chlorophyll (RC/ABS)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.11. Density of active reaction centers (RC/CS_0)

Mean values with standard errors for measured of density active reaction centers (RC/CS_0) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 17. A). Analysis of variance for the same parameter in 2013 has shown the

same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$) and significance of Treatment×Genotype interaction was lower than in 2013 ($p < 0.05$) (Table 17. B).

Table 17. Analysis of variance for measured **density of active reaction centers (RC/CS_0)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

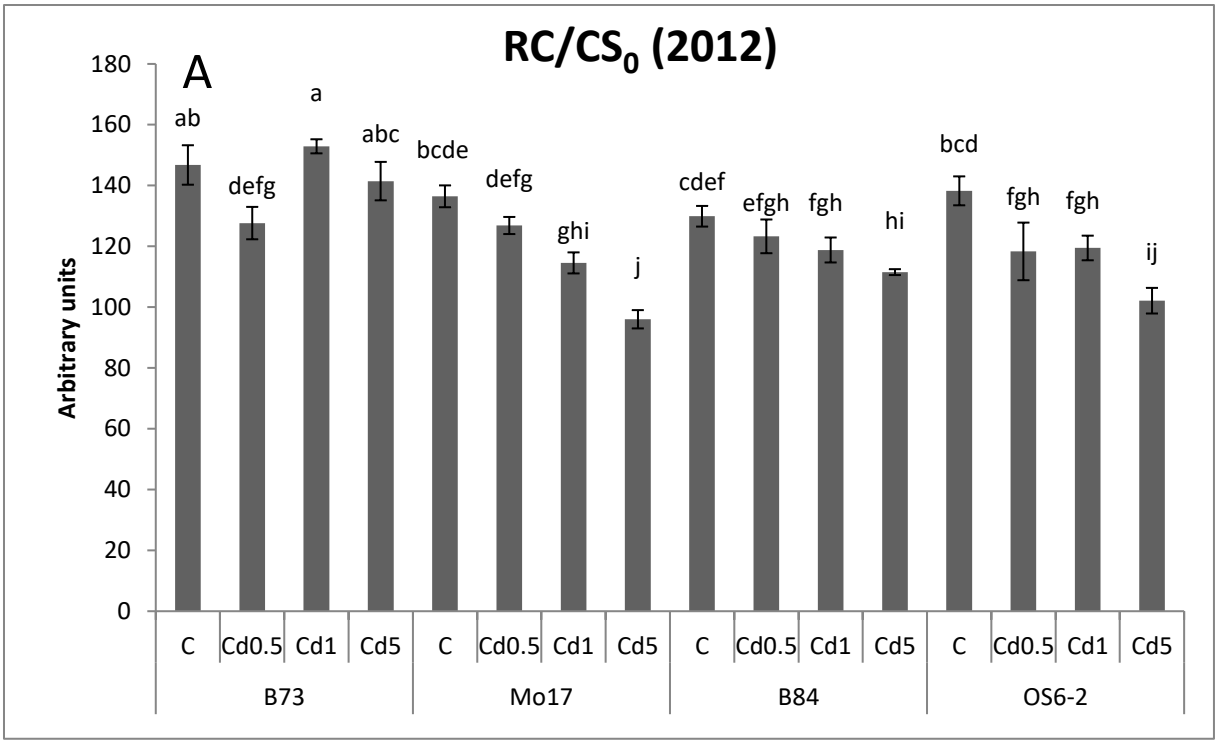
Source of variation	Degrees of freedom	F value
Treatment	3	18.265***
Genotype	3	22.164***
Treatment×Genotype	9	3.972***
Replication	15	0.739

B

Source of variation	Degrees of freedom	F value
Treatment	3	27.467***
Genotype	3	6.679***
Treatment×Genotype	9	2.777*
Replication	15	0.584

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of RC/CS_0 between different genotypes and that there are significant differences in mean values between different treatments. In Figure 24 A, B mean values and results of LSD test are shown for electron transport flux per active reaction center (RC/CS^0) measured on ear-leaves of four maize genotypes in 2012 and 2013. Values of RC/CS_0 in 2012 were slightly higher than in 2013. In general, in both years values of RC/CS_0 were lowest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in RC/CS_0, and in both years there were no significant differences in RC/CS_0 between treatments for that genotype (except in Cd0.5 treatment) (Figure 24. A, B). Other three genotypes responded to increasing cadmium content in soil with decreasing RC/CS_0 values.



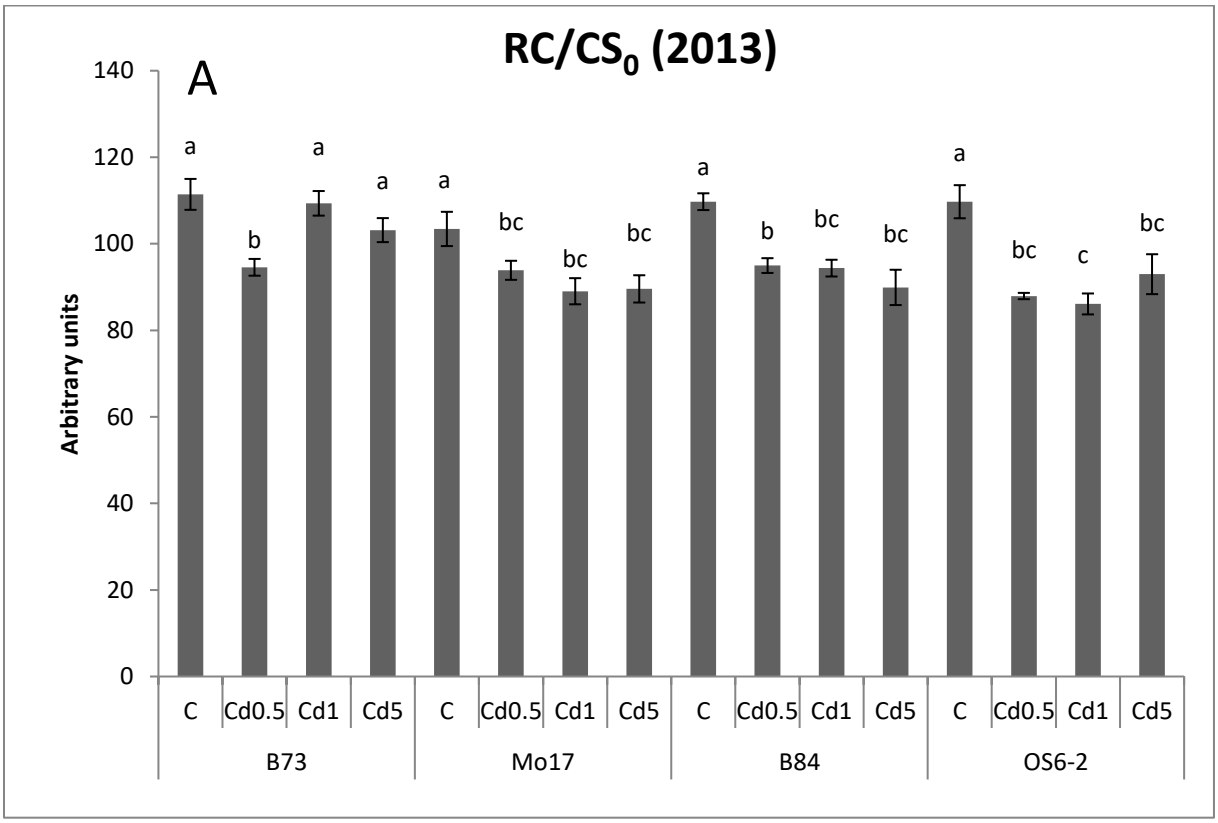


Figure 24. A, B Mean values, standard errors and results of LSD test for **density of active reaction centers (RC/CS$_0$)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.12. Time to reach maximal fluorescence intensity (t_{max})

Mean values with standard errors for measured time to reach maximal fluorescence intensity (t_{max}) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 18. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$ and $p < 0.01$, respectively) and their interaction ($p < 0.001$) (Table 18. B). Unlike RC/CS_0 there were no significant effects of Treatment×Genotype interaction in both years.

Table 18. Analysis of variance for measured **time to reach maximal fluorescence intensity (t_{max})** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	8.844***
Genotype	3	4.064*
Treatment×Genotype	9	1.201
Replication	15	0.941

B

Source of variation	Degrees of freedom	F value
Treatment	3	10.885***
Genotype	3	4.030*
Treatment×Genotype	9	1.763
Replication	15	2.309

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of t_{max} between different genotypes and that there are significant differences in mean values between different treatments. In Figure 25 A, B mean values and results of LSD test are shown for time to reach maximal fluorescence intensity (t_{max}) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of t_{max} were lowest at highest cadmium concentration in soil (Cd5 treatment). According to $LSD_{0.05}$ test lowest values were in Os6-2 and B84, followed by Mo17 and B73. Inbred line B73 showed the smallest changes in t_{max} and in both years there were no significant differences in t_{max} between, except in Cd0.5 treatment for 2013 which was significantly lower than control (Figure 25 A, B). Other three genotypes responded to increasing cadmium content in soil with decreasing t_{max} values.

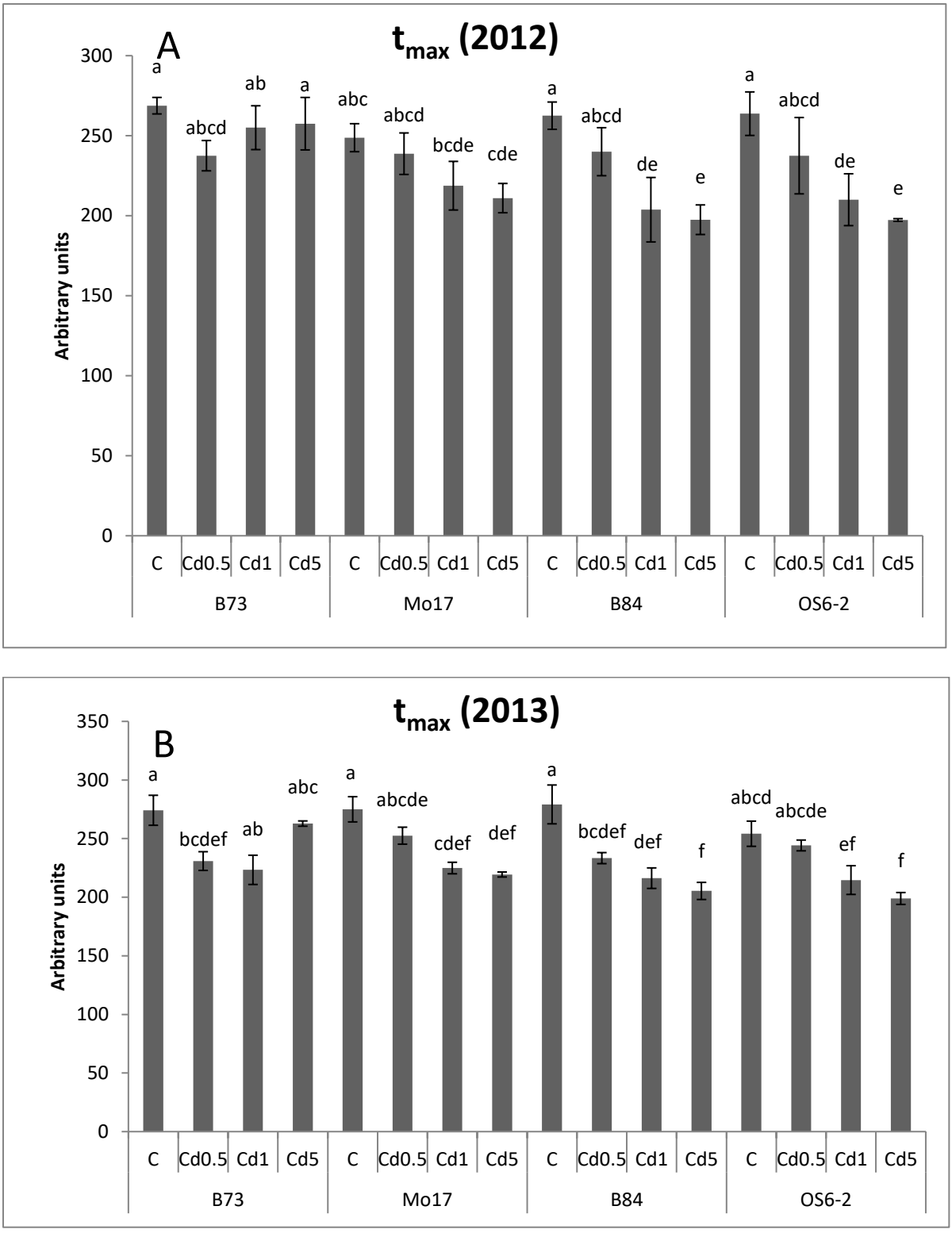


Figure 25. A, B Mean values, standard errors and results of LSD test for **time to reach maximal fluorescence intensity (t_{max})** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.13. Maximum quantum yield of photosystem II (TR_0/ABS)

Mean values with standard errors for measured maximum quantum yield of photosystem II (TR_0/ABS) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of

variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 19. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment, Genotype and their interaction ($p < 0.001$) (Table 19. B).

Table 19. Analysis of variance for **maximum quantum yield of photosystem II (TR_0/ABS)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

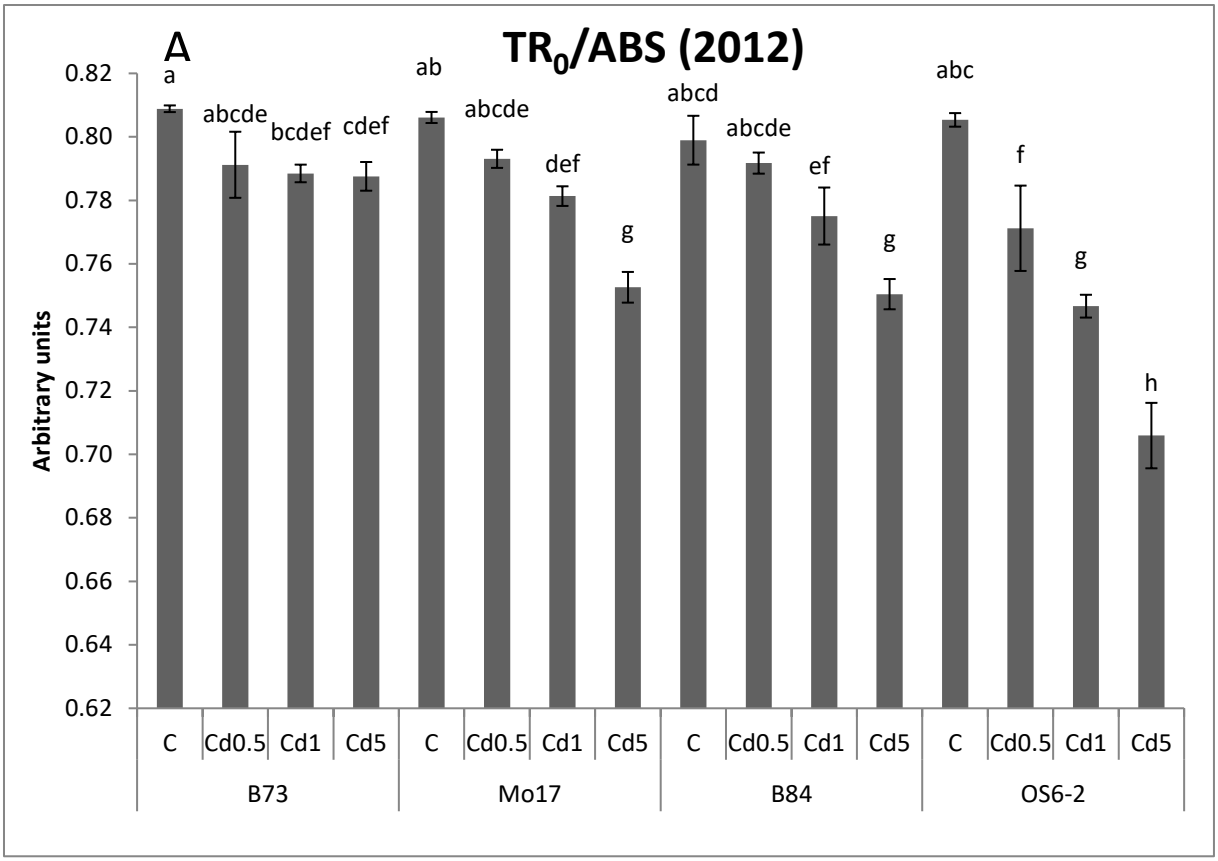
Source of variation	Degrees of freedom	F value
Treatment	3	62.459***
Genotype	3	20.056***
Treatment×Genotype	9	6.562***
Replication	15	2.715

B

Source of variation	Degrees of freedom	F value
Treatment	3	61.716***
Genotype	3	26.097***
Treatment×Genotype	9	6.475***
Replication	15	1.666

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of TR_0/ABS between different genotypes and that there are significant differences in mean values between different treatments. In Figure 26 A, B mean values and results of LSD test are shown for maximum quantum yield of photosystem II (TR_0/ABS) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of TR_0/ABS were lowest at lowest cadmium concentration in soil (Cd5 treatment) and there is a clear decreasing trendline. Significantly lowest values were in Os6-2, followed by B84, Mo17 and B73. Inbred line B73 showed the smallest changes in TR_0/ABS it is the only genotype that does not show a clear decreasing trendline with increasing cadmium concentrations in soil (Figure 26. A, B). Other three genotypes responded to increasing cadmium content in soil with decreasing TR_0/ABS values with large differences between control and Cd5 treatment.



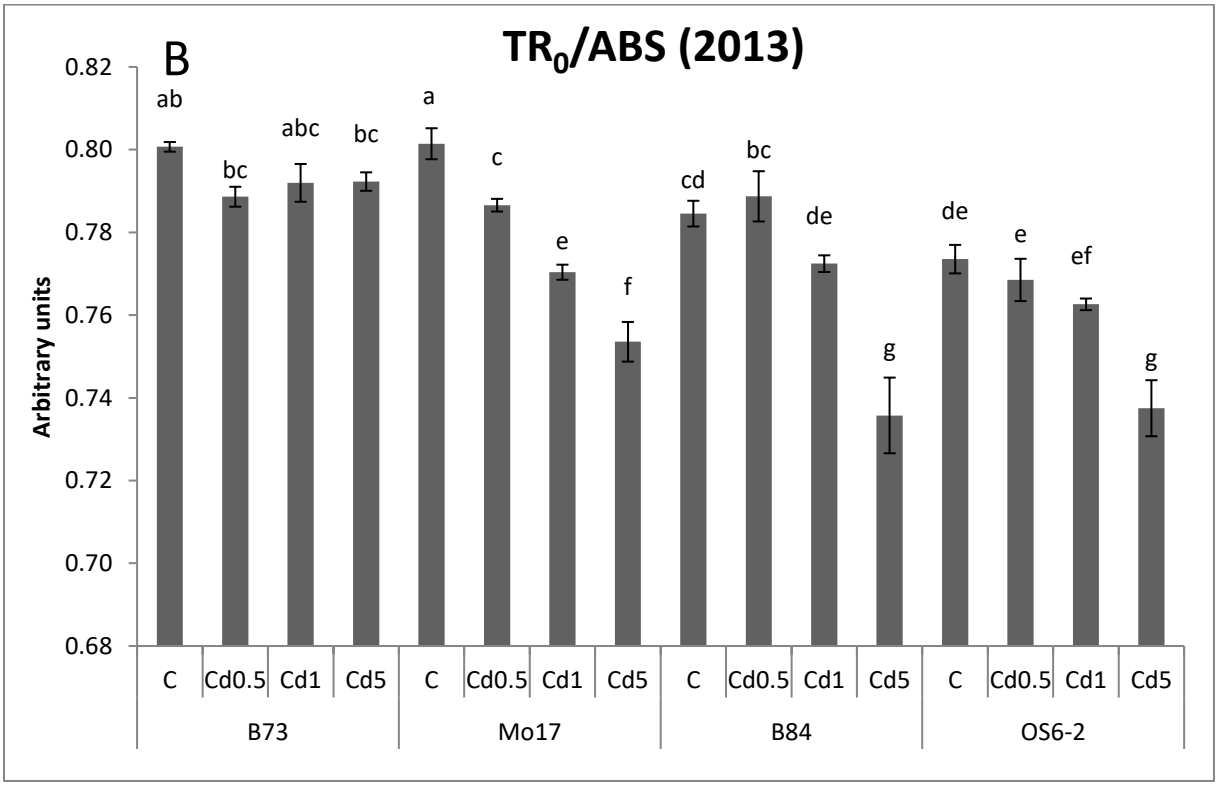


Figure 26. A, B Mean values, standard errors and results of LSD test for **maximum quantum yield of photosystem II (TR_0/ABS)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.14. Quantum yield for electron transport (ET_0/ABS)

Mean values with standard errors for measured quantum yield for electron transport (ET_0/ABS) in July 2012 and 2013 are shown in Table 27 in the Appendix. Similarly to TR_0/ABS, analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 20. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$) and a higher level of significance for their interaction ($p < 0.001$) (Table 20. B).

Table 20. Analysis of variance for **quantum yield for electron transport (ET_0/ABS)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	34.449***
Genotype	3	11.536***
Treatment×Genotype	9	2.493*
Replication	15	1.336

B

Source of variation	Degrees of freedom	F value
Treatment	3	61.452***
Genotype	3	15.567***
Treatment×Genotype	9	6.386***
Replication	15	0.375

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of ET_0/ABS between different genotypes and that there are significant differences in mean values between different treatments. In Figure 27 A, B mean values and results of LSD test are shown for quantum yield for electron transport (ET_0/ABS) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of ET_0/ABS were lowest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in ET_0/ABS and in 2012 there were no significant differences in ET_0/ABS from the control for that genotype (except in Cd0.5 treatment) (Figure 27 A). In 2013valuies of ET_0/ABS decreased in B73 but there was no gradual decrease in values like in other genotypes (Figure 27 B). B84, Mo17 and Os6-2 genotypes responded to increasing cadmium content in soil with a gradual decrease in ET_0/ABS values. Genotypes B84 and Os6-2 had largest decreases in both years, while B73 had the smallest.

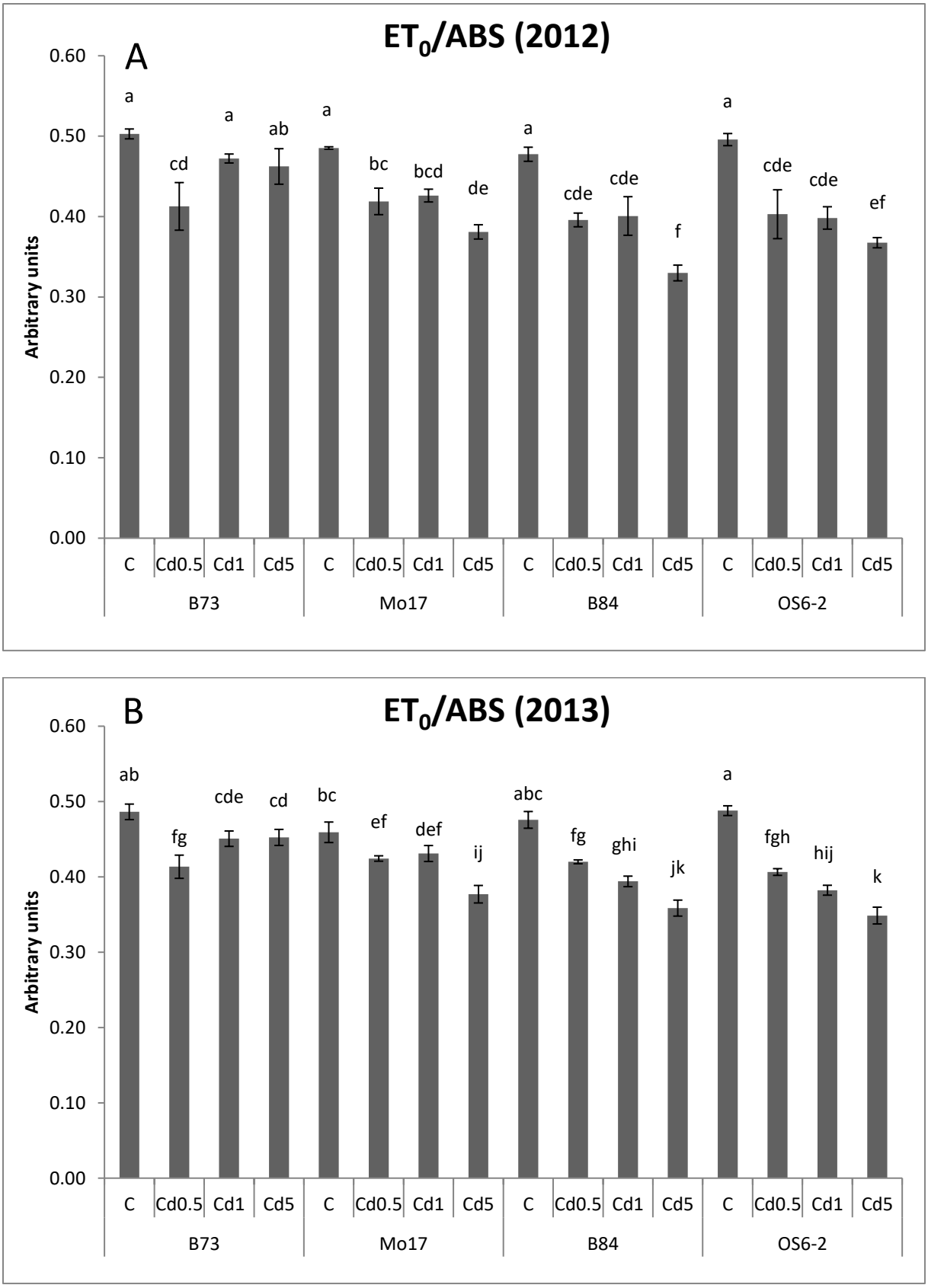


Figure 27. A, B Mean values, standard errors and results of LSD test **for quantum yield for electron transport (ET_0/ABS)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.15. Probability for electron transport (ET_0/TR_0)

Mean values with standard errors for measured probability for electron transport (ET_0/TR_0) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 ($p < 0.001$) (Table 21. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p<0.001$) and also for their interaction ($p < 0.001$) (Table 21. B).

Table 21. Analysis of variance for **probability for electron transport (ET_0/TR_0)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	26.471***
Genotype	3	7.837***
Treatment×Genotype	9	2.023
Replication	15	1.049

B

Source of variation	Degrees of freedom	F value
Treatment	3	40.434***
Genotype	3	10.637***
Treatment×Genotype	9	5.486***
Replication	15	0.109

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of ET_0/TR_0 between different genotypes and that there are significant differences in mean values between different treatments. In Figure 28 A, B mean values and results of LSD test are shown for probability for electron transport (ET_0/TR_0) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of ET_0/TR_0 were lowest at highest cadmium concentration in soil (Cd5 treatment) with the exception of B73 inbred line. Inbred line B73 showed the smallest changes in ET_0/TR_0 and in both years there were no significant differences in ET_0/TR_0 from the control for that genotype (except in Cd0.5 treatment) (Figure 28 A, B). Other three genotypes responded to increasing cadmium content in soil with decreasing ET_0/TR_0 values.

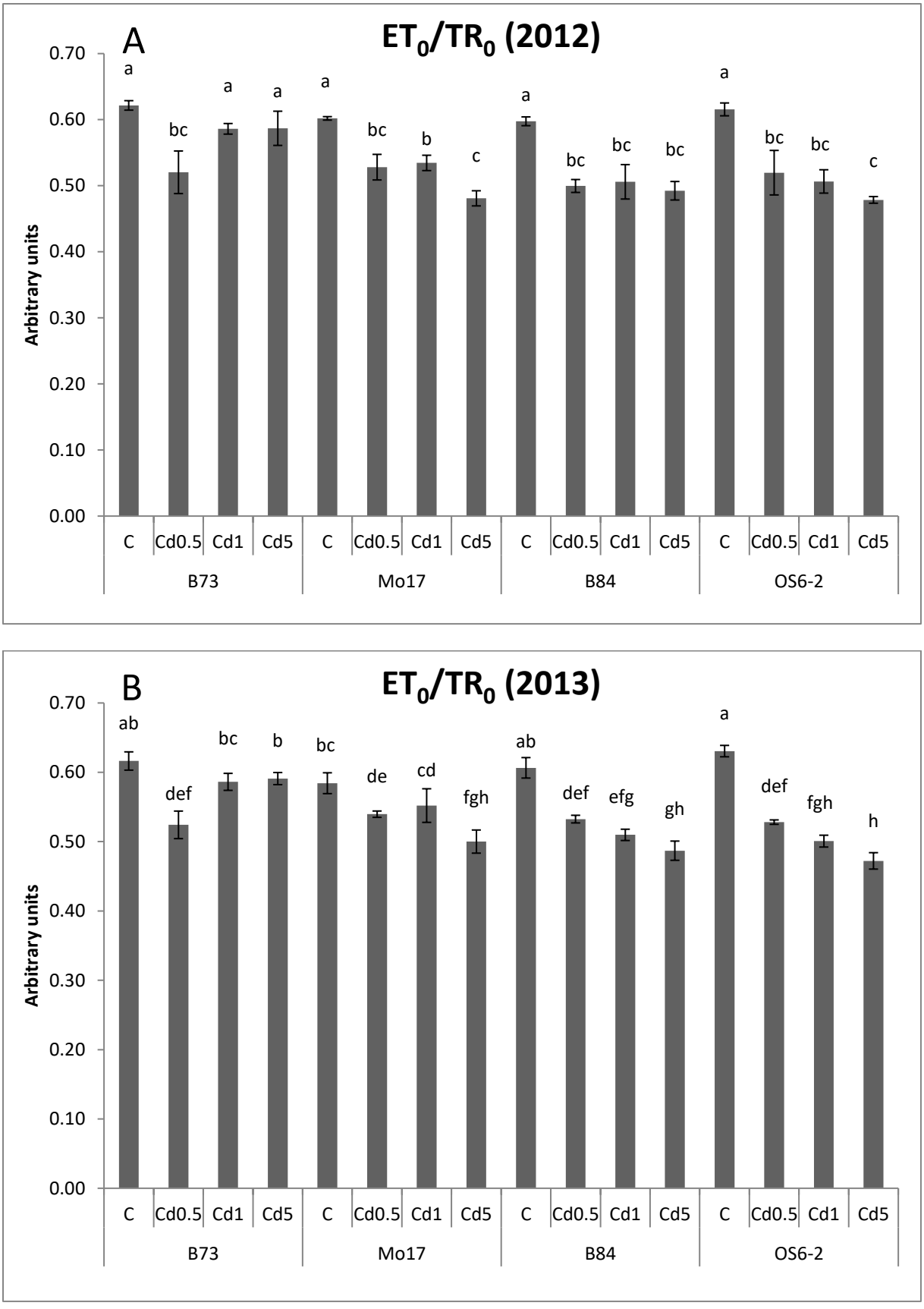


Figure 28. A, B Mean values, standard errors and results of LSD test for **probability for electron transport (ET_0/TR_0)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.16. Ratio of trapped photons and energy dissipation (TR_0/DI_0)

Mean values with standard errors for the ratio of trapped photons and energy dissipation (TR_0/DI_0) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 22. A). Analysis of variance for the same parameter in 2013 has shown the same level of significance as in 2012 for Treatment ($p<0.001$) and a higher level of significance for Genotype ($p < 0.001$) (Table 22. B).

Table 22. Analysis of variance for the **ratio of trapped photons and energy dissipation (TR_0/DI_0)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	10.862***
Genotype	3	5.282**
Treatment×Genotype	9	1.030
Replication	15	2.459

B

Source of variation	Degrees of freedom	F value
Treatment	3	82.220***
Genotype	3	10.440***
Treatment×Genotype	9	1.400
Replication	15	2.390

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of TR_0/DI_0 between different genotypes and that there are significant differences in mean values between different treatments (Table 22. A, B). In Figure 29 A, B mean values and results of LSD test are shown for the ratio of trapped photons and energy dissipation (TR_0/DI_0) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, there is a decreasing trend in TR_0/DI_0 values with increasing cadmium content in soil, and the decrease is especially visible in Cd1 and Cd5 treatments in both years (Figure 29. A, B). Lowest values recorded were in B84 and Os6-2 genotypes in Cd5 treatments. B73 genotype showed smallest changes in TR_0/DI_0 values and in both yearsCd1 and Cd5 treatments did not differ significantly from the control.

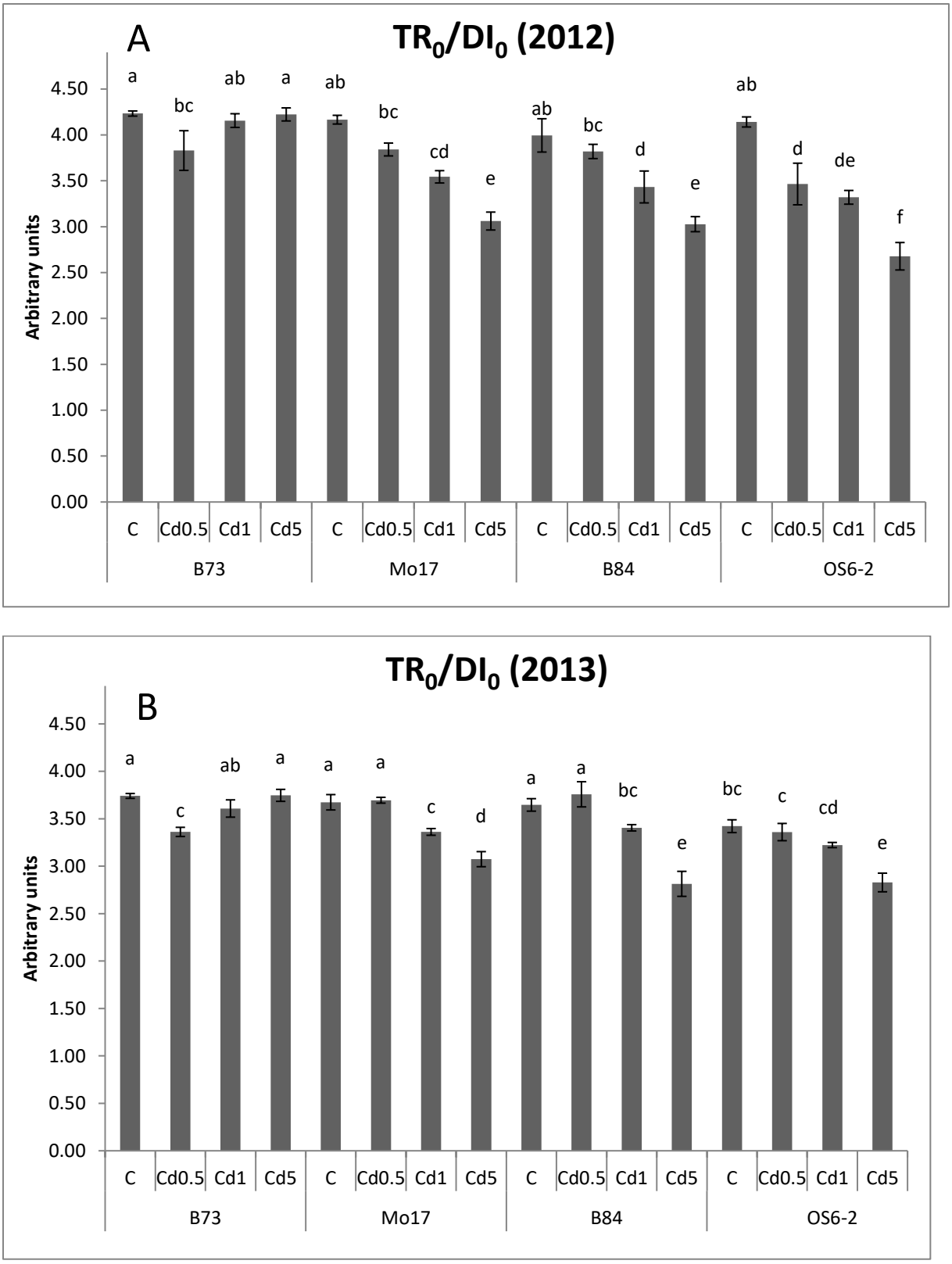


Figure 29. A, B Mean values, standard errors and results of LSD test for **the ratio of trapped photons and energy dissipation (TR_0/DI_0)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.17. Electron transport further than primary acceptor Q_A ($ET_0/(TR_0-ET_0)$)

Mean values with standard errors for electron transport further than primary acceptor Q_A ($ET_0/(TR_0-ET_0)$) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 23. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment, Genotype and their interaction ($p < 0.001$, $p < 0.001$, $p < 0.01$, respectively) (Table 23. B).

Table 23. Analysis of variance for the **electron transport further than primary acceptor Q_A ($ET_0/(TR_0-ET_0)$)** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	28.994***
Genotype	3	6.681***
Treatment×Genotype	9	2.867**
Replication	15	0.988

B

Source of variation	Degrees of freedom	F value
Treatment	3	37.880***
Genotype	3	6.766***
Treatment×Genotype	9	3.649**
Replication	15	0.630

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of ($ET_0/(TR_0-ET_0)$) between different genotypes and that there are significant differences in mean values between different treatments. In Figure 30 A, B mean values and results of LSD test are shown for electron transport further than primary acceptor Q_A ($ET_0/(TR_0-ET_0)$) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of ($ET_0/(TR_0-ET_0)$) were significantly lower in highest cadmium concentration in soil (Cd5 treatment) than in control. Exception is inbred line B73 in 2012 which showed smallest decreases between treatments and control and in 2012 there was no difference between C and Cd0.5 treatment (Figure 30. A), while in 2013 mean value of ($ET_0/(TR_0-ET_0)$) in Cd5 treatment was significantly lower than in control, although the decrease was quite small (Figure 28. B). In other genotypes, in both years values of ($ET_0/(TR_0-ET_0)$) were significantly lower in Cd0.5 treatment than in control (Figure 30. A, B).

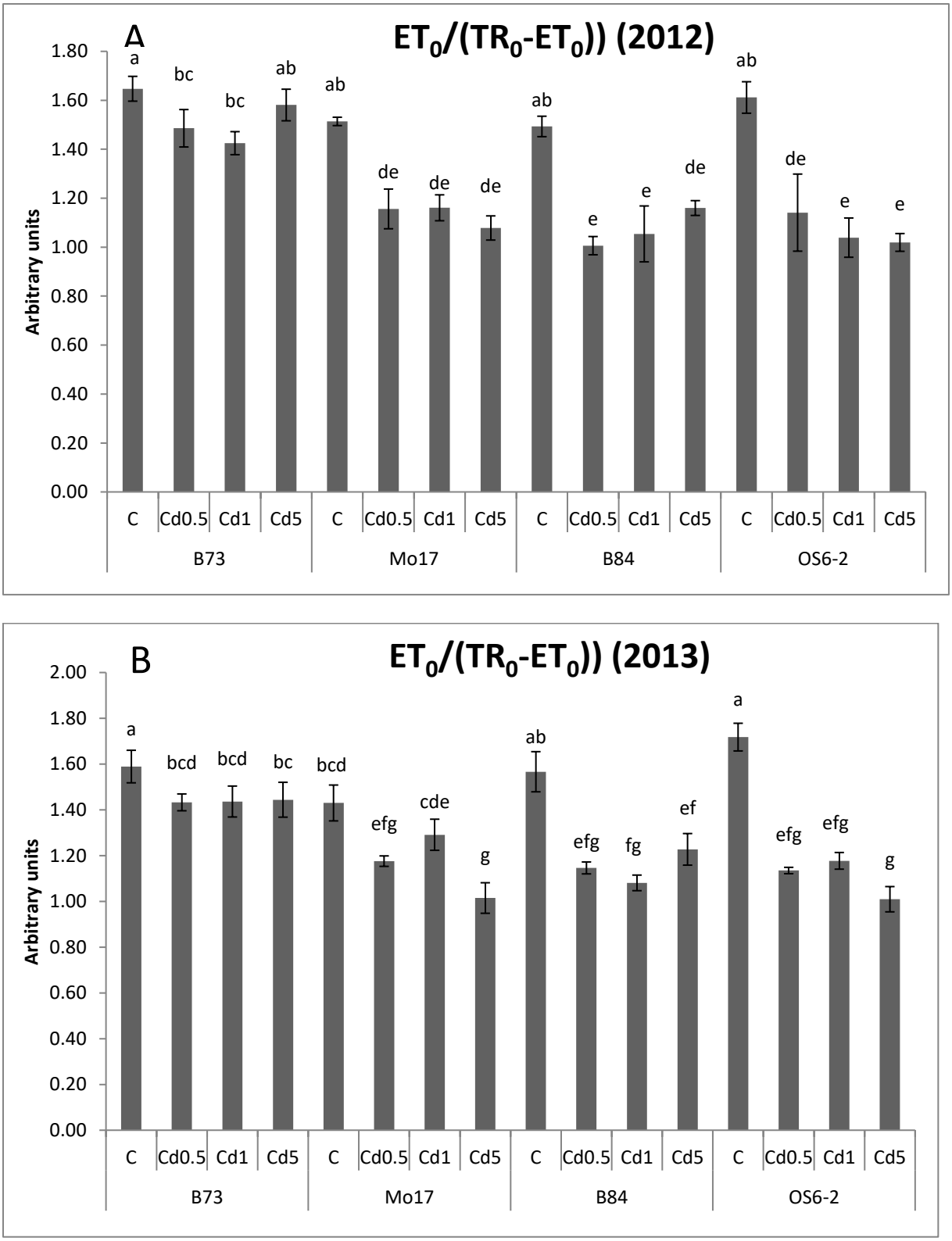


Figure 30. A, B Mean values, standard errors and results of LSD test for **electron transport further than primary acceptor Q_A ($ET_0/(TR_0-ET_0)$)** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.18. Performance index for energy conservation from exciton to the reduction of intersystem electron acceptors (PI_{ABS})

Mean values with standard errors for performance index (PI_{ABS}) in July 2012 and 2013 are shown in Table 27 in the Appendix. Analysis of variance for this parameter has shown significant effects of cadmium treatment, genotype and their interaction in 2012 (Table 24. A). Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment, Genotype and their interaction ($p < 0.001$, $p < 0.001$, $p < 0.05$, respectively) (Table 24. B).

Table 24. Analysis of variance for **performance index (PI_{ABS})** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	22.406***
Genotype	3	55.897***
Treatment×Genotype	9	2.674*
Replication	15	0.455

B

Source of variation	Degrees of freedom	F value
Treatment	3	10.677***
Genotype	3	48.851***
Treatment×Genotype	9	2.725*
Replication	15	0.905

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of PI_{ABS} between different genotypes and that there are significant differences in mean values between different treatments. In Figure 31 A, B mean values and results of LSD test are shown for performance index (PI_{ABS}) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, PI_{ABS} values were slightly higher PI_{ABS} than in 2013. In both years values of PI_{ABS} were lowest at highest cadmium concentration in soil (Cd5 treatment). The exception is inbred line B73 in which PI_{ABS} values were not significantly different in Cd5 and control in both years (Figure 31. A, B). Other three genotypes showed decreasing trend in PI_{ABS} values with increasing cadmium content in soil.

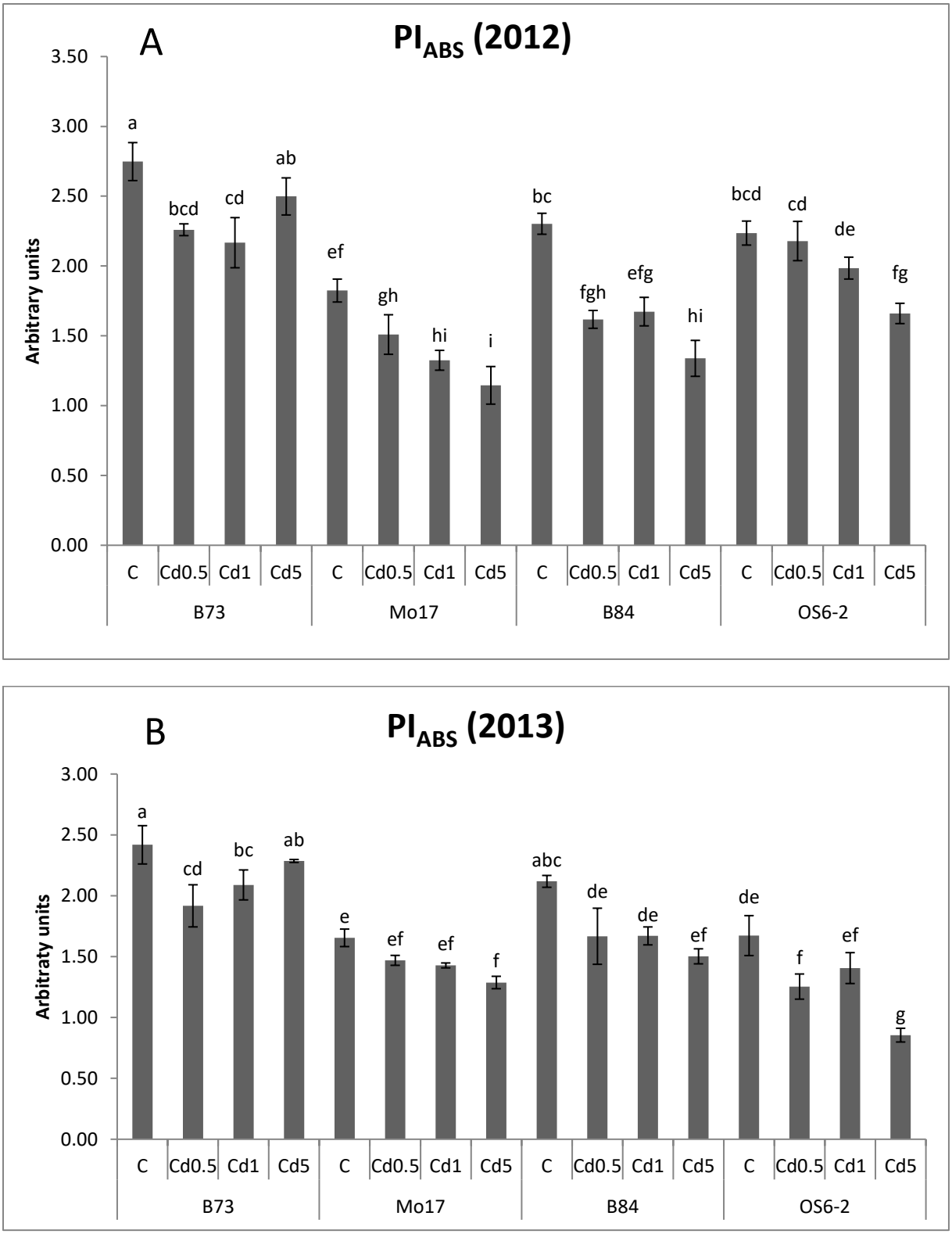


Figure 31. A, B Mean values, standard errors and results of LSD test for **performance index (PI_{ABS})** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.5.19. Performance index for energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total})

Mean values with standard errors for performance index for energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total}) in July 2012 and 2013 are shown in Table 27 in the Appendix. As for PI_{ABS}, analysis of variance for this parameter has shown significant effects of cadmium treatment and genotype in 2012 (Table 25. A), but not for interaction. Analysis of variance for the same parameter in 2013 has shown the same levels of significance as in 2012 for Treatment and Genotype ($p < 0.001$, $p < 0.001$, respectively) (Table 25. B). Similarly as in 2012 interaction effect was not significant.

Table 25. Analysis of variance for **energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total})** in ear-leaves measured in July 2012 (A) and 2013 (B).

A

Source of variation	Degrees of freedom	F value
Treatment	3	13.415***
Genotype	3	11.366***
Treatment×Genotype	9	0.600
Replication	15	0.512

B

Source of variation	Degrees of freedom	F value
Treatment	3	19.149***
Genotype	3	16.586***
Treatment×Genotype	9	1.675
Replication	15	0.658

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Results of ANOVA and LSD test have shown that there are differences in mean values of PI_{total} between different genotypes and that there are significant differences in mean values between different treatments. In Figure 32 A, B mean values and results of LSD test are shown for performance index for energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total}) measured on ear-leaves of four maize genotypes in 2012 and 2013. In general, in both years values of PI_{total} were lowest at highest cadmium concentration in soil (Cd5 treatment). Inbred line B73 showed the smallest changes in PI_{total} and in 2012 there were no significant differences in PI_{total} values from the control in any of the treatments (Figure 32 A). In 2013 PI_{total} values of inbred line B73 decreased compared to control, but there were not any significant differences in mean values between treatments (Figure 32 B). Other three genotypes responded to increasing cadmium content in soil with decreasing PI_{total}

values. Largest decreases in PI_{total} values occurred in inbred line Os6-2 in both years (Figure 32. A, B).

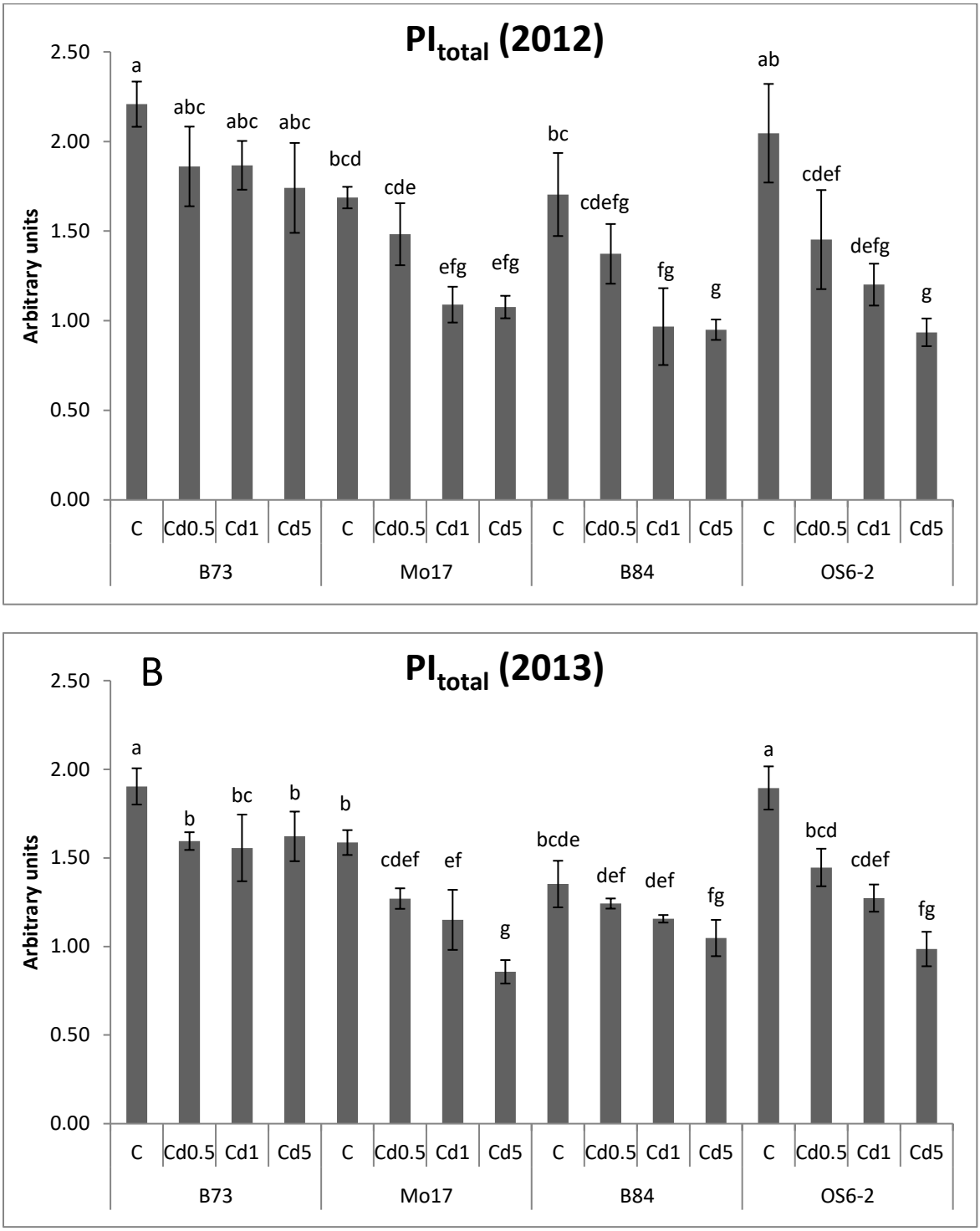


Figure 32. A, B Mean values, standard errors and results of LSD test for **performance index for energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total})** in four maize genotypes determined measured on ear-leaves in 2012 and 2013. Bars with different letters represent significant differences ($LSD_{0.05}$).

4.6. Correlations between OJIP-test parameters and cadmium and zinc content in ear-leaves

Table 26. Correlations of OJIP-test parameters and cadmium and zinc content in ear-leaves of four maize genotypes (B73, Mo17, B84, Os6-2) challenged by four different levels of cadmium (C, Cd0.5, Cd1, Cd5). Results are displayed for 2012 (A) and 2013 (B).

A

Parameters	F_0	F_m	F_{300}	t_{max}	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC
Cd mg kg^{-1}	0.716***	-0.64***	0.604***	-0.433***	0.113n.s.	0.003n.s.	-0.469***	0.507***
Zn mg kg^{-1}	0.566***	-0.476***	0.388**	-0.291*	0.183n.s.	0.056n.s.	-0.319*	0.497***
Parameters	TR_0/ABS	ET_0/ABS	Et_0/Tro	TR_0/DI_0	$ET_0/(TR_0-ET_0)$	PI_{ABS}	PI_{total}	Zn mg kg^{-1}
Cd mg kg^{-1}	-000.770***	-0.451***	-0.491***	-0.427***	-0.260*	-0.449***	-0.445***	0.781***
Zn mg kg^{-1}	-0.616***	-0.301*	-0.296*	-0.438***	-0.116n.s.	-0.339**	-0.289*	1
Parameters	V_J	V_I						
Cd mg kg^{-1}	0.640***	0.660***						
Zn mg kg^{-1}	0.510***	0.510***						

B

Parameters	F_0	F_m	F_{300}	t_{max}	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC
Zn mg kg^{-1}	0.405***	-0.594***	0.473***	-0.265*	-0.071n.s.	0.330**	-0.252*	0.322**
Cd mg kg^{-1}	0.599***	-0.763***	0.534***	-0.437***	-0.039n.s.	0.280*	-0.409***	0.503***
Parameters	TR_0/ABS	ET_0/ABS	Et_0/Tro	TR_0/DI_0	$ET_0/(TR_0-ET_0)$	PI_{ABS}	PI_{total}	Zn mg kg^{-1}
Zn mg kg^{-1}	-0.402**	-0.368**	-0.349**	-0.344**	-0.193n.s.	-0.541***	-0.433***	1
Cd mg kg^{-1}	-0.597***	-0.556***	-0.501***	-0.523***	-0.249*	-0.612***	-0.484***	0.864***
Parameters	V_J	V_I						
Cd mg kg^{-1}	0.610***	0.630***						
Zn mg kg^{-1}	0.370**	0.570***						

*,**,*** significance levels at the 0.05, 0.01, 0.001 probability levels, respectively

Significant correlations were found between OJIP-test parameters and ICP-OES analysis for ear-leaf cadmium and zinc concentrations for both years of the experiment and are more or less the same for both years (Table 26 A, B). Quantum yields (TR_0/ABS, ET_0/ABS, ET_0/TR_0) were negatively correlated leaf Cd concentration, likewise specific flux for electron transport (ET_0/RC) and electron transport beyond Q_A^- ($ET_0/(TR_0-ET_0)$) were also negatively correlated with leaf Cd concentration (Table 24. A, B). Related to suggested Q_A^- accumulation are significant strong and positive correlations of V_J and V_I parameters with cadmium leaf accumulation. Both performance indexes (PI_{ABS}, PI_{total}) were negatively correlated with Cd leaf concentration. Negative correlations were also observed between density of reaction centers on chlorophyll *a* basis (RC/ABS) and leaf Cd and between density of reaction centers per excited cross section (RC/CS_0) and cadmium content in soil. Largest positive correlations of cadmium content in soil and OJIP-test parameters were in F_0, F_{300} and DI_0/RC, which are all parameters that suggest impaired functionality of PSII. Correlations of zinc content in ear-leaves and OJIP-test parameters were very similar to the ones of cadmium, due to high correlation between cadmium and zinc uptake (correlations were 0.781 ($p < 0.001$) for 2012, and 0.864 ($p < 0.001$) for 2013).

5. Discussion

Heavy metal pollution is one of the largest ecological threats and large industries cause serious problems due to disposal of pollutants containing heavy metals directly into the agroecosystem (Wang and Chen 2009). Another large source of heavy metals is the use of phosphate fertilizers that contain heavy metals. Vrommann et al. (2010) have shown that 60 % of cadmium uptake in Belgian adult population comes from cereal products and potato. Heavy metals have a large impact on plant homeostasis and the exposure of plants to i.e. cadmium leads to alterations cellular processes as photosynthetic activity, antioxidant activity, and plant water status (Perfus-Barbeoch et al. 2002, Ortega-Villasante et al. 2005, Ekmekçi et al. 2008).

Plant response to stress is complex and involves numerous physiological, molecular, and cellular adaptations. Decrease in photosynthetic activity can indicate unfavorable conditions that plants are exposed to. By monitoring changes in photosynthetic activity in different genotypes it is possible to discover sensitive and tolerant genotypes. Chlorophyll *a* fluorescence is an efficient and non-destructive method for assessing the effect of various abiotic stress factors, including heavy metals (Maxwell and Johnson 2000, Burzyński and Żurek 2007, Żurek et al. 2014). It provides the data on PSII functionality, which can indicate stress even before any visual symptoms occur. With the use of OJIP test parameters that quantify the stepwise flow of energy through PSII plant vitality and efficiency can be assessed (Strasser et al. 2004).

In this research, changes in photochemical mechanisms in ear-leaves of four maize genotypes challenged by different cadmium levels were investigated through the use of chlorophyll *a* fluorescence (OJIP-test and chlorophyll *a* fluorescence transient curves). Ear-leaves were selected as they are one of the most important leaves in maize; they affect plant morphology and yield (Zheng and Liu 2013). Effects of different levels of cadmium in soil on photosynthetic parameters were assessed in different maize genotypes and related to cadmium and zinc content in ear-leaves determined by ICP-OES analysis.

5.1.ICP-OES analysis of cadmium and zinc content in ear-leaves

Biological effects of individual metals on plants are mostly known but the effects of combinations of heavy metals and their interactions are still mostly unexplained. Cadmium and zinc are common companions in the environment and, being chemically similar, plants can uptake them as divalent cations. Mechanisms of Cd uptake and translocation haven't been properly elucidated. Tudoreanu and Phillips (2002) stated that cadmium accumulation, besides pH, is affected largely by genotype (Cd excluders and non-excluders), variation in acidification of root rhizosphere between species, soil temperature, level of evapotranspiration and also by chemical and physical parameters of the soil in the experiment which differs from soil in the field. Primary mechanism for cadmium intracellular immobilization is through formation of Cd-pyhtochelatin complexes. Cadmium is deposited in vacuoles and Cd ions are translocated by xylem and phloem, where translocation is genotype specific (Tudoreanu and Phillips 2002). Studies on accumulation of cadmium and zinc reveal mostly antagonistic interaction (Wu and Zhang 2002, Balen et al. 2011), but synergistic interactions are reported as well (Moraghan 1993, Nan et al. 2002, Larbi et al. 2002). As shown by Smilde et al. (1992)

synergistic effects could be observed in loam soil where zinc uptake increased with applied cadmium and Mckenna et al. (1993) reported that cadmium stimulated the uptake of zinc in young leaves of *Lactuca sativa* L. Zha et al. (2004) in a research on *Thlaspi caerulescens* grown in hydroponic culture reported an increase in zinc accumulation with cadmium treatments with significant strong positive correlation. They also suggested that the difference in cadmium (and also zinc) accumulation between two investigated ecotypes is caused by more than one gene, although in maize cadmium accumulation is probably controlled by only one gene as suggested by Sorić et al. (2009), and that accumulation and tolerance are genetically independent traits. Similarly, in our research there is a synergistic relationship between cadmium and zinc uptake (Figure 10 A, B, Figure 11 A, B) and the connection between uptake of these two metals can be seen from their correlation which is strong, positive and significant (for 2012 $r = 0.781$, $p < 0.001$, and for 2013 $r = 0.864$, $p < 0.001$) (Table 25 A, B). Florijn et al. (1993) classified maize plants as cadmium excluders and cadmium non-excluders. By that classification, B73 and B84 would be cadmium excluders and Mo17 and Os6-2 would be cadmium non-excluders and this difference in cadmium accumulation can be seen between these two groups in Figure 10 A, B. In the research of Florijn et al. (1993), cadmium excluder was B73 line (Stiff stalk heterotic group) and cadmium non-excluder was H98 line (Lancaster heterotic group). In our research, ICP-OES analysis of cadmium accumulation in leaf revealed also a separation of genotypes that seems to be based on heterotic groups: Stiff stalk (B73, B84) as cadmium excluder and Lancaster (Mo17, Os6-2) as cadmium non-excluder.

5.2.Chlorophyll *a* fluorescence transients

Increased levels of cadmium in soil caused concentration and genotype dependent changes in photosynthetic machinery that is detectable through chlorophyll *a* fluorescence transients (Figure 12 A, B, C, D and Figure 13 A, B, C, D). Chlorophyll *a* fluorescence transients can be separated in two groups: (i) cadmium treatments caused discernible changes in the shape of OJIP transient curves (Mo17, B84, Os6-2) and (ii) cadmium treatments caused no/or minimal changes in the shape of OJIP transient curves (B73). In genotypes sensitive to cadmium (B84, Os6-2, Mo17) differences from the control treatment were visible mostly in J and I steps showing a gradual increase with the increase of cadmium content in soil. Related to ICP-OES analysis, cadmium non-excluders (Mo17, Os6-2) exhibited cadmium induced changes in chlorophyll fluorescence transients while in Cd excluders group B73 did not exhibit changes. The B84 showed changes similar to cadmium non-excluder group even though it accumulated very small amounts of cadmium (for cadmium content in ear-leaves see Figure 10 A, B, for chlorophyll *a* fluorescence transients see Figure 12 A, B, C, D and Figure 13 A, B, C, D).

OJIP curve represents the reduction of all electron acceptors in PSII. Fluorescence intensity increases from minimal (F_0) to maximal fluorescence intensity (F_m) with two intermediate steps: J step at ~2 ms, and I step at 30 ms (Strauss et al. 2006). When recorded data points of chlorophyll *a* fluorescence are plotted on a logarithmic time scale, a polyphasic curve is obtained. O to J rise represents the photochemical phase and it is a result of Q_A reduction. J step represents the maximum level of Q_A reduction reached and I step represents further Q_A to Q_B reduction. JI and IP rises represent non photochemical phase (Strauss et al. 2006). P step is

corresponds to the maximum accumulation of Q_A^- and Q_B^{2-} and PQH_2 and it represents the phase when all reaction centers are closed (Govindjee 2004). Shape of the OJIP curve is very sensitive to stressful changes in the environment (Strasser et al. 2004.). Chlorphyll *a* fluorescence transient curves normalized between steps O and P provide relative variable fluorescence at time t and it is a measure of the portion of closed RCs of PSII (Strasser et al. 2004). JI phase of the transient represents changes in PQ-pool reduction (Schreiber et al. 1989). Increase in J step reflects the start of Q_A re-oxidation by Q_B (Strasser et al. 1995) so an increase in J step would suggest a problem in Q_A re-oxidation and a consequent build-up of reduced Q_A. Direct effect of cadmium on the electron transfer between Q_A and Q_B could be explained through interaction with non-heme iron involved in this step (Ciscato et al. 1999). Increase in I step suggests accumulation of reduced plastoquinone which is unable to transfer electrons to dark reactions (Kalaji et al. 2014). The largest increases in J step were in Os6-2 in both years, whereas the increase in J step in Mo17 was slightly less than in Os6-2. J step in B84 was even smaller although obvious, and in B73 the increase was practically non-existent (Figure 12 A, B, C, D and Figure 13 A, B, C, D). Cd5 treatment caused largest increases in J step in all genotypes (except in B73 in which there was no increase). Increases in J step are in agreement with decreases of TR_0/ABS and ET_0/ABS (Figure 26, Figure 27) which suggest that electron transport further than Q_A is impaired and Q_A^- to Q_B electron transport is disrupted. Increases in I step are expressed in all genotypes (except B73) and most pronounced increase was in Os6-2 in 2013 (Figure 13 D). According to Lazár (2006), relative height of the I-step (plateau) is a measure of the relative amount of the Q_B–nonreducing PSIIs. Also, the inactivation of the ferredoxin-$NADP^+$–oxidoreductase (FNR) has been suggested as a factor that could contribute to the appearance of the I step (Schansker et al. 2003). This is also in concordance with decreases in TR_0/ABS, ET_0/ABS and increases in ABS/RC increase in ABS/RC which could have happened due to inhibition of electron Q_A^- to Q_B transfer and transformation of RCs to "silent" RCs (Yusuf et al. 2010) and it would lead to increases in both J and I steps.

Appearance of the K step, which was usually hidden in the OJ rise but seen as increase in fluorescence at 300 μs (F_{300}) (Figure 16 A, B), suggests an imbalance in the electron donor and acceptor sides of PSII and could be related to deactivation of oxygen evolving center (OEC) (Strasser et al. 1994, Jiang et al. 2006). Activity of OEC is often inhibited under stress which leads to the blockage of electron transfer from the electron donor side to the electron acceptor side. This imbalance was confirmed by the increase in J step which reflects the start of Q_A re-oxidation by Q_B. The B73 was the only genotype that did not express the appearance of K step (or increases in F_{300}, Figure 16 A, B) which indicates that the OEC or the donor side of PSII, of B73 was functional in all cadmium treatments. Appearance of K step under heavy metal stress has been previously reported on barley (lead) and tall fescue (cadmium) (Kalaji and Loboda 2007, Huang et al. 2017).

5.3.OJIP-test parameters

Minimal or initial fluorescence (F_0) is the fluorescence intensity measured in dark adapted conditions when all PSII reaction centers are opened. Increase in F_0 values with increased

cadmium content in soil can be attributed to a reduction in energy transfer from antennae to the reaction center (Ralph and Burchett 1998) and F_0 increase can also be provoked by dissociation of LHCII from the PSII core complex (Misra et al. 2007). It can be noticed from Figure 14 A, B that in both years of the experiment F_0 values were highest in the Cd1and Cd5 treatments: increasing trend can be noticed with increasing cadmium content in soil. Statistical analysis revealed that there were significant differences between treatments, but also between genotypes (Table 7). Only exception from this was B73 inbred line in which this increase was not clear as in other three genotypes. This genotype reacted differently from cadmium non-excluder genotypes (Mo17 and Os6-2) but also differently from B84 genotype which is a cadmium excluder. This suggests a different mechanism of light harvesting system of B73 line to cope with excess cadmium.

Maximum fluorescence intensity (F_m) represents fluorescence intensity when all reaction centers are closed. Decrease in F_m could indicate the inhibition of OEC (Lazar, 1999). Also decrease of F_m with increasing cadmium content in soil could indicate changes in ultrastructure of thylakoid membranes which could affect electron transport rate (Ekmekçi et al. 2008). F_m values decreased with increasing cadmium content in soil in both years of the experiment. In the Cd5 treatment, all genotypes except B73 had lowest values of F_m (Figure 15 A, B). In the B73, there were no differences in F_m values between control and Cd1 and Cd5 treatments in both years of the experiment. Decrease in F_m values was mostly noticeable in the genotype Os6-2 where increasing cadmium content in soil gradually decreased F_m values. Two cadmium non-excluder genotypes (Mo17, Os6-2) showed the same decreasing pattern, while two cadmium excluder genotypes (B73, B84) showed different responses to increased cadmium content in soil. This suggests that OEC functioning was not inhibited by excess cadmium in B73 as it was in B84 or other two cadmium non-excluder genotypes.

Impairment of the OEC is confirmed by increases in F_{300} values in both years of the experiment (Figure 16 A, B). F_{300}, or the fluorescence intensity at 300 μs, indicates the appearance of the K step. It suggests an imbalance in the electron donor and electron acceptor side of PSII that relates to the deactivation of the OEC (Strasser et al. 1994, Jiang et al. 2006) and this can be attributed to inhibition of electron transfer to secondary electron donor of PSII (YZ) (Nash et al. 1985). Bertamini and Nedunchezhian (2003) found that the activity of OEC is often inhibited under stress which leads to the blockage of electron transfer from the electron donor side to the electron acceptor side. In our study, it is evident that increasing cadmium content in soil caused increase in F_{300} values and that the highest cadmium concentration caused the highest increase (Figure 16 A, B). This is true for all genotypes in both years, except B73. Increases in these values did occur in B73 but were very small and not significant. Results suggest that the OEC of the B73 genotype stays functional even in highest cadmium treatment.

Variable fluorescence at J step is the probability at which a trapped exciton moves an electron into the electron transport chain further from primary acceptor (Q_A). Increase in V_J values occur when Q_A reoxidation is limited which leads to accumulation of reduced QA and decrease in electron transport (Strasser et al. 2004). Increases in variable fluorescence at I step (V_I) along with increases in variable fluorescence at J step suggest the acuumulation of

reduced Q_A and plastoquinone which are unable to transfer electrons to dark reactions (Kalaji et al. 2014). Both V_J and V_I parameters show more or less increased values with increasing cadmim levels in soil, reaching highest levels at Cd5 treatment (Figure 17 A, B and Figure 18 A, B). Mo17 and Os6-2 were affected more than B84, which suggests that these three genotypes have problems in reoxidation of Q_A and plastoquinone due to inability to transfer electrons further in the electron transport chain. Genotype B73 practically showed no change in V_J or V_I values in any of the treatments suggesting its Q_A and plastoquinone reoxidation was intact and electron transport beyond Q_A is not affected. This is in concordance with ET_0/ABS values.

Increased cadmium content in soil caused increases in ABS/RC values compared to control and increases were largest in Cd5 treatment (Figure 19 A, B). Increase in ABS/RC values suggest that a fraction of active reaction centers was inactivated or that the apparent antenna size has increased (Lichtenthaler et al. 1982). ABS/RC parameter is conditioned by the number of active and inactive reaction centers. The genotypes B84, Os6-2 and Mo17 had similar responses to increasing cadmium content but B73 responded differently. Increases in ABS/RC in the Cd5 treatment were very small and they were not significantly different from control in 2012 (Figure 19 A). The Cd0.5 treatment seems to have more effect on the increase of ABS/RC in this genotype. Our results indicate that B84 genotype which is a cadmium excluder responded to increased cadmium content in soil as Cd non-excluder genotypes (Mo17, Os6-2), while B73 had different response (or the lack of any significant response).

Increase in ABS/RC values was accompanied by increase in TR_0/RC. TR_0/RC represents maximum speed of exciton trapping by RC, or in other words it represents the trapped photon flow by RC (Stirbet and Strasser 1996). Increase in TR_0/RC (Figure 20 A, B) accompanied by decrease in TR_0/ABS (Figure 26 A, B) suggests inactivation of a portion of RCs most probably due to inactivation of OEC complexes, and partly due to transformation of some reaction centers to “silent” centers which dissipate energy in the form of heat and not fluorescence (Strasser et al. 2004, Yusuf et al. 2010). In our study, the cadmium excluder B84 responded to increased cadmium content in soil in a similar way to the Cd non-excluder genotypes(Mo17and Os6-2, while B73 had different response (or the lack of any significant response in the Cd1 and Cd5 treatments). From ABS/RC and TR_0/ABS data, it seems that in B73 genotype OEC and RCs are less sensitive to cadmium than the ones of B84.

Electron transport per active reaction center is represented by ET_0/RC. Decreases in ET_0/RC suggest decreased reduction ability beyond Q_A. It has been previously shown that cadmium ions impair the electron transport system on the reducing side of PSII indicating problems in electron transfer from Q_A to Q_B (Atal et al. 1991) Excess of manganese has also been shown to negatively affect electron transport in *Citrus grandis* (Li et al. 2010). Due to decreased electron transport only a fraction of absorbed light energy can be utilized in photosynthetic processes and excess energy is accumulated. Photosynthetic machinery protects itself from this excess energy that is accumulated by dissipating it. B84, Mo17 and Os6-2 genotypes showed a gradual decrease in electron transport with increasing cadmium content in soil reaching lowest values in the Cd5 treatment (Figure 21 A, B). Electron transport of B84 and Os6-2 were impacted slightly more than Mo17, especially in 2013 (Figure 21 B). Decreases in

ET_0/RC are in concordance with the increases in DI_0/RC and TR_0/RC. The B73 genotype showed very small changes in ET_0/RC values: there were no significant changes compared to control in Cd1 and Cd5 treatments in 2012, while in 2013 electron transport increased slightly in the Cd1 and Cd5 treatments. The small increase in ET_0/RC could be attributed to thermal activation of dark reactions (Strasser et al. 2000).

Ratio of total dissipation energy from all RCs and the number of active reaction centers is represented by DI_0/RC. It depends on the balance of active and inactive RCs; larger number of inactive RCs leads to a larger number of photons that are not trapped and this excess of photons is registered as dissipation energy. Energy dissipation is a defense mechanism that protects leaves from photo-oxidative damage by serving as a valve that releases excess energy. Increase in dissipation energy suggests that absorbed energy was not utilized to reduce Q_A^-, but instead it was dissipated. Increase in dissipation energy has previously been shown to occur in heavy metal treated plants, including cadmium (Zhou et al. 2005, Begović et al. 2016). In this research, increasing cadmium concentration in soil caused increases in DI_0/RC. Highest values of DI_0/RC in 2012 and 2013 were in OS6-2 genotype, while lowervalues were in B73 in both years (Figure 22 A, B). ABS/RC and TR_0/RC data suggested B73 genotype differs from other three genotypes and the same is confirmed with DI_0/RC. As results of ABS/RC, TR_0/RC and DI_0/RC suggested, reaction centers of B73 inbred line are much less affected by cadmium than B84, Mo17 or Os6-2. RCs in B73 do not inactivate or turn "silent" (or do, but in a much smaller fraction than other three genotypes) and functionality of its OEC is much less affected by cadmium. Structural transformation of active RCs to "silent" RCs is suggested by TR_0/RC increases (Strasser et al. 2004), and confirmed with increases in DI_0/RC.

Increases in dissipation energy (DI_0/RC) due to probable transformation of some of the RCs to "heat sinks" that dissipate excess excitation energy (Strasser et al. 2000) is corroborated with the decrease of Q_A-reducing RCs per PSII antenna chlorophyll (RC/ABS). Decrease in the number of active RCs has been previously listed as the main target of cadmium toxicity, along with damage to the OEC (Gonzalez-Mendoza et al. 2007). Our results show that largest decreases in RC/ABS values were at highest cadmium content in soil (Figure 23 A, B). Most affected was genotype Os6-2, which also accumulated the most cadmium in ear leaves, where values significantly decreased in the Cd1 and Cd5 treatments in both years. In other genotypes significant decreases occurred only in Cd5 treatment. Smallest decreases were in B73 in both years, and in this genotype Cd0.5 seems to have a larger effect on RC/ABS decrease than Cd5 treatment (Figure 23 A, B). B84 inbred line, being cadmium excluder, seems to be equally sensitive to RC inactivation as Mo-17 and Os6-2 which are cadmium non-excluders.

Density of active reaction centers (RC/CS_0) decreased with increasing cadmium content in soil and decreases were larger in 2012 than in 2013 (Figure 24 A, B). The largest decreases were in Cd1 and Cd5 treatments in all genotypes except B73. In B73 there were no significant differences between control and Cd1 and cd5 treatments, although Cd0.5 treatment seems to have the largest effect on RC/CS_0 decrease. Largest decreases in RC/CS_0 values were in Os6-2 and Mo17 inbred lines. As with RC/ABS, B84 inbred line showed different pattern from B73, which was similar to cadmium non-excluder genotypes (Os6-2, Mo17). Decreases in

RC/CS_0 values were reported previously on *Avicennia germinans* L. exposed to cadmium stress (Gonzalez-Mendoza et al. 2007). Decreases in RC/ABS and RC/CS_0 followed by increases in DI_0/RC and ABS/RC suggest inactivation of reaction centers. Likewise, increase in TR_0/RC suggest that inactive RCs antennas release absorbed energy non-photochemically, which is registered as increase in DI_0/RC (Nussbaum et al. 2001). Release of energy in this way indicates that some of the reaction centers are "silent" (Q_A non-reducing) (Strasser et al. 2004).

Time to reach maximal fluorescence intensity (t_{max}) showed decreases with increased cadmium content in soil (Figure 25 A, B). Inbred lines B84, Mo17 and Os6-2 showed more or less similar decreasing trend in t_{max} values with increasing cadmium content in soil in both years, while inbred line B73 showed the smallest decreases which were not significant (only significant difference was in Cd0.5 treatment in 2013). Time to reach maximal fluorescence intensity decreases with the energy needed for closure of all reaction centers. With the decreases in needed energy for closure of all RCs fewer electrons are transported from Q_A^- to electron transport chain. It is accepted that F_m expresses the state of PSII at which all Q_A´s are reduced (Mallick and Mohn 2003) hence a decrease in t_{max} suggests that the pool of Q_A´s available for reduction has decreased and that plants are under stressful conditions.

Maximum quantum yield of PSII (TR_0/ABS) is extracted from minimal and maximal fluorescence intensity (F_0 and F_m) and it represents the efficiency of PSII primary photochemistry (Strasser et al. 2000). Values in the range of 0.75 – 0.85 suggest normal functionality of PSII (Bolhar-Nordenkampf et al. 1989). All values of TR_0/ABS in both years were in the range of 0.77-0.80 (Figure 26 A, B). TR_0/ABS values decreased gradually with the increase of cadmium content in soil in all genotypes in both years. Large decreases were evident in the Cd1 and Cd5 treatments (especially in Os6-2 genotype in 2012, Figure 26 B). In the inbred line B73, decreases were the smallest and there were no significant differences between treatments and control in 2013. in 2012, the Cd1 and Cd5 were significantly different from control but there were no significant differences between treatments. Efficiency of PSII primary photochemistry in B73 inbred line was not compromised even in the Cd5 treatment. The inbred line B84 expressed decreases in a similar way to Mo17 and Os6-2 which accumulated far more cadmium: this shows the sensitivity of the B84 inbred line to cadmium exposure. Decreases in TR_0/ABS in plants exposed to heavy metals have been reported and discussed previously (Turnau et al. 2008, Jiang et al. 2008) and have been attributed to photoinhibition caused by excess of heavy metals. Decrease in TR_0/ABS and increase in TR_0/RC (Figure 26 A, B and Figure 20 A, B, respectively) suggest inactivation of some of the RCs. This is probably in part due to inactivation of OEC (which is suggested by decrease in F_m values, Figure 15 A, B) and partly due to transformation of some of the RCs to "silent" RCs which is suggested by increases in DI_0/RC (Figure 20 A, B) (Strasser et al. 2004). This is also backed by increase in ABS/RC, which occurred due to inhibition of electron Q_A^- to Q_B transfer (suggested by V_J and V_I increases) and transformation of RCs to "silent" RCs (Yusuf et al. 2010). Increase in F_0 which occurred (Figure 14 A, B) along with decrease of TR_0/ABS suggests dissociation of LHC complexes and RCs of PSII which results in a blockage of electron transport to PSII RCs (Mathur et al. 2011).

Quantum yield of electron transport (ET_0/ABS) and the probability for electron transport (ET_0/TR_0) expressed lower values with increasing cadmium content in soil (Figure 27 A, B and Figure 28 A, B). Decreases in ET_0/TR_0 indicate that electron transport further than Q_A is reduced. Decreased values of these two parameters suggest that electron transport further than Q_A is impaired and that there are more Q_B non-reducing RCs which disrupts Q_A^- to Q_B electron transport (which is backed by increases in V_I parameter). Decreases in acceptor side dependent yields (ET_0/ABS, ET_0/TR_0), which describe electron transport efficiency, have been previously reported to occur under cadmium stress and linked to photoinhibitory damage of cadmium to PSII (Pagliano et al. 2006). The B73 inbred line showed the smallest decreases in ET_0/ABS values: in 2012 there were no significant differences between control and the Cd1 and Cd5 treatments while in 2013 decreases were small but significant (Figure 27 A, B). The Cd0.5 seemed to have a larger effect on this genotype than Cd1 or Cd5 which could suggest that B73 inbred line has different mechanisms for managing lower and higher cadmium levels in soil. Other three genotypes expressed a gradual decrease with increasing cadmium content in soil. The genotypes B84 and Os6-2 were a little more affected than Mo17 which can be seen from lowest values in both years in the Cd5 treatment. Similarly, the B73 inbred line showed little change in ET_0/TR_0 even in the Cd5 treatment: there were no significant differences in both years between control and the Cd1 and Cd5 treatments (Figure 28 A, B). Only significant decrease was in the Cd0.5 treatment. In other three genotypes, values similarly decreased as cadmium content in soil increased and values in the Cd5 treatment were similarly low. In both of these parameters, the inbred line B73 showed different behavior from other three inbred lines. The inbred line B84 accumulated low amounts of cadmium; it can be grouped with Mo17 and Os6-2 – cadmium nonexcluders that accumulated high amounts of cadmium in ear-leaves. Based on the results, the B73 inbred line does not seem to struggle with electron transport further than Q_A, even in the Cd5 treatment the probability for the electron transport beyond Q_A^- and maximum yield of electron transport beyond Q_A did not change significantly or were only slightly decreased. TR_0/ABS and ET_0/TR_0 of the inbred line B84, on the other hand, are shown to be very sensitive to cadmium content in soil with decrease being significant even in the Cd0.5 treatment (Figure 26 A, B and Figure 28 A, B).

Ratio of trapped photons and energy dissipation (TR_0/DI_0) showed a decrease with increasing cadmium content in soil (Figure 29 A, B) in all genotypes, except B73. Lowest values were in B84 and Os6-2 in the Cd5 treatment in both years. In B73, there were no significant differences between control and the Cd1 and Cd5 treatments. Decrease TR_0/DI_0 is an indication of lowered driving force of light reactions, and it is related to quantum yield of primary photochemistry. It is likely that decreases in this parameter can be linked with larger increases in dissipation energy DI_0/RC that can be due to inactivation of some of the RCs and transformation of RCs to "silent" RCs that act as heat sinks (Strasser et al. 2000).

Decreases in electron transport further than primary acceptor ($ET_0/(TR_0-ET_0)$) were significant in all genotypes and both years except in B73: decreases were smaller in B73 than in other genotypes, especially in the Cd1 and Cd5 treatments (Figure 30 A, B). This parameter is related to dark reactions after Q_A^- and decrease could suggest that cadmium treatment

caused an increase in CO_2 assimilation, since a relationship between photosynthetic electron transport and CO_2 assimilation has been established (Krall et al. 1992). The results suggest that B73 can transport electrons further than Q_A with slight decreases in efficiency even in highest cadmium concentration (Cd5 treatment). The inbred line B84 seems to be more susceptible and it responded to cadmium treatments in the same manner as Mo-17 and Os6-2 which are cadmium non-excluders.

Performance index on absorption basis (PI_{ABS}) is an indicator of plant vitality and photosynthetic efficiency which has previously been used in plant stress research (Strauss et al. 2006, Christen et al. 2007). Performance index is based on the Nernst equation and it is derived from three components. First component is RC/ABS related to the force generated by RC concentration per antenna chlorophyll. Second component is the force of light reactions related to quantum yield of primary photochemistry (TR_0/ABS). Third component is related to force of dark reactions (after Q_A^-) (ET_0/TR_0) (for derivations of PI_{ABS} see Strasser et al. 1999).

Since PI_{ABS} utilizes RC/ABS and ET_0/TR_0 values it is more sensitive for stress detection than F_v/F_m (TR_0/ABS). This can be seen from Figure 31 A, B for PI_{ABS} and Figure 26 A, B for TR_0/ABS where differences between treatments were better pronounced, especially in the non-sensitive B73. All three parameters that are used to calculate performance index (PI_{ABS}) showed a decrease with decreasing cadmium content in soil, hence they all contributed to decrease of PI_{ABS}. Although, largest contribution to this decrease probably comes from decrease in primary photochemistry yield (TR_0/ABS). Decreases in PI_{ABS} were the largest in the Cd5 treatment, although there is an notable decline in PI_{ABS} values from control to the Cd5. Exception was in B73 genotype where the Cd1 and Cd5 treatments caused a small decrease (in 2012 not significant). The Cd0.5 treatment seems to have more effect on the decrease in PI_{ABS} values than the Cd5 and Cd1 treatments which can suggest different mechanisms of management with different cadmium levels in soil depending on their concentrations being high or low. By the decrease in PI_{ABS} values cadmium excluder genotype B84 can be grouped with cadmium non-excluders (Mo17, Os6-2) which indicates sensitivity of B84 to cadmium. Significant negative correlations were detected between PI_{ABS} and cadmium content in ear-leaves in both years of the experiment (Table 26 A, B). Decreases in PI_{ABS} under heavy metal stress have been reported previously (Żurek et al. 2014, Begović et al. 2016). Decrease in PI_{ABS} under heavy metal stress has been reported in tall fescue by Huang et al. (2017) and in perennial grasses by Zurek et al. (2014).

Performance index for energy conservation from exciton to the reduction of PSI end-electron acceptors (PI_{total}) is an extension of PI_{ABS} which includes the calculation of the efficiency with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side. Inclusion of this calculation increases sensitivity of PI_{total} compared to PI_{ABS}. Its relation PSII RC density, quantum efficiency of primary photochemistry, efficiency of conversion of excitation energy to electron transport and performance due to quantum efficiency of the reduction of end electron acceptors make it the most sensitive JIP-test parameter which can be correlated with plant growth and survival rate (Yusuf et al. 2010). Decreases in PI_{total} have previously been previously used in plant biotic and abiotic stress research, including cadmium (Huang et al 2017, Li et al. 2014, Perboni et al.

2012, Shen et al. 2015). In our research values of PI_{total} have decreased in all genotypes with increasing cadmium content in soil. The Cd5 treatment caused the largest decreases in PI_{total} values in B84, Os6-2 and Mo17 (Figure 32 A, B). In the genotype B73, PI_{total} mean values of treated plants decreased compared to control but the decreases were small and was not significant in 2012 (Figure 32 A). In both years, there were no significant differences between cadmium treatments for B73 genotype. Due to smallest decreases in PI_{total} it can be suggested that B73 inbred line has better operating RCs, primary photochemistry, electron transport and reduction of end electron acceptors than other three genotypes used in the experiment. The inbred line B84, being a cadmium excluder like B73, seems to be sensitive to cadmium even though it accumulated small amounts of cadmium in ear-leaves even at highest cadmium concentration in soil (Cd5) (Figure 10 A, B). The results demonstrate that all components of PI_{total} decreased in this genotype, showing its sensitiveness to cadmium content in soil (For PI_{total} see Figure 32 A, B, for TR_0/ABS see Figure 26 A, B, for ET_0/TR_0 Figure 28 A, B and for RC/ABS see Figure 23 A, B). Significant negative correlations of cadmium content in leaves and PI_{total} were observed in both years of the experiment (Table 26 A, B). In general, significant negative correlations were detected between cadmium content in ear-leaves and OJIP-test parameters that contribute to normal PSII functioning (TR_0/ABS, ET_0/ABS, ET_0/TR_0, PI_{ABS}, PI_{total}), while significant positive correlation were detected between cadmium content in ear-leaves and OJIP-test parameters that contribute to decrease in PSII functionality (F_{300}, DI_0/RC) (Table 26 A, B).

5.4 Implications for maize breeding programs

This research was focused on the accumulation of cadmium from cadmium treated soil in ear-leaves of different maize genotypes. Focus was also given to the changes in the functionality of photosystem II of those selected genotypes that was caused by cadmium that accumulated in ear-leaves. Our results demonstrate that there are significant differences in accumulation of cadmium in ear-leaves of selected genotypes and according to cadmium accumulation, they can be divided in two groups – cadmium excluders (B73, B84) and cadmium non-excluders (Mo17, Os6-2). This distinction is based on heterotic groups – low cadmium accumulating Stiff stalk (B73, B84) and Lancaster (Mo17, Os6-2). Photosynthetic response of selected genotypes followed the same pattern and there were two groups. First group showed almost no changes in photosynthetic activity even at highest cadmium concentration in soil, and only member of this group is cadmium excluder B73 inbred line. It showed minimal changes in practically all measured chlorophyll *a* fluorescence parameters, even in the most sensitive PI_{ABS} and PI_{total}. Other three genotypes (B84, Mo17, Os6-2) fall in the second group which showed, in most parameters, cadmium concentration dependent response which resulted in the decreases of two performance indexes (PI_{ABS}, PI_{total}). Two members of this group are cadmium non-excluders (Mo17, Os6-2) and decreases in these performance indexes are expected due to high accumulation of cadmium in ear-leaves. The B84 inbred line accumulated low amounts of cadmium, even in the highest cadmium concentration level in soil, and showed practically the same level of sensitivity as genotypes that accumulated large amounts of cadmium in ear-leaves.

Maize has been widely used for cadmium phytomanagement due to its high biomass and cadmium accumulation capacity. Appropriate selection of cultivars and use of agronomic practices could increase effectiveness of remediation of cadmium contaminated soils with maize. Due to risk of cadmium contamination of food chain with maize grains acquired from cadmium contaminated soils Rizwan et al. (2017) suggested cultivation of maize on low- and medium-grade cadmium contaminated soils if grain is required. For cadmium polluted soil, authors suggested maize cultivation only for biomass production for energy production. With high cadmium accumulation Mo17 and Os6-2 genotypes (Lancaster heterotic group) could be used in maize breeding to develop hybrids for phytoextraction of cadmium and the resulting biomass could be used for energy production purposes. For maize production on cadmium contaminated soils B73 and B84 genotypes (BSSS heterotic group) could be used to cultivate maize on cadmium contaminated soil due to their low cadmium accumulation.

6. Conclusions

Based on the results of cadmium accumulation in maize ear-leaves and chlorophyll *a* fluorescence measurements it can be concluded:

- There are significant differences in cadmium accumulation in ear leaves of the four preselected genotypes – maize inbred lines: B73, Mo17, B84, Os6-2. This accumulation of cadmium was concentration-dependent in all genotypes; highest levels of cadmium in soil resulted in highest cadmium accumulation in ear-leaves.
- Based on cadmium accumulation in ear-leaves, genotypes can be separated in two groups: (i) cadmium low accumulating or cadmium excluders (B73, B84) and (ii) cadmium high accumulating or cadmium non-excluders (Mo17, Os6-2). This grouping coincides with heterotic grouping of these genotypes: BSSS (B73, B84) and Lancaster (Mo17, Os6-2)
- Cadmium caused changes in PSII functionality that was detectable through the use of chlorophyll *a* fluorescence. All chosen parameters showed sensitivity in detection of cadmium induced changes except ABS/RC and TR_0/RC which were less informative
- Negative effects of cadmium on PSII functionality can be mostly seen through the impairment of OEC functionality (appearance of K step and increase in F_{300}), reduction in energy transfer from antennae to the reaction center and transformation of some of the RCs to "silent" RCs that dissipate energy (Increase in F_0, ABS/RC, TR_0/RC, DI_0/RC and decrease in RC/ABS and RC/CS_0), and problems in Q_A^- to Q_B electron transport (decrease in ET_0/RC, TR_0/ABS, ET_0/ABS, increases in V_J and V_I)
- The smallest deviation from the control was expressed by B73 genotype with almost identical curves in all treatments compared to control in both years. Other three genotypes showed similar responses that were cadmium treatment related, and the changes in OJIP curves were visible as increases in J and I steps in both years which is also backed by increases in V_J and V_I values
- Decreases in PI_{ABS} values were cadmium concentration dependent. Highest cadmium level in soil caused the largest decreases in PI_{ABS} in all genotypes, except in B73 which was much less sensitive to cadmium than other three genotypes. Decreases in PI_{ABS} were due to decrease in all three components of PI_{ABS}.
- Due to smallest decreases in PI_{total} B73 inbred line seems to have better operating RCs, primary photochemistry, electron transport and reduction of end electron acceptors than other three genotypes used in the experiment exhibiting similar responses to cadmium in all treatments.
- Cadmium accumulation in ear leaves of cadmium non-excluder genotypes (Mo17, Os6-2) had almost the same negative effect in all measured chlorophyll *a* fluorescence parameters. Cadmium excluder genotypes showed totally different responses of OJIP-test parameters. The B84 inbred line showed practically the same level of sensitivity to cadmium as cadmium non-excluders indicating that PSII of B84 is sensitive even to small amounts of cadmium accumulated in ear-leaves. On the other hand, inbred line B73 showed to be almost insensitive to cadmium, where only small decreases in both performance indexes (PI_{ABS}, PI_{total}) could be seen even at highest cadmium concentration in soil.

Maize genotypes that were grown in the same conditions under same treatments accumulated different amounts of cadmium in ear-leaves and expressed different photochemical sensitivity to accumulated cadmium. As the most cadmium sensitive genotype, the B84 inbred line showed negative effects of cadmium on all measured parameters despite being cadmium excluder and accumulating small amounts of cadmium in all treatments. Compared to B84, the inbred line B73 can be considered cadmium tolerant according to minor decreases in PI_{ABS} and PI_{total} , especially at high levels of cadmium in soil. Genotypes Mo17 and Os6-2 accumulated significantly higher levels of cadmium than B73 or B84 in all treatments and decreases in plant vitality indexes as expected. Results of this research could be used in maize breeding for development of high cadmium accumulation genotypes for phytoextraction and for production of low cadmium accumulation maize on cadmium contaminated soil.

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8. Summary

Maize (*Zea Mays* L.) is one of the most important cereal crops in the world as it is a major staple food and a source for many industrial applications. It has also been widely used as a model organism for basic, translational and applied research. Various abiotic stress conditions such as heat, drought, salinity, heavy metal stress and low temperatures affect agricultural production worldwide and can cause extensive agronomic and economic losses. Heavy metals such as cadmium, mercury and lead, generally have no role in the metabolism and when present in excess amounts in soil can become extremely toxic. Cadmium, as a non-essential metal, causes many adverse effects in plant functionality. Cadmium causes disturbances and impairments of photosynthetic activity, antioxidant activity, ion channels, plant water status and redox imbalance and reduction of cell proliferation and growth. It is generally assumed that heavy metals enter plant cells through transporters of essential metals and heavy metal uptake is in competition with essential metals uptake, such as potassium, calcium, magnesium, iron, manganese. There is a significant variation in affinity for heavy metals accumulation among as well as within plant species. Temperate maize inbred lines B84 and Os6-2 have been designated as different according to their respective ionomic profiles and leaf cadmium accumulation.

Photosynthesis, as a process, is very sensitive to stressful conditions. Decrease in photosynthetic activity can be an early indicator of the unfavorable conditions plants are currently exposed to. Variability in photosynthetic parameters can be used in order to select cadmium sensitive or tolerant maize genotypes. Chlorophyll *a* fluorescence measurements were used in this research to assess negative effects of different levels of cadmium in soil on four maize genotypes: B73, Mo17, B84 and Os6-2. Photosynthetic performance was determined through the use of polyphasic rise in chlorophyll *a* fluorescence (OJIP test) which reveals changes in photosystem II photochemical performance. Cadmium content in ear-leaves was measured by ICP-OES analysis and related to changes in photosynthetic parameters.

Results for ICP-OES analysis show that investigated maize genotypes can be divided in two groups based on cadmium accumulation – cadmium excluders (B73, B84) and cadmium non-excluders (Mo17, Os6-2). It seems that this division is based on heterotic groups – low cadmium accumulating Stiff stalk (B73, B84) and Lancaster (Mo17, Os6-2).

Based on the photosynthetic response of selected genotypes there were also two groups. First group showed almost no changes in photosynthetic activity even at highest cadmium concentration in soil, and only member of this group is cadmium excluder B73 inbred line. It showed minimal changes in practically all measured chlorophyll *a* fluorescence parameters, even in the most sensitive PI_{ABS} and PI_{total}. B84, Mo17, Os6-2 fall in the second group which showed, in most parameters, cadmium concentration dependent response which resulted in the decreases of two performance indexes (PI_{ABS}, PI_{total}). B84 inbred line accumulated low amounts of cadmium, even in the highest cadmium concentration level in soil, and showed practically the same level of sensitivity as genotypes that accumulated large amounts of cadmium in ear-leaves. B84 inbred line can be considered the most cadmium sensitive genotype as it showed negative effects of cadmium on all measured parameters despite being

cadmium excluder and accumulating small amounts of cadmium in all treatments. Compared to B84, inbred line B73 can be considered cadmium tolerant especially based on minor decreases in PI_{ABS} and PI_{total} at high levels of cadmium in soil.

Results of this research could be used in maize breeding for development of high cadmium accumulation genotypes for phytoextraction and for production of low cadmium accumulation maize on cadmium contaminated soil.

Keywords: Maize, heavy metal stress, cadmium, chlorophyll *a* fluorescence, OJIP test, ICP-OES

9. Sažetak

Kukuruz (*Zea Mays* L.) je jedna od najvažnijih žitarica na svijetu kao osnovna hrana i kao sirovina u raznim vrstama industrije. Također je široko korišten kao modelni organizam za osnovna, interdisciplinarna i primijenjena istraživanja. Razni abiotski stresovi kao što su toplina, suša, niske temperature , solni stres i stres uzrokovan teškim metalima utječu na poljoprivrednu proizvodnju u cijelom svijetu, te mogu uzrokovati ogromne agronomske i ekonomske gubitke. Teški metali, kao kadmij, živa i olovo, u pravilu nemaju nikakvu ulogu u metabolizmu te njihova prisutnost u tlu može uzrokovati toksičnost. Kadmij, kao neesencijalni metal, ima brojne negativne učinke na biljnu funkcionalnost. Kadmij uzrokuje poremećaje i oštećenja fotosintetske aktivnosti, antioksidativne aktivnosti, ionskih kanala, vodnog statusa biljke, neuravnoteženost redoks statusa biljke, smanjenu proliferaciju i rast stanica. Prihvaćeno je da teški metali uglavnom ulaze u biljne stanice preko transportera esencijalnih metala, te da je unos teških metala u kompeticiji s unosom esencijalnih metala poput kalija, kalcija, magnezija, željeza i mangana. Postoji vrlo velika razlika u afinitetu za unos pojedinih teških metala između, ali i unutar, biljnih vrsta.

Fotosinteza je kao proces vrlo osjetljiva na stresne uvjete. Smanjenje fotosintetske aktivnosti može biti rani pokazatelj nepovoljnih uvjeta u kojima se biljka nalazi. Varijabilnost parametara fotosinteze može biti upotrijebljena za odabir genotipova kukuruza osjetljivih ili tolerantnih na kadmij. U ovom istraživanju korištena je fluorescencija klorofila *a* da bi se procijenili negativni učinci različitih koncentracija kadmija u tlu na četiri genotipa kukuruza: B73, Mo17, B84 i Os6-2. Fotosintetska učinkovitost je procijenjena upotrebom fluorescencije klorofila *a* (OJIP test) koja otkriva promjene u fotokemijskoj učinkovitosti fotosustava II. Sadržaj kadmija u listovima ispod klipa je određen pomoću ICP-OES analize i povezan s promjenama fotosintetskih parametara.

Rezultati ICP-OES analize su pokazali da korišteni genotipovi kukuruza mogu biti podijeljeni u dvije grupe prema usvajanju kadmija u listove ispod klipa – mali unos kadmija (B73, B84) I velik unos kadmija (Mo17, Os6-2). Ovakva podjela odgovara podjeli temeljenoj na heterotičnim grupama – Stiff stalk (B73, B84) i Lancaster (Mo17, Os6-2).

Odabrani genotipovi se mogu podijeliti u dvije grupe i prema fotosintetskom odgovoru. Prvu grupu uključuje minimalne promjene u fotosintetskoj aktivnosti čak i pri najvišoj koncentraciji kadmija u tlu, te je jedini predstavnik te grupe inbred linija B73. Ona je pokazala minimalne promjene u gotovo svim mjerenim parametrima fluorescencije klorofila *a*, čak i kod najosjetljivijih parametara (PI_{ABS} i PI_{total}). B84, Mo17 i Os6-2 spadaju u drugu grupu koja pokazuje promjene u većini parametara, koje su povezane sa koncentracijom kadmija u tlu. Promjene su rezultirale smanjenima dvaju indeksa učinkovitosti (PI_{ABS}, PI_{total}). Inbred linija B84 je usvojila male količine kadmija, čak i pri najvišoj koncentraciji kadmija u tlu, a pokazale je gotovo istu razinu osjetljivosti kao i linije koje su usvojile velike količine kadmija u listovima ispod klipa (Mo17, Os6-2). Inbred linija B84 se prema tome može smatrati osjetljivom na kadmij, jer su negativni učinci kadmija bili vidljivi u gotovo svim izmjerenim parametrima unatoč tome što je usvojila male količine kadmija u svim tretmanima. U usporedbi s inbred linijom B84, inbred linija B73 se može smatrati tolerantnom

na kadmij s obzirom na minimalna smanjenja PI_{ABS} and PI_{total} vrijednosti pri visokim koncentracijama kadmija u tlu.

Rezultati ovog istraživanja mogu se upotrijebiti u oplemenjivanju kukuruza za razvoj genotipova s visokom akumulacijom kadmija za fitoekstrakciju ili za proizvodnju kukuruza s niskom akumulacijom kadmija na tlima zagađenima kadmijem.

Ključne riječi: Kukuruz, teški metali, kadmij, stres, fluorescencija klorofila *a*, OJIP test, ICP-OES analiza

10.Appendix

Table 26. Mean values and standard errors of selected OJIP test parameters across genotypes and treatments.

Treatment	Genotype	F_0	F_m	F_{300}	V_J	V_I	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC
B73	C	328.021±18.055	1647.33±119.593	608.181±28.839	0,381±0,007	0,726±0,004	2.553±0.032	2.039±0.026	1.259±0.01	0.514±0.013
	Cd0.5	323.521±16.971	1547.194±90.054	660.603±36.062	0,386±0,006	0,726±0,005	2.934±0.074	2.314±0.043	1.201±0.031	0.587±0.017
	Cd1	355.104±14.980	1648.268±103.917	651.709±34.690	0,377±0,006	0,732±0,009	2.598±0.028	2.045±0.020	1.323±0.016	0.578±0.016
	Cd5	347.167±16.875	1606.842±125.620	649.453±27.900	0,392±0,009	0,732±0,007	2.775±0.049	2.132±0.038	1.297±0.015	0.575±0.009
B84	C	330.313±13.744	1596.323±86.983	606.943±33.683	0,398±0,007	0,751±0,009	2.758±0.029	2.183±0.023	1.312±0.016	0.575±0.016
	Cd0.5	320.813±16.299	1538.417±85.559	664.305±37.908	0,394±0,004	0,752±0,009	2.952±0.026	2.331±0.016	1.201±0.019	0.621±0.014
	Cd1	366.229±12.360	1524.825±81.082	701.798±45.757	0,409±0,011	0,762±0,007	2.853±0.056	2.228±0.035	1.127±0.020	0.641±0.007
	Cd5	405.417±13.306	1331.82±93.981	764.967±44.725	0,437±0,009	0,802±0,012	3.403±0.042	2.807±0.027	1.033±0.009	0.807±0.028
Mo17	C	333.563±18.422	1651.132±122.297	629.899±41.024	0,376±0,01	0,743±0,005	2.788±0.039	2.218±0.032	1.313±0.019	0.569±0.015
	Cd0.5	326.208±18.658	1558±98.924	662.718±42.648	0,396±0,008	0,745±0,006	2.959±0.028	2.337±0.02	1.246±0.017	0.622±0.01
	Cd1	365.375±15.368	1538.637±98.698	742.872±30.938	0,424±0,012	0,774±0,008	2.888±0.048	2.262±0.031	1.193±0.016	0.678±0.01
	Cd5	406.754±16.353	1327.056±112.868	863.081±19.688	0,442±0,011	0,829±0,005	3.252±0.044	2.793±0.039	1.177±0.015	0.698±0.011
Os6-2	C	334±14.558	1610.278±112.593	592.092±37.818	0,377±0,006	0,716±0,009	2.704±0.033	2.134±0.027	1.328±0.007	0.57±0.020
	Cd0.5	314.438±18.216	1394.964±100.010	640.106±30.286	0,476±0,015	0,729±0,009	2.717±0.039	2.386±0.056	1.238±0.017	0.729±0.015
	Cd1	362.167±18.654	1297.579±89.493	692.147±53.412	0,489±0,009	0,771±0,011	2.931±0.042	2.266±0.029	1.136±0.021	0.745±0.017
	Cd5	421.692±20.123	1122.508±100.009	763.954±51.58	0,522±0,008	0,819±0,012	3.782±0.144	2.932±0.049	1.069±0.010	0.844±0.031

Genotype	Treatment	RC/ABS	RC/CS_0	t_{max}	TR_0/ABS	ET_0/ABS	ET_0/TR_0	TR_0/DI_0	$ET_0/(TR_0-ET_0)$	PI_{ABS}	PI_{total}
B73	C	0.392±0.005	129.096±7.508	271.458±6.466	0.805±0.002	0.495±0.006	0.619±0.007	3.987±0.095	1.618±0.042	2.583±0.115	2.056±0.095
	Cd0.5	0.342±0.008	111.109±6.782	234.167±5.867	0.79±0.005	0.413±0.015	0.522±0.018	3.596±0.135	1.459±0.041	2.088±0.105	1.728±0.117
	Cd1	0.385±0.004	131.131±8.405	239.167±10.456	0.79±0.003	0.461±0.007	0.586±0.007	3.882±0.117	1.431±0.038	2.249±0.095	1.711±0.122
	Cd5	0.361±0.006	122.312±7.912	260.167±7.725	0.79±0.002	0.457±0.012	0.589±0.013	3.985±0.100	1.512±0.053	2.392±0.074	1.681±0.135
B84	C	0.363±0.004	119.82±4.218	270.833±9.188	0.792±0.005	0.477±0.007	0.602±0.008	3.821±0.111	1.53±0.047	2.21±0.054	1.528±0.14
	Cd0.5	0.339±0.003	109.131±5.988	236.667±7.387	0.79±0.003	0.408±0.006	0.516±0.008	3.789±0.072	1.076±0.034	1.643±0.111	1.308±0.082
	Cd1	0.351±0.006	106.594±5.073	210±10.437	0.774±0.004	0.397±0.012	0.508±0.013	3.419±0.082	1.068±0.055	1.672±0.058	1.062±0.106
	Cd5	0.294±0.004	100.729±4.521	201.417±5.65	0.743±0.006	0.344±0.009	0.49±0.009	2.921±0.082	1.194±0.037	1.421±0.073	0.999±0.057
Mo17	C	0.359±0.005	119.948±6.715	261.875±8.113	0.804±0.002	0.472±0.008	0.593±0.008	3.92±0.103	1.472±0.04	1.739±0.06	1.637±0.047
	Cd0.5	0.338±0.003	110.368±6.450	245.625±7.353	0.79±0.002	0.422±0.008	0.534±0.009	3.768±0.045	1.166±0.039	1.489±0.069	1.376±0.094
	Cd1	0.347±0.006	101.798±5.272	221.819±7.48	0.776±0.003	0.429±0.006	0.543±0.013	3.453±0.049	1.226±0.047	1.376±0.039	1.12±0.092
	Cd5	0.308±0.004	92.8±2.358	215.188±4.627	0.753±0.003	0.379±0.007	0.491±0.01	3.068±0.058	1.047±0.04	1.216±0.072	0.967±0.059
Os6-2	C	0.37±0.005	123.995±6.088	258.958±8.225	0.789±0.006	0.492±0.005	0.623±0.007	3.782±0.142	1.665±0.046	1.954±0.137	1.97±0.142
	Cd0.5	0.369±0.006	103.136±7.237	240.833±11.313	0.77±0.007	0.405±0.014	0.524±0.016	3.413±0.115	1.138±0.073	1.716±0.193	1.449±0.137
	Cd1	0.342±0.005	102.787±6.674	212.313±9.446	0.755±0.004	0.39±0.008	0.504±0.009	3.272±0.041	1.108±0.048	1.555±0.173	1.237±0.066
	Cd5	0.267±0.010	97.554±3.369	198.115±2.4	0.722±0.008	0.358±0.007	0.475±0.006	2.753±0.088	1.014±0.031	1.258±0.158	0.96±0.058

11.Curriculum vitae

PERSONAL INFORMATION

Name and surname	Mario Franić
Date and place of birth	29.01.1986. Požega, Croatia
Address	Umaška5, 31 000 Osijek
Phone	+385 98 682 894
E-mail	mario.franic@poljinos.hr
Citizenship	Croatian

WORK EXPERIENCE

Date (from – until)	2012- present
Institution	Agricultural institute Osijek
Position	Research fellow
Work field	Biology/Agriculture, Abiotic stress research
Date	May 2011 – February 2012
Institution	Elementary school Filip Korajac
Position	Biology teacher

EDUCATION

Date	2012 - present
Place	Osijek, Croatia
Institution	University J.J. Strossmayer, Postgraduate studies of molecular biosciences
Qualification awarded	to be PhD in molecular biosciences
Date	2004 - 2010
Place	Osijek, Croatia

Institution	University J.J. Strossmayer, Department of Biology
Qualification awarded	mag. educ. biol. et chem.

PERSONAL SKILLS AND COMPETENCES

Languages	Croatian (mother language); English (fluent)
Computer skills	Excellent general knowledge of computer based software, and usage of Microsoft Windows Office Suite programs, R programming language.
Other	Type B driver's license

TRAINING

Year	2016
Place	Novi Sad, Serbia
Institution	Institute of filed and vegetable crops, Novi Sad in cooperation with UC Davis Plant Breeding Academy
Subject and skills covered	Plant breeding and trial design and analysis

Year	2014
Place	Not applicable (internet course)
Institution	Coursera, Johns Hopkins University
Subject and skills covered	Data science management in R programming language

Year	2012 – 2015 (multiple stays for one month)
Place	Zagreb, Croatia
Institution	Ruđer Bošković institute, Laboratory for molecular plant biology and biotechnology
Subject and skills covered	Molecular techniques (PCR, tagging, use of restriction enzymes, DNA cloning with plasmid vectors)

RESEARCH AND PROJECTS

2014 – present, researcher: Genetics and physiology of multiple-stress tolerance in maize, HRZZ project no. 5707

2007 - 2013, researcher: Genetic analysis of mineral concentrations in maize kernel (073-0730463-0203); MZOS RH

PUBLICATIONS

Franić, Mario; Galić, Vlatko; Mazur, Maja; Šimić, Domagoj. Effects of excess cadmium in soil on JIP-test parameters, hydrogen peroxide content and antioxidant activity in two maize inbreds and their hybrid. // Photosynthetica. (2017)
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