Revista Caatinga
ISSN: 0100-316X
ISSN: 1983-2125
Universidade Federal Rural do Semi-Árido

Sacramento, Bárbara Lima do; Azevedo Neto, André Dias de; Alves, Andréia Teixeira; Moura, Silvany Cardim; Ribas, Rogério Ferreira

PHOTOSYNTHETIC PARAMETERS AS PHYSIOLOGICAL INDICATORS OF TOLERANCE TO CADMIUM STRESS IN SUNFLOWER GENOTYPES1

Revista Caatinga, vol. 31, no. 4, October-December, 2018, pp. 907-916
Universidade Federal Rural do Semi-Árido

DOI: 10.1590/1983-21252018v31n413rc

Available in: http://www.redalyc.org/articulo.oa?id=237158245013

Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252018v31n413rc

PHOTOSYNTHETIC PARAMETERS AS PHYSIOLOGICAL INDICATORS OF TOLERANCE TO CADMIUM STRESS IN SUNFLOWER GENOTYPES[1]

BÁRBARA LIMA DO SACRAMENTO[2]*, ANDRÉ DIAS DE AZEVEDO NETO[3], ANDRÉIA TEIXEIRA ALVES[2], SILVANY CARDIM MOURA[2], ROGÉRIO FERREIRA RIBAS[2]

ABSTRACT - The objective of the present study was to evaluate the tolerance to cadmium (Cd) of sunflower genotypes grown in greenhouse conditions, and the effectiveness of using photosynthetic parameters as physiological indicators of this tolerance. Seeds of two sunflower genotypes previously identified as tolerant (H358) and Cd-sensitive (AG960) to Cd were used. The seeds were germinated in plastic cups containing plant substrate; after 9 days, the seedlings were transplanted to plastic basins containing a nutrient solution with 0 or 10 μM of Cd, where they remained for 16 days. Samples of the plants were harvested every 5 days. The experiment was carried out in a randomized complete design, using a 4×2×2 factorial arrangement (4 days of grown in a nutrient solution with Cd, 2 sunflower genotypes, and 2 Cd levels) with four replications. Cd stress decreased CO_2 net assimilation, stomatal conductance, carboxylation efficiency, photosynthetic pigment contents, potential quantum yield (F_v/F_m), and effective quantum yield of plants of the two evaluated genotypes. The decrease in photosynthetic rates of these plants was caused by both stomatal and non-stomatal limitations. Plants of the AG960 genotype showed more pronounced deleterious effects due to Cd stress than those of the H358 genotype. Thus, CO_2 net assimilation rate, stomatal conductance, and chloroplast pigment content are good physiological indicators of sunflower tolerance to Cd and can at least in part, explain the greater tolerance of the H358 genotype to Cd stress when compared to the AG960 genotype.

Keywords: Stomatal conductance. CO_2 net assimilation. Photosynthesis. *Helianthus annuus*.

EFICÁCIA DOS PARÂMETROS FOTOSSINTÉTICOS COMO INDICADORES FISIOLÓGICOS DA TOLERÂNCIA AO CÁDMIO EM GIRASSOL

RESUMO - O presente estudo foi conduzido em casa de vegetação objetivando-se demonstrar a tolerância ao cádmio (Cd) em genótipos de girassol e avaliar a eficácia dos parâmetros fotossintéticos como indicadores fisiológicos dessa tolerância. Para isto, foram utilizadas sementes de dois genótipos de girassol previamente identificados como tolerante (H358) e sensível (AG960) ao Cd.As sementes foram germinadas em copos plásticos contendo substrato vegetal e, após 9 dias, as plântulas foram transferidas para bacias plásticas com solução nutritiva contendo 0 ou 10 μM de Cd , onde permaneceram por 16 dias. As plantas foram coletadas a cada 5 dias. O delineamento experimental foi o inteiramente casualizado, em um arranjo fatorial 4 (dias de exposição) × 2 (genótipos) × 2 (níveis de Cd), com quatro repetições. A exposição ao Cd reduziu a taxa de assimilação líquida do CO_2, a condutância estomática e a eficiência de carboxilação, bem como os teores de todos os pigmentos fotossintéticos, a razão F_v/F_m e o Y_{II} das plantas de ambos os genótipos estudados. Demonstrando que a redução na taxa fotossintética dessas plantas foi ocasionada tanto por limitações estomáticas como não-estomáticas. Os efeitos deletérios do Cd foram mais pronunciados no genótipo AG960 em comparação com o H358. Assim, as variáveis *A*, *gs* e os teores de pigmentos mostraram-se bons indicadores fisiológicos da tolerância do girassol ao estresse por Cd e podem, ao menos em parte, explicar a maior tolerância do genótipo H358 em relação ao AG960.

Palavras-chave: Condutância estomática. Assimilação líquida do CO_2. Fotossíntese. *Helianthus annuus*.

*Corresponding author

[1]Received for publication in 03/27/2017; accepted in 04/19/2018.

Paper extracted from the dissertation of the first author.

[2]Center of Agrarian, Environmental and Biological Sciences, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil; barbaralimads@gmail.com - ORCID: 0000-0002-2101-2479, dea_alves106@yahoo.com.br - ORCID: 0000-0003-4079-8515, silvany_cardim@hotmail.com - ORCID: 0000-0002-7715-9605, ecofisiologia@gmail.com - ORCID: 0000-0003-0766-825X.

[3]Center of Exact and Technological Sciences, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil; andre@ufrb.edu.br - ORCID: 0000-0002-4073-8726.

INTRODUCTION

Sunflower (*Helianthus annuus*) is an oleaginous plant of the Asteraceae family that is native to North America. It produces an oil of excellent nutritional quality and has great capacity of adaptation, with good responses to several environmental conditions due to its diversity of genotypes (CAPONE et al., 2012).

Several studies have shown the capacity of sunflower crops to retain several heavy metals (CHAVES; ESTRELA; SOUZA , 2011; GAJDOS et al., 2012; NASCIMENTO et al., 2014), such as cadmium (Cd), whose presence in soils has receiving considerable attention due to its increasing rates in the soil and impacts on human health throughout the food chain.

Once absorbed, Cd tends to exhibit high mobility in plants; thus, part of the amount of this metal that is absorbed by the roots is translocated to the shoot, reaching their photosynthetic apparatus (KÜPPER; ANDRESEN, 2016). Cd causes inactivation and denaturation of enzymes, blocking of metabolically important functional groups, translocation or substitution of essential metal ions of biomolecules and functional cell units, changes in protein conformation, and membrane integrity disorders (HOSEINI; ZARGARI, 2013).

This toxicity is due to the affinity of Cd to sulfhydryl groups in proteins, which inhibits their activity or ruptures their structures. Therefore, these effects alter cellular metabolism, affecting essential processes, such as respiration and photosynthesis of plants grown in environments with this metal (BENAVIDES; GALLEGO; TOMARO, 2005).

Chlorophylls are the main pigments responsible for the uptake of solar radiation to produce chemical energy (ATP and NADPH) (TAIZ; ZEIGER, 2013), while carotenoids are accessory pigments in the light-harvesting complexes and photoprotectors of the photosynthetic apparatus (PARMAR; KUMARI; SHARMA, 2013).

The energy from the light absorbed by the chlorophyll molecules in a leaf can be used in the photosynthesis process, re-emitted as fluorescence, or dissipated as heat (BAKER, 2008). These three processes occur in competition, therefore, changes in photosynthetic rates and heat loss will cause additional changes in fluorescence emission. Thus, chlorophyll-*a* fluorescence analysis can detect safely and reliably the effects of stress and injuries caused by biotic or abiotic factors on the photosynthetic process (CHAVES, 2015).

Gas exchange and chlorophyll-*a* fluorescence are variables of rapid determination that allow the noninvasive evaluation of the plant development, providing qualitative and quantitative information on the physiological conditions of photosynthetic processes (KALAJI et al., 2014). These variables have been widely used to select tolerant genotypes to various abiotic and biotic stresses (AZEVEDO NETO et al., 2011; CHAVES, 2015; SILVA et al., 2014).

In this context, the objective of the present study was to evaluate the tolerance to cadmium (Cd) of sunflower genotypes grown in greenhouse conditions, and the effectiveness of using photosynthetic parameters as physiological indicators of this tolerance by analyzing the gas exchanges, chlorophyll-*a* fluorescence, and pigment content of leaves of these plants.

MATERIAL AND METHODS

Two experiments were developed in a greenhouse for this study. The first experiment consisted of germination of seeds of 28 genotypes in 200-mL plastic cups containing plant substrate, using daily irrigation with distilled water. Nine days after germination, each seedling was transplanted to 12-L plastic basin containing nutrient solution (FURLANI , 1999), where they remained for seven days for acclimation.

Subsequently, these plants were subjected to the treatments: 1 – control (nutrient solution without Cd), and 2 - stress (nutrient solution containing Cd $(NO_3)_2$ at 10 μM). Preliminary experiments using various concentrations of Cd (0 to 50 μM) showed that concentrations up to 10 μM are sublethal but induce significant effects on plants in a short term (FERREIRA et al., 2016). The nutrient solution levels were replenished daily with distilled water and its pH was maintained at 6.0±0.5. The aeration of the nutrient solution was performed by an air compressor. The mean temperature, relative air humidity, and photosynthetically active radiation (at noon) were 27°C, 65%, and 1200 μmol m^{-2} s^{-1}, respectively.

Samples of the plants were harvested after 8 days of treatment and the plant material was packed in paper bags and placed in a forced air circulation oven at 65ºC for 72 h to determine their total dry mass (TDW).

The effect of Cd on biomass production was evaluated by the relative production of dry weight (RP) of each genotype in the stress treatment in relation to respective control, using the formula: $RP = (TDWs / TDWc) \times 100$, where *TDWs* is the total dry weight of plants in the Cd stress, and *TDWc* is the total dry weight of plants in the control. The plants that presented higher RP were classified Cd-tolerant, and those with lower RP as Cd-sensitive.

A randomized complete experimental design was used, in a factorial arrangement 28 (genotypes) × 2 (cadmium levels), with four replications. The data were submitted to variance analysis (ANOVA) and the means compared by the Scott Knott test at 5% probability.

The second experiment was carried out using

seeds of one Cd-tolerant (H358) and one Cd-sensitive (AG960) sunflower genotype. Growth conditions and Cd concentrations were the same as those used in the first experiment. Samples were harvest at 1, 6, 11, 16 days after the beginning of the Cd treatment. Chlorophyll-*a* (Cl*a*), chlorophyll-*b* (Cl*b*) and carotenoid (Car) contents were determined by spectrophotometry at 664.1, 648.6 and 470 nm, respectively, in ethanolic extract, according to the methodology described by Lichtenthaler and Buschmann (2001).

CO_2 net assimilation rate (*A*, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}), transpiration (*E*, mmol m^{-2} s^{-1}), and instantaneous carboxylation efficiency (*A/Ci*) were measured in a portable gas exchange measurement system (LI-6400, LI-COR Biosciences, Nebraska, USA), equipped with a blue/red light source (LI-6400-02B). The measurements were performed on the first pair of fully expanded leaves from the apex, every 5 days, from 9 to 11 h, under artificial saturating light of 1,000 μmol photons m^{-2} s^{-1}, mean temperature of 27.0±2.1°C, mean CO_2 concentration of 389±4 ppm, and mean water vapor pressure of 0.74±0.2 kPa.

Chlorophyll-*a* fluorescence was measured in the same leaves and time used for evaluation of the gas exchanges, using an OS5p modulated portable fluorometer (Opti-Sciences, Hudson, USA). After 30 minutes of dark adaptation, the parameters of minimum fluorescence (F_0), maximum fluorescence (F_m), and potential quantum yield (F_v/F_m) of photosystem II (PSII) were evaluated. The yields of the competitive pathways of the energy absorbed in PSII: effective quantum yield (Y_{II}); quantum yield of regulated (Y_{NPQ}) and non-regulated (Y_{NO}) energy loss were calculated according to the methodology described by Kramer et al. (2004) and Klughammer and Schreiber (2008).

A randomized complete experimental design was used, in a 4×2×2 factorial arrangement - 4 days of days of grown in a nutrient solution with Cd × 2 genotypes × 2 Cd rates - with four replications. The data were subjected to analysis of variance (ANOVA) and the means were compared by the Tukey's test at 5% of probability.

RESULTS AND DISCUSSION

Considering the variables related to the effects of soil contaminants on plant growth, biomass production has been an important parameter for the selection of metal-tolerant plants (BAVI; KHOLDEBARIN; MORADSHAHI, 2011). Plants grown in environments with heavy metals have several physiological and metabolic changes (HOSEINI; ZARGARI, 2013). Cd causes inhibition of cell division, chlorosis, and changes in nitrogen and carbohydrate metabolism, photosynthesis, mineral nutrition, water balance, and membrane structure and permeability (GAJDOS et al., 2012). These factors, singly or combined, reduce plant growth.

The relative production (dry weight) (RP) of the sunflower genotypes evaluated varied in the first experiment (Table 1). The highest RP were found for the H358 (76%), BRS323 (73%), IAC-Uruguai (76%), and Olisun-5 (78%) genotypes; and the lowest RP were found for the AG963 (26%), and AG960 (24%) genotypes. These data classified the genotypes H358, BRS323, IAC-Uruguai, and Olisun -5 as Cd-tolerant, and AG963, and AG960 as Cd-sensitive.

Table 1. Relative production of dry weight (RP) of sunflower plants grown for 8 days in nutrient solution with 10 μM of Cd.

Genotypes	RP (%)		Genotypes	RP (%)	
H358	75.53	A	HLA860HO	46.39	C
BRS323	73.02	A	H863	48.57	C
IAC-Uruguai	75.56	A	EXP60050	46.13	C
Olisun 5	78.33	A	EXP44-63	41.11	D
BRS322	54.53	B	BRS324	42.25	D
AG975	59.24	B	BRS321	39.70	D
AG962	52.30	B	Catisol	40.69	D
AG967	54.39	B	AG972	35.00	E
Olisun 3	53.83	B	TC rola 8122	36.56	E
BRS-G27	52.80	B	HLA211	31.22	E
H250	53.53	B	EXP44-49	36.84	E
H360	58.45	B	EXP11-26	33.76	E
EXP887	56.89	B	AG963	25.52	F
H251	46.81	C	AG960	23.84	F

The second experiment showed a negative effect of Cd on the gas exchanges parameters. However, this effect was less pronounced in plants of the Cd-tolerant (H358) than in plants of the Cd-

sensitive (AG960) genotype.

The stomatal conductance (*gs*) decreased 44% at 6, 21% at 11, and 7% at 16 days of Cd stress in H358 plants (Figure 1C), and decreased 36% at 6, 75% at 11 and 48% at 16 days of Cd stress in AG960 plants (Figure 1D).

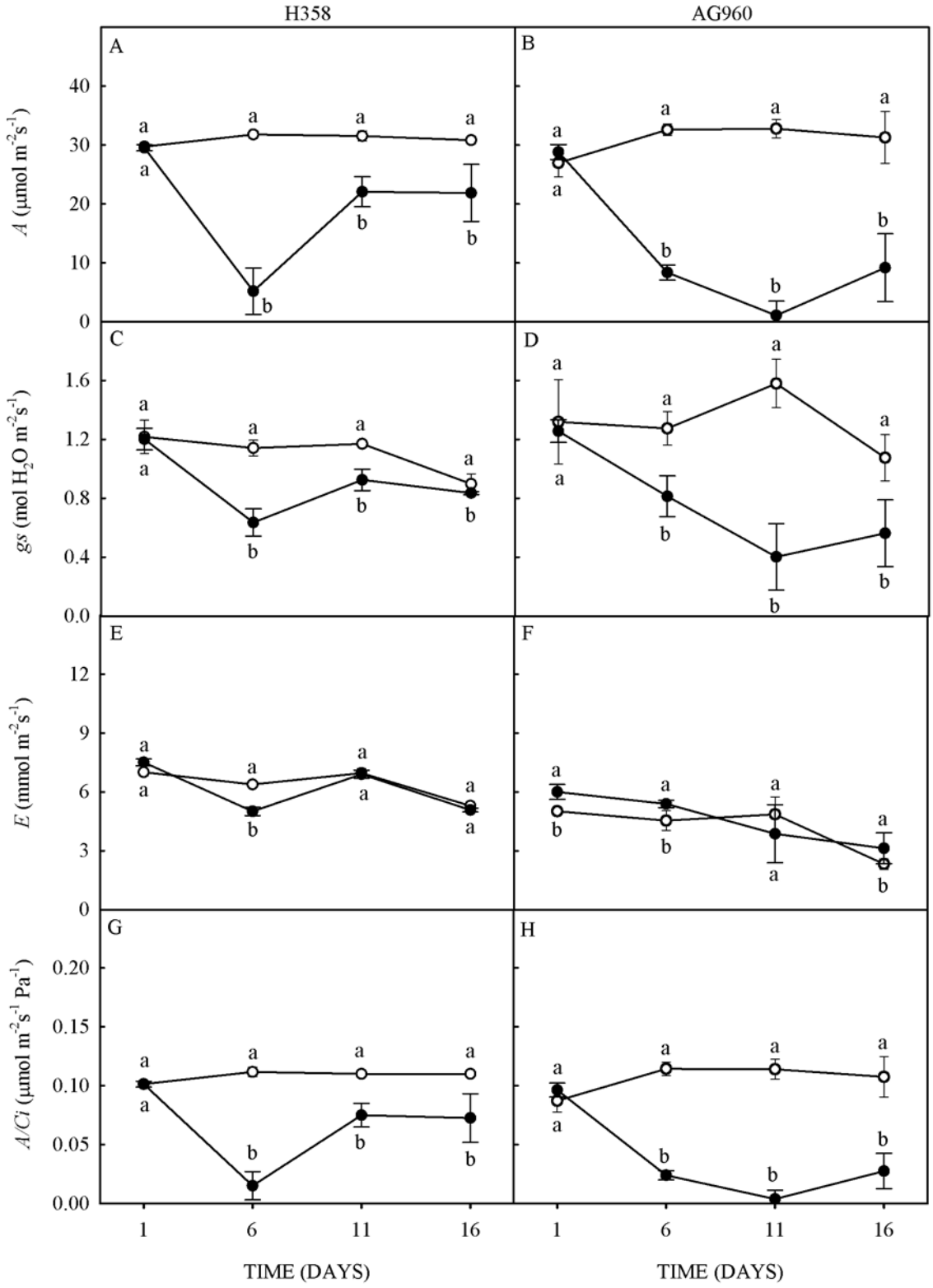

Figure 1. CO_2 net assimilation rate (A and B), stomatal conductance (C and D), transpiration rate (C and D), and carboxylation efficiency (G and H) of a Cd-tolerant (H358) and a Cd-sensitive (AG960) sunflower genotypes, grown in the absence (○) or presence (●) of 10 μM of cadmium in the nutrient solution. Plants were harvest after 1, 6, 11 and 16 days of Cd stress. Means followed by the same letters in each harvest day do not differ by the Tukey's test at 5% probability.

Cd changes water relations of plants, decreasing the turgescence pressure (BENAVIDES; GALLEGO; TOMARO, 2005), and affects translocations of K, Ca, and abscisic acid in guard cells and, consequently, decreases stomatal conductance (PIETRINI et al., 2010), as observed in the present study. The CO_2 net assimilation rate (*A*) of both genotypes decreased when compared to the controls, because of the low *gs* induced by Cd. The *A* decreased 84% at 6, 30% at 11, and 29% at 16 days of Cd stress in H358 plants (Figure 1A), and 74% at 6, 97% at 11, and 71% at 16 days of Cd stress in AG960 plants (Figure 1B). Similarly, the carboxylation efficiency (*A/Ci*) decreased after 6 days of Cd stress in both genotypes, with decreases of 87% at 6, 34% at 11, and 35% at 16 days of Cd stress in H358 plants (Figure 1G), and 79% at 6, 97% at 11, and 75% at 16 days of Cd stress in AG960 plants (Figure 1H). Inhibition of photosynthetic rate due to Cd was observed in different plant species, such as *Robinia pseudoacacia* (DEZHBAN et al., 2015), *Ricinus communis* (LIU et al., 2011) and *Triticum aestivum* (MOUSSA; EL-GAMAL, 2010).

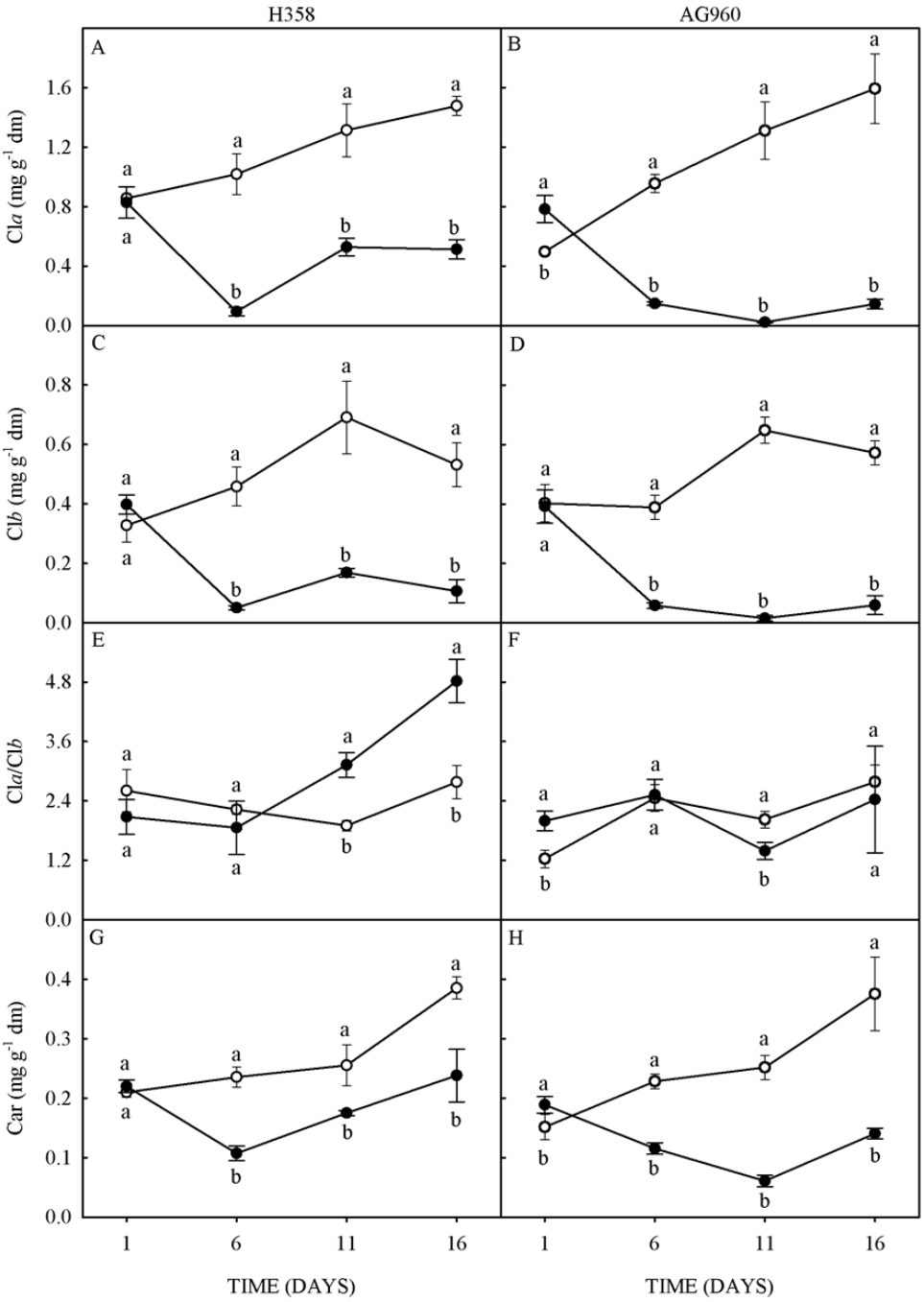

Figure 2. Contents of chlorophyll-*a* (Cl*a*) (A and B) and chlorophyll-*b* (Cl*b*) (C and D), Cl*a* to Cl*b* ratio (E and F), and content of carotenoids (G and H) of a Cd-tolerant (H358) and a Cd-sensitive (AG960) sunflower genotype, grown in the absence (○) or presence (●) of 10 µM of cadmium in the nutrient solution.

In control conditions, the transpiration rates (*E*) of the evaluated genotypes followed the same trend of the *gs*. *E* decreased 21% only at 6 days of Cd stress in H358 plants (Figure 1E), and increased 19% at 1, 18% at 6, and 34% at 16 days of Cd stress in the AG960 (Figure 1F). The tolerant genotype (H358) presented a transient reduction in photosynthetic parameters at 6 days of Cd stress, which was not observed in the sensitive genotype (AG960), suggesting a more efficient adaptation of the H358 genotype to Cd stress.

Non-stomatal factors, such as the reduction of photosynthetic pigment contents, damage of photosynthetic proteins, and inhibition of the Calvin cycle may also have reduced the photosynthetic rate in plants exposed to Cd (TRAN; POPOVA, 2013).

Cd may reduced photochemical activity, because its phytotoxicity causes denaturation or conformational changes in the structures of some proteins of the photosystems (PARMAR; KUMARI; SHARMA, 2013). The activity of several enzymes of the Calvin cycle may also be affected by the substitution of a metal of its structure by Cd, or binding of Cd to sulfhydryl groups, leading to an incorrect folding or interfering with redox-enzymatic regulation (HOSEINI; ZARGARI, 2013). Chlorophyll-*a* (Cl*a*) contents of plants of the H358 (Cd-tolerant) genotype decreased 91% at 6, 60% at 11, and 65% at 16 days of Cd stress, when compared to the control (Figure 2A). In the AG960 (Cd-sensitive) genotype there was an increase of 58% at 1 day of Cd stress, followed by a decrease of 85% at 6, 98% at 11, and 91% at 16 days of Cd stress (Figure 2B).

The chlorophyll-*b* (Cl*b*) decreased 89% at 6, 76% at 11, and 80% at 16 days of Cd stress in H358 plants (Figure 2C), and decreased 85% at 6, 98% at 11, and 90% at 16 days of Cd stress in AG960 plants (Figure 2D). Cl*a*/Cl*b* increased 64% at 11, and 73% at 16 days of Cd stress in H358 plants (Figure 2E), and 62% at 1, and 31% at 11 days of Cd stress in AG960 plants (Figure 2F). These increases were due to a more pronounced deleterious effect of Cd on Cl*b* than on Cl*a*. Considering that stressed plants of the H358 genotype had higher Cl*a*/Cl*b* than AG960, and the fact that reaction centers do not have Cl*b*, a higher Cl*a*/Cl*b* may indicate an acclimation of the antenna to reaction center ratio in order to reduce the energy input in the H358.

The carotenoid contents decreased 54% at 6, 31% at 11, and 38% at 16 days of Cd stress in H358 plants (Figure 2G). In AG960 increased 25% at 1 day of Cd stress, and decreased at 49% at 6, 76% at 11, and 63% at 16 days of Cd stress (Figure 2H).

Chlorophyll degradation in older leaves and biosynthesis inhibition in young leaves have been the main cause of leaf chlorosis in plants grown in soils with Cd (HOSEINI; ZARGARI, 2013).

The reductions in pigment content were probably due to the affinity of Cd to sulfhydryl groups, forming associations that inactivate some enzymes, and can inhibit the synthesis of 5-aminolevulinic acid (chlorophyll precursor molecule), besides inducing deficiencies of magnesium (Mg), iron (Fe) and manganese (Mn), which are necessary for the activity of enzymes involved in the biosynthesis of these pigments (MARQUES et al., 2011). Decreases in Fe, Mn and Mg contents have been attributed to competitive inhibition between Cd and these nutrients for absorption sites in the plasma membrane (HOSEINI; ZARGARI, 2013).

In addition, the Cd-induced decreases in pigment contents can be due to the increased activity of chlorophyllase, enzyme responsible for chlorophyll degradation, that play a key role in the net loss of chlorophyll content in plants exposed to Cd (BENAVIDES; GALLEGO; TOMARO, 2005).

Other studies also found a Cd-induced decrease in chlorophyll content in plants of *Eucalyptus camaldulensis* (MARQUES et al., 2011), *Pisum sativum* L. (JANUŠKAITIEN, 2012) and *Medicago sativa* L. (MAHMOOD et al., 2014).

The carotenoids were the less affected pigments in plants of the two genotypes. This indicates a greater preservation of the mechanism of loss of excess light energy. These molecules are also antioxidant agents that protect the membrane lipids from oxidative stress generated in plants grown in environments with abiotic stresses (FALK; MUNNÉ-BOSCH, 2010).

Regarding the fluorescence parameters, F_0 increased 18% at 1 day of Cd stress and decreased 17% at 6 days of Cd stress in H358 plants (Figure 3A). In AG960 plants, decreased 29% at 11, and 33% at 16 days of Cd stress (Figure 3B). Similarly, the maximum fluorescence (Fm) decreased 59% at 6, 35% at 11, and 35% at 16 days of Cd stress in H358 plants (Figure 3C), and 47% at 6, 55% at 11, and 65% at 16 days of Cd stress in AG960 plants (Figure 3D).

The F_v/F_m of plants under Cd stress of both genotypes also decreased, indicating that Cd stress causes a decrease in the photochemical efficiency of PSII and a disturbance or damage in the photosynthetic apparatus of these plants. In H358 plants, the reductions were 24% at 6, and 17% at 11 days of Cd stress (Figure 3E); and in AG960, 18% at 6, 16% at 11, and 25% at 16 days of Cd stress (Figure 3F).

The F_v/F_m ratio represents the maximum efficiency at which the light absorbed by the PSII antenna is converted into chemical energy, and is an indicator of photoinhibition in plants subject to stress (BAZIHIZINA et al., 2015).

Fv/Fm decreases combined with decreases in F_0 and Fm have been strongly correlated to activation of thermal loss mechanisms, which can be measured by non-photochemical quenching of chlorophyll fluorescence (NPQ) (TAIZ; ZEIGER,

2013).

The reduction of F_0 values in plants under stress can be related to the smaller size of the light collecting antennas. This hypothesis is confirmed by the greatest reduction of F_0 in plants of the AG960 genotype, which had the greatest decrease in chlorophyll-*a*, chlorophyll-*b*, and carotenoids levels.

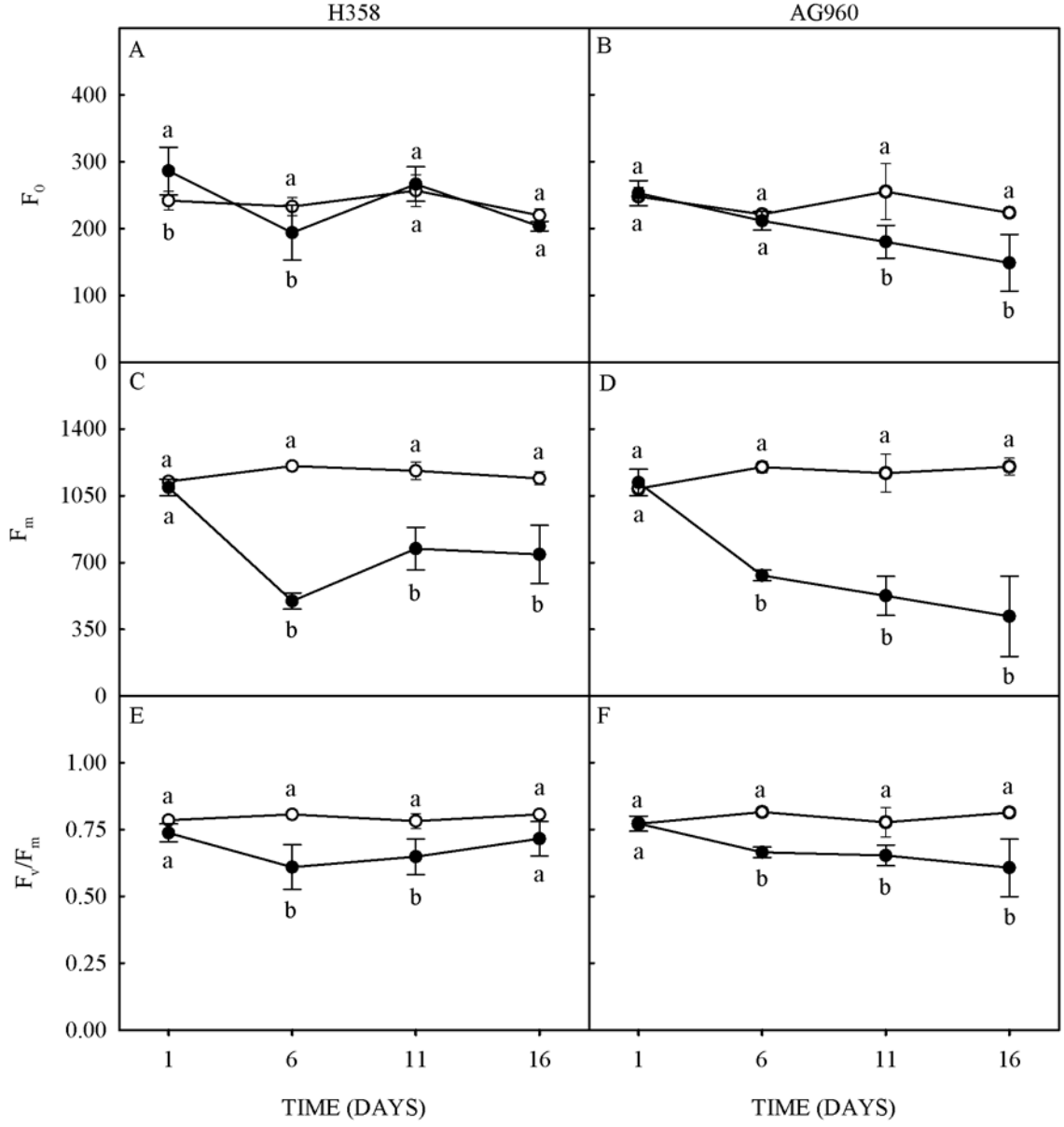

Figure 3. Initial fluorescence (A and B), maximum fluorescence (C and D) and maximum quantum yield of PSII (E and F) of a Cd-tolerant (H358) and a Cd-sensitive (AG960) sunflower genotype, grown in absence (○) or presence (●) of 10 µM of cadmium in the nutrient solution.

The Cd stress decreased the effective quantum yield (Y_{II}) in plants of both genotypes; with decreases of 70% at 6, and 20% at 11 days of Cd stress in H358 plants (Figure 4A), and 65% at 6, 50% at 11, and 22% at 16 days of Cd stress in AG960 plants (Figure 4B). These results confirm the reduction of photochemical efficiency of these plants due to Cd stress. Y_{II} represents the fraction of energy absorbed by the chlorophyll in the PSII that was used in photochemical activity, thus, it informs the number of electrons transported and is an indicative of the photochemical activity (BAKER, 2008).

The quantum yield of regulated (Y_{NPQ}) and non-regulated (Y_{NO}) energy loss (Figure 4C and 4E) was not significantly changed by the Cd stress in plants of the H358 genotype. On the other hand, the Y_{NO} of plants of the AG960 genotype increased 79% at 6, 77% at 11, and 127% at 16 days of Cd stress (Figure 4D). These data indicate that the AG960 plants had a greater loss of energy, which was non-constitutively dissipated in PSII antennas, either as heat during excitation transfer, or by fluorescence emission, which may be associated with photoinhibition (BAZIHIZINA et al., 2015).

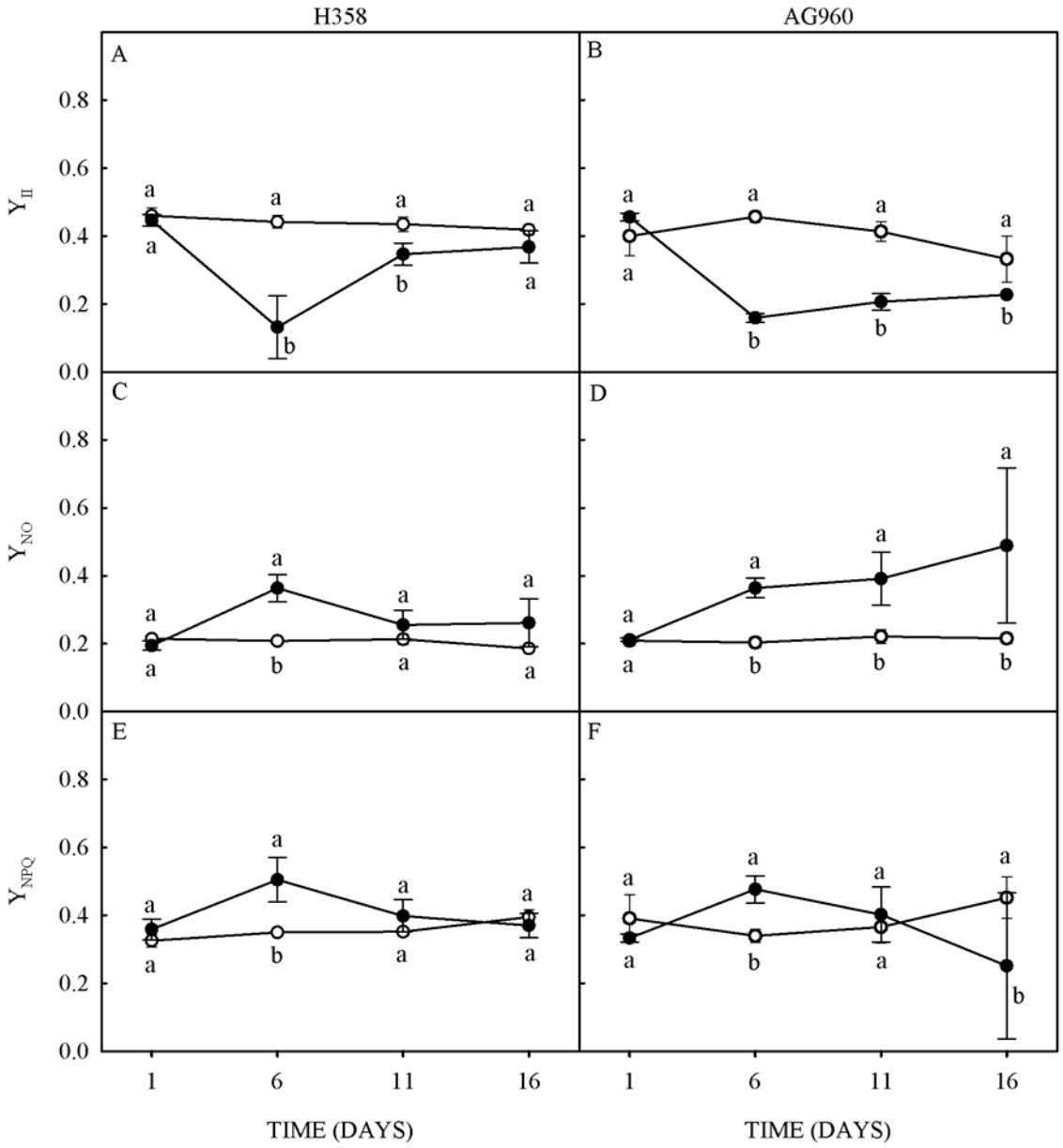

Figure 4. Effective quantum efficiency of PSII (A and B), and quantum yield of non-regulated (C and D) and regulated (E and F) energy loss of a Cd-tolerant (H358) and a Cd-sensitive (AG960) sunflower genotype, grown in absence (○) or presence (●) of 10 µM of cadmium in the nutrient solution.

Y_{NPQ} represents the fraction of dissipated energy as heat through the regulated photoprotective mechanism (xanthophyll cycle). Thus, a high Y_{NPQ} indicates high photoprotective capacity; and a high Y_{NO} followed by a low Y_{NPQ} indicates inability of photoprotective reactions, which may eventually cause damage to the photosynthetic apparatus (KLUGHAMMER; SCHREIBER, 2008).

Figures 4E and 4F show a transient increase in Y_{NPQ} in both genotypes at 6 days of Cd stress, indicating an activation of the thermal dissipation mechanism that allows the chlorophyll de-excitation throughout the xanthophyll cycle. The Y_{NPQ} decreased 44% at 16 days of Cd stress in AG960 plants, suggesting that the prolongation of the stress may have reduced the photoprotection capacity of this genotype against excess radiation. The reduced carotenoid contents and increased Y_{NO} of these plants support this hypothesis.

The combined evaluation of gaseous exchange, chlorophyll-*a* fluorescence, and chloroplast pigment content indicates that, in sunflower plants, the reduction in photosynthetic rates induced by Cd is due to both stomatal and non-stomatal limitations. The results also show that the variables *A*, *gs*, and chloroplast pigment content can be used as indicators of Cd tolerance in sunflower plants.

CONCLUSIONS

The Cd stress affects all variables related to photosynthesis in sunflower plants due to stomatal and biochemical limitations.

The variables CO_2 net assimilation rate, stomatal conductance, and chloroplast pigment contents are good physiological indicators of sunflower tolerance to Cd stress and can be used to diagnose the Cd effects on the integrity of the photosynthetic apparatus in this plant species.

REFERENCES

AZEVEDO NETO, A. D. et al. Fluorescência da clorofila como uma ferramenta possível para seleção de tolerância à salinidade em girassol. **Revista Ciência Agronômica**. Fortaleza, v. 42, n. 4, p. 893-897, 2011.

BAKER, N. R. Chlorophyll fluorescence: a probe of photosynthesis in vivo. **Annual Review of Plant Biology**. Palo Alto, v. 59, s/n., p. 89-113, 2008.

BAVI, K.; KHOLDEBARIN, B.; MORADSHAHI, A. Effect of cadmium on growth, protein content and peroxidase activity in pea plants. **Pakistan Journal Botany.** Karachi, v. 43, n. 3, p. 1467-1470, 2011.

BAZIHIZINA, N. et al. Photosynthesizing on metal excess: Copper differently induced changes in various photosynthetic parameters in copper tolerant and sensitive *Silene paradoxa* L. populations. **Plant Science**. Amsterdam, v. 232, n. 2, p. 67-76, 2015.

BENAVIDES, M. P.; GALLEGO, S. M.; TOMARO, M. L. Cadmium toxicity in plants. **Brazilian Journal of Plant Physiology**, Goytacazes, v. 17, n. 1, p. 21-34, 2005.

CAPONE, A. et al. Desempenho agronômico de cultivares de girassol no sul do Estado de Tocantins. **Journal Biotechnology and Biodiversity**, Gurupi, v. 3, n. 3, p. 13-23, 2012.

CHAVES, L. H. G; ESTRELA, M. A; SOUZA, R. S. Effect on plant growth and heavy metal accumulation by sunflower. **Journal of Phytology**, Kerala, v. 3, n. 12, p. 4-9, 2011.

CHAVES, V. V. **Trocas gasosas e fluorescência da clorofila a em genótipos de cana-de-açúcar infestados por cigarrinha-das-raízes *Mahanarva fimbriolata***. 2015. 33 f. Dissertação (Mestrado em Fitotecnia: Área de Concentração em Produção Vegetal) – Universidade Federal de Viçosa, Viçosa, 2015.

DEZHBAN, A. et al. Cadmium and lead effects on chlorophyll fluorescence, chlorophyll pigments and proline of *Robinia pseudoacacia.* **Journal of Forest Research**, Harbin, v. 26, n. 2, p. 323-329, 2015.

FALK, J.; MUNNÉ-BOSCH, S. Tocochromanol functions in plants: antioxidation and beyond. **Journal of Experimental Botany**, Lancaster, v. 61, n. 6, p. 1549-1566. 2010.

FERREIRA, M. M. A. A. S. et al. Cadmium effects on sunflower growth and mineral nutrition. **African Journal of Agricultural Research**, Nairobi, v. 11, n. 37, p. 3488-3496, 2016.

FURLANI P. R. **Cultivo hidropônico de plantas.** Campinas: IAC, 1999. 52 p. (Boletim técnico, 180).

GAJDOS, E. et al. Effects of Biofertilizers on Maize and Sunflower Seedlings under Cadmium Stress. **Communications in Soil Science and Plant Analysis**, London, v. 43, n. 1-2, p. 272-279, 2012.

JANUŠKAITIENĖ, I. The effect of cadmium on several photosynthetic parameters of pea (*Pisum sativum* L.) at two growth stages. **Žemdirbystė-Agriculture**, Kėdainiai, v. 99, n. 1, p. 71-76, 2012.

HOSEINI, S. M.; ZARGARI, F. Cadmium in Plants: A Review. **International Journal of Farming and Allied Sciences**, Peshawar, v. 2, n. 17, p. 579-581, 2013.

KALAJI, H. M. et al. Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. **Plant Physiology and Biochemistry**, Paris, v. 81, n. 1, p. 16-25, 2014.

KLUGHAMMER, C.; SCHREIBER, U. Complementary PSII quantum yield calculated from simple fluorescence parameters measured by PAM fluorometry and saturation pulse method. **PAM Application Notes**, Effeltrich, v. 1, n. 7, p. 27-35, 2008.

KRAMER, D. M. et al. New fluorescence parameters for the determination of QA redox stat and excitation energy fluxes. **Photosynthesis Research**, Baton Rouge, v. 79, n. 2, p. 209-218, 2004.

KÜPPER, H.; ANDRESEN, E. Mechanisms of metal toxicity in plants. **Metallomics**, Cambridge, v. 8, n. 3, p. 269-285, 2016.

LICHTENTHALER H. K.; BUSCHMANN C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. **Current Protocols in Food Analytical Chemistry**, Hoboken, v. 1, n. 1, p. F4.3.1-F4.3.8, 2001.

LIU, C. Effects of cadmium and salicylic acid on

growth, spectra reflectance and photosynthesis of castor bean seedlings. **Plant and Soil**, Subiaco, v. 344, n. 1-2, p. 131-141, 2011.

MAHMOOD, S. et al. Biometric and biochemical attributes of alfalfa seedlings as indicators of stress induced by excessive cadmium, **Journal of Soil Science and Plant Nutrition**, Temuco, v. 14, n. 3, p. 546-553, 2014.

MARQUES, T. C. L. L. S. M. et al. Respostas fisiológicas e anatômicas de plantas jovens de eucalipto expostas ao cádmio. **Revista Árvore**, Viçosa, v. 35, n. 5, p. 997-1006, 2011.

MOUSSA H.; EL-GAMAL, S. Effect of salicylic acid pretreatment on cadmium toxicity in wheat. **Biologia Plantarum**, Praga, v. 54, n. 2, p. 315-320, 2010.

NASCIMENTO, A. L. et al. Teores de metais pesados no solo e em girassol adubado com lodo de esgoto. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 18, n. 3, p. 294-300, 2014.

PARMAR, P.; KUMARI, M.; SHARMA, V. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. **Botanical Studies**, London, v. 54, n. 1, p. 45-50, 2013.

PIETRINI, F. et al Spatial distribution of cadmium in leaves and on photosynthesis: examples of different strategies in willow and poplar clones. **Plant Biology**, New York, v. 12, n. 2, p. 355-363, 2010.

SILVA, L. A. et al. Mecanismos fisiológicos em híbridos de citros sob estresse salino em cultivo hidropônico. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 18, Sup., p. 1-7, 2014.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. 5. ed. Porto Alegre, RS: Artmed, 2013. 954 p.

TRAN, T. A.; POPOVA, L. P. Functions and toxicity of cadmium in plant recent advances and future prospects. **Turkish Journal of Botany**, Trebizonda, v. 37, n. 1, p. 1-13, 2013.