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Physiological, nutritional, and biochemical indicators of lead tolerance in sunflower genotypes

Indicadores fisiológicos, nutricionais e bioquímicos de tolerância ao chumbo em genótipos de girassol

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Highlights

Selection of lead-tolerant sunflower genotypes for phytoremediation.
Lead stress induces nutritional changes in macro- and micronutrient contents.
Pb-sensitive genotypes have higher energy costs for osmoregulation.
Genotype BRS-G27 can be recommended for phytoremediation of Pb contaminated soils.

Abstract

This study aimed to select and classify sunflower genotypes tolerant to lead (Pb) stress and evaluate their capacity of phytoextraction based on physiological, nutritional, and biochemical responses. Two experiments were carried out under lead stress. In the first experiment, out of 21 genotypes studied three showed higher relative biomass yield and were characterized as Pb-tolerant and five showed lower relative biomass production and were considered Pb-sensitive. In the second experiment, one Pb-tolerant (BRS-G27) and two Pb-sensitive (H251 and AG963) genotypes were studied. In this experiment, Pb stress reduced the growth and contents photosynthetic pigments in all genotypes, but more pronouncedly in sensitive genotypes. There were no substantial changes in micronutrient levels in the leaves and stem, but the levels of Cu and Mn in the stressed roots of sensitive genotypes were much lower than in BRS-G27.

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The contents of organic solutes in the roots suggest that sensitive genotypes have higher energy costs for osmoregulation by carbohydrates and amino acids synthesis. However, the accumulation of proline may be related to a greater Pb tolerance. Considering the results of dry mass yield, transfer coefficient, translocation factor, and tolerance index, the BRS-G27 genotype can be recommended for use in phytoremediation of Pb-contaminated soils.

Key words: Mineral nutrition. Organic solutes. Pigments. Toxic metal.

Resumo

Este estudo teve como objetivo selecionar e classificar genótipos de girassol tolerantes ao estresse por chumbo (Pb) e avaliar sua capacidade de fitoextração com base nas respostas fisiológicas, nutricionais e bioquímicas. Dois experimentos foram realizados sob estresse de Pb. No primeiro experimento, dos 21 genótipos estudados, três apresentaram maior produção relativa de biomassa e foram caracterizados como tolerantes ao Pb e cinco apresentaram menor produção relativa de biomassa e foram considerados sensíveis ao Pb. No segundo experimento foram estudados um genótipo tolerante ao Pb (BRS-G27) e dois sensíveis ao Pb (H251 e AG963). Neste experimento, o estresse por Pb reduziu o crescimento e os teores de pigmentos fotossintéticos em todos os genótipos, porém, mais pronunciado em genótipos sensíveis. Não houve mudanças substanciais nos níveis de micronutrientes nas folhas e no caule, mas os níveis de Cu e Mn nas raízes estressadas de genótipos sensíveis foram muito mais baixos do que o BRS-G27. Os conteúdos de solutos orgânicos nas raízes sugerem que genótipos sensíveis apresentam maiores custos energéticos para osmorregulação por carboidratos e síntese de aminoácidos. No entanto, o acúmulo de prolina pode estar relacionado a uma maior tolerância ao Pb. Considerando os resultados de produção de massa seca, coeficiente de transferência, fator de translocação e índice de tolerância, o genótipo BRS-G27 pode ser recomendado para uso em fitorremediação de solos contaminados com Pb.

Palavras-chave: Nutrição mineral. Solutos orgânicos. Pigmentos. Metal tóxico.

Introduction

Pollution by heavy metals has become one of the major environmental issues faced by mankind (Malar et al., 2014). Environmental contamination can occur due to metallurgical, industrial, and agroindustrial activities, which may cause toxic effects on plants and animals (Bassegio et al., 2020), for example, the application of steel slag as a corrective of soil acidity can increase contents of heavy metals in soil beyond critical limits for agriculture (Caetano et al., 2016). These metal pollutants make it particularly difficult to reclaim the soil, water, and air, because, unlike an organic pollutant, which gets degraded with time into small harmless molecules, toxic elements such as lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu) and zinc (Zn) are immutable by biochemical reactions (Malar et al., 2014).

Lead, even at low concentrations, can be cytotoxic or genotoxic (Bassegio et al., 2020). In plants, stress responses vary widely depending on species, toxic elements, duration of the exposure and environmental conditions (Bassegio et al., 2020; Dalyan et al., 2020). In the case of Pb, the most common symptoms of toxicity include reduction of photosynthetic pigments, loss of membrane integrity, reduction of enzyme and photosynthetic activities, disruption of hormonal, ion, and water homeostasis, root browning, and nutritional disorders, inhibition in seed germination, leading to reduced plant growth (Hussain et al., 2013; Abreu et al., 2016; Bassegio et al., 2020; Dalyan et al., 2020).

High Pb concentration in the soil causes the replacement of elements such as potassium (K) and calcium (Ca) in aluminosilicates, especially feldspars, and metals in sulfides (Bosso & Enzweiler, 2008). Despite the diversity of metals in the soil, Pb uptake by plants depends mainly on its bioavailable fraction (Kim et al., 2015) in the form of divalent cation (Pb^{2+}) and the uptake by passive mechanism (Kabata-Pendias, 2010).

Some authors have reported Pb-induced changes in the physiological and biochemical parameters of plants (Abreu et al., 2016; Song et al., 2020). However, responses related to plant growth, accumulation of nutrients and organic solutes, and the Pb distribution in the different organs directly depend on the genotypic characteristics and the Pb concentration in the root environment (Ashraf et al., 2020). Due to the diversity of plant responses associated with genotypic variations, screening of plants of the same species to abiotic stress has been done through multivariate analyses, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Azevedo et al., 2020). The sunflower crop is quite versatile and has important agronomic characteristics, such as drought tolerance, adaptation to different soil and climatic conditions, and potential for phytoremediation (Alaboudi et al., 2018). However, little is known about the damage caused by Pb on the physiological and biochemical processes in contrasting sunflower genotypes.

The knowledge and determination of heavy metal toxicity mechanisms are of great importance for the exploration of food and economic crops (Onakpa et al., 2018). Therefore, this study aimed to select and classify sunflower genotypes tolerant to lead stress and indicate their capacity of phytoextraction based on physiological, nutritional, and biochemical responses.

Material and Methods

Experimental conditions and treatments

The experiments were carried out in a greenhouse, and the plant tissue analyses were performed in the Biochemistry Laboratory of the Center of Exact and Technological Sciences (CETEC) of the Federal University of Recôncavo of Bahia, in the municipality of Cruz das Almas - BA (12º 40′ 19″ S; 39º 06′ 23″ W; altitude ~220 m). According to the Köppen-Geiger classification, the region has a humid tropical climate (Af), with rain almost every month of the year (Alvares et al., 2013).

The study was divided into two experiments, both in a completely randomized design, with four replicates. The first experiment was performed in a factorial arrangement with 21 sunflower genotypes × two levels of lead (0 and 0.65 mM) in the nutrient solution. The second experiment was conducted in a factorial arrangement with three sunflower genotypes × two levels of lead (0 and 0.65 mM). This level of lead was based on recommendation of Abreu et al. (2016), who indicated that sunflower tolerates Pb up to this level and the soil contamination with Pb usually does not exceed this limit.

Experiment 1: Selection of sunflower genotypes differing in Pb tolerance

In this experiment, the biomass of 21 sunflower genotypes obtained from different companies were evaluated: Atlântica Sementes (Olisum 3, Olisum 5); Ceapar (AG960, AG963, AG975); Coordenadoria de Assistência Técnica Integral (Catissol); Embrapa Soja (BRS321, BRS322, BRS323, BRS-G27), Helianthus do Brasil (EXP11-26, EXP44-49, EXP44-63, EXP60050, H250, H251, H360, H863, HLA860HO, TC8122), and Instituto Agronômico de Campinas (IAC-Iarama).

The genotypes were seeded in 200 mL plastic cups, using washed sand as substrate, and daily irrigated with distilled water. The seedlings were transferred to containers with 12 L of aerated Hoagland and Arnon (1950) nutrient solution, after 10 days of emergence. The volume of the nutrient solution was adjusted daily with distilled water and the pH was maintained at 5.5 ± 0.5 with the addition of 1.0 M NaOH or HCl, throughout the experiment. The plants remained for eight days in acclimation. After this period, the treatments with two lead concentrations (0 or 0.65 mM Pb, in the form of lead nitrate [$Pb(NO_3)_2$]) in the nutrient solution were initiated. The lead applied in the nutrient solution was EDTA chelated in order to avoid its precipitation with sulfate and phosphate ions. The nutrient solution was kept under intermittent aeration of 15 minutes every hour, by means of an air compressor coupled to a timer.

The plants of all treatments were carefully removed from the nutrient solution, 10 days after Pb addition. The roots were washed with distilled water and plants were separated into leaves, stems, and roots. The dry masses of the leaf (LDM), stem (SDM), and root (RDM) were determined, after drying at 65 °C in forced air circulation oven for 72 h. The shoot dry mass (ShDM) and total dry mass (TDM) of the plants were calculated by summation.

Experiment 2: Assessment of physiological, nutritional, and biochemical parameters and phytoextraction capacity of Pb-tolerant and Pb-sensitive sunflower genotypes

This experiment was carried out using seeds of three genotypes selected from the first experiment, one (BRS-G27) classified as Pb-tolerant and two (H251 and AG963) classified as Pb-sensitive. The production of seedlings, cultivation system, Pb treatments, and the management of nutrient solutions were the same as those of Experiment 1.

After 10 days under Pb stress, fresh leaf samples were collected for the determination of pigments (chlorophyll *a* - Chl*a*, chlorophyll *b* - Chl*b*, and carotenoids - Car). Later, plants were harvested and separated into leaves, stems, and roots, frozen and lyophilized for the determination of nutrients (K, Ca, Mg, Mn, Zn, Cu, Fe), Pb, and content of organic solutes. The extraction of pigments was performed with 95% ethanol solution, and the determination of Chl*a*, Chl*b*, and Car contents was performed by spectrophotometry at 470, 649, and 664 nm, respectively, according to the methodology described by Lichtenthaler and Buschmann (2001). From Chl*a* and Chl*b* contents, the total chlorophyll content (Chl*t*) and the Chl*a*/Chl*b* and Chl*t*/Car ratios were calculated.

The extracts used in the determination of nutrients and Pb contents were prepared according to the procedures recommended by Jones (2001) by acid digestion in a mixture of 5 mL of concentrated nitric acid (HNO_3) and 3.0 mL of 30% hydrogen peroxide (H_2O_2). The volume of digested samples was adjusted to 50 mL with a solution of hydrochloric acid (1:10 v/v) for the determination of K, Ca, Mg, Mn, Zn, Cu, Fe, and Pb. The concentrations of K, Ca, Mg, Mn, Zn, Cu, Fe, and Pb were quantified simultaneously by inductively coupled plasma optical emission spectroscopy (ICP-OES), model 710-ES (Varian, Mulgrave, Victoria, Australia).

The extracts used for the analyses of organic solutes were prepared by grinding 0.1 g of lyophilized leaf and root tissues in 5 mL of 100 mM potassium phosphate buffer pH 7.0 containing 0.1 mM EDTA. After homogenization, the mixture was filtered on muslin tissue and centrifuged at 12,000 × g for 15 min. The supernatant was stored in an ultra-freezer (-80 °C) for determinations of organic solutes.

Soluble carbohydrate content was determined by spectrophotometry at 490 nm by the phenol-sulfuric acid method using D-(+)-glucose as standard (Dubois et al., 1956). Free proline was determined by spectrophotometry at 520 nm using ninhydrin as a specific reagent and pure proline as standard (Bates et al., 1973). Total free amino acids were determined by spectrophotometry at 570 nm by the ninhydrin method using pure L-leucine as standard (Yemm & Cocking, 1955). Soluble proteins were determined by spectrophotometry at 595 nm by the dye-binding method using pure bovine serum albumin as standard (Bradford, 1976).

For assessment of the translocations of heavy metals into the growing plant's tissues, the transfer coefficient (TC), translocation factor (TF), and tolerance index (TI) were calculated using the following equations (Bassegio et al., 2020).

$$TC = C_{shoot} / C_{nutrient\ solution} \qquad (1)$$

where C_{shoot} (mg L^{-1}) and $C_{nutrient\ solution}$ (mg L^{-1}) represent the concentrations of the metal in shoots and nutrient solution, respectively.

$$TF = C_{shoot} / C_{root} \qquad (2)$$

where C_{shoot} (mg L^{-1}) and C_{root} (mg L^{-1}) represent the concentrations of the metal in shoots and roots, respectively.

$$TI = DM_{contaminated} / DM_{control} \qquad (3)$$

where $DM_{contaminated}$ (g $plant^{-1}$) and $DM_{control}$ (g $plant^{-1}$) represent the dry mass of the plants in treatment with Pb and dry mass of the plants in control treatment, respectively.

Statistical analysis

In Experiment 1, the results were subjected to principal components analysis (PCA) and hierarchical cluster analysis (HCA) for traits of this experiment (ShDM, RDM, and TDM) using the Past v.3.4 Software (Hammer et al., 2001).

In Experiment 2, the results were subjected to analysis of variance (F-test), and the means were compared by Tukey test at 0.05 probability, using the Sisvar 5.6 statistical software (Ferreira, 2019).

Results and Discussion

Experiment 1: Selection and classification of 21 sunflower genotypes with respect to Pb stress

Lead stress reduced the relative dry mass yield of all plant parts of the studied sunflower genotypes (Table 1). The genotypes that simultaneously showed the highest values of ShDM, RDM, and TDM were AG975, BRS-G27 and H863. In contrast, IAC-Iarama, BRS322, AG963, H251, and H360 were the genotypes that had the lowest relative biomass production in all organs of the plant.

Table 1

Dry weight yield, expressed as percentage of the control, of shoot (ShDM), root (RDM), and total (TDM) of 21 sunflower genotypes grown under greenhouse conditions for 10 days in nutrient solution containing 0.65 mM of Pb

	ShDM	RDM	TDM		ShDM	RDM	TDM
Genotypes	----- % of the control-----			Genotypes	---- % of the control ----		
Olisun 3	21.15d	33.67d	23.30d	AG975	36.95a	62.51a	41.70a
HLA860HO	21.56d	40.60c	25.06d	AG963	12.73e	17.29e	13.69e
Olisun 5	30.85b	42.63c	32.84b	BRS-G27	43.06a	56.37a	46.25a
Catisol	17.02e	27.04d	18.98e	EXP60050	20.36d	30.14d	22.43d
TC8122	27.71c	58.02a	33.36b	H251	9.50e	14.52e	10.52e
H250	25.90c	58.31a	32.63b	H863	40.72a	64.07a	45.33a
EXP44-69	21.82d	53.37a	27.54c	AG960	23.24d	18.37e	22.18d
IAC-Iarama	14.68e	12.33e	14.19e	H360	14.85e	17.12e	15.39e
BRS323	21.21d	29.27d	23.03d	EXP44-63	27.16c	50.49b	32.61b
BRS321	21.32d	24.16d	21.92d	EXP11-26	23.59d	55.32a	28.22c
BRS322	14.46e	21.26e	15.84e	-	-	-	-

Means followed by the same letters in a column do not significantly differ by the Scott-Knott's test ($p \leq 0.05$).

Biomass yield has been considered as the most important among the traits regarding the effects of the contaminant on plant growth (Bassegio et al., 2020) and, consequently, considered an important parameter in the selection of metal-tolerant plants.

Principal component analysis (PCA) showed that PC1 and PC2 together explained 99.96% of the variance of data (Figure 1A). PC1 explained the largest variance observed in the data (93.64%), while PC2 accounted for 6.32% of the total variance.

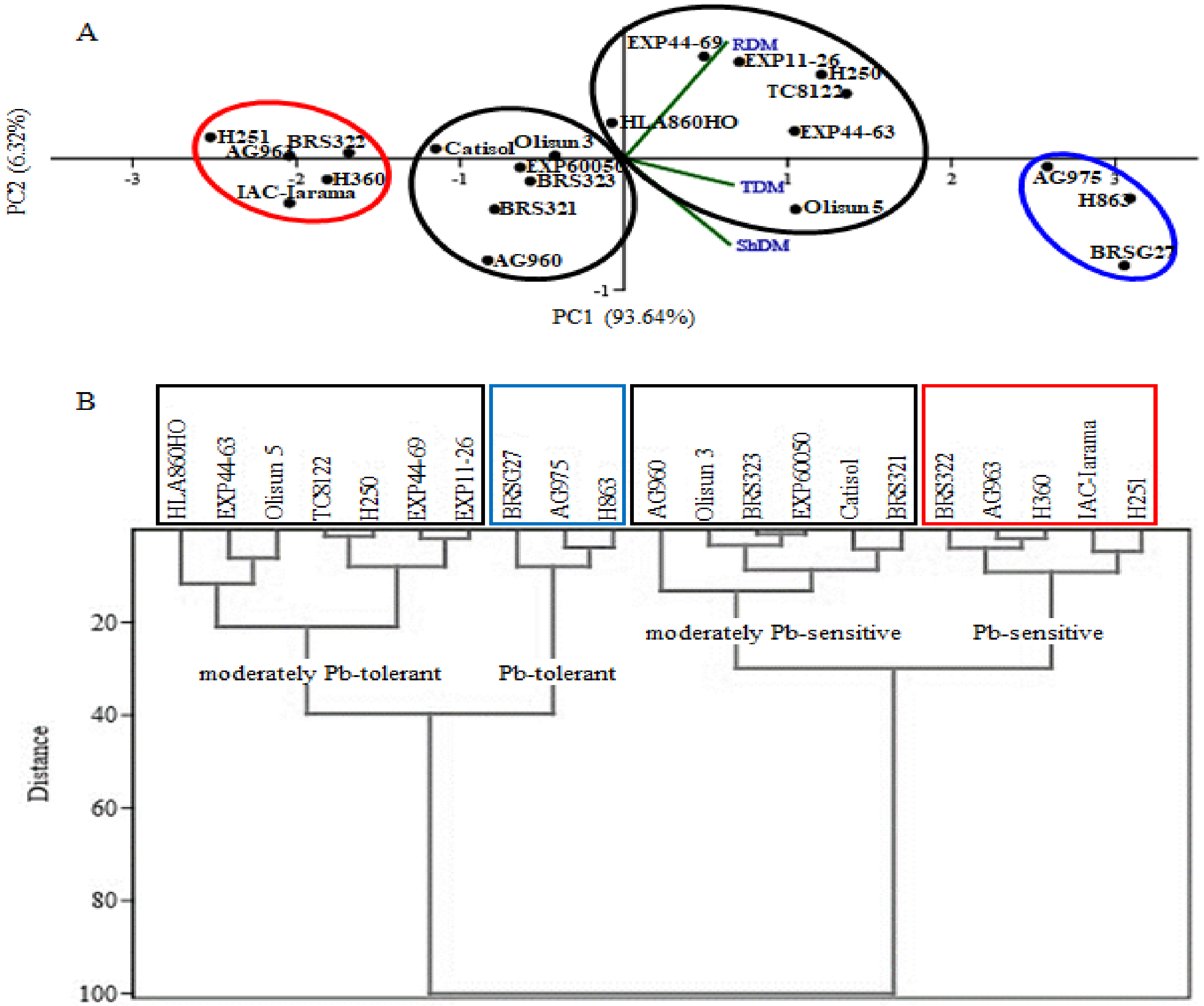

Figure 1. Principal component analysis (PCA) (A) and dendrogram of hierarchical cluster analysis (HCA) (B) using the relative yield of shoot dry mass (ShDM), root dry mass (RDM), and total dry mass (TDM) of 21 sunflower genotypes grown under greenhouse conditions for 10 days in nutrient solution containing 0.65 mM Pb.

By examining the scores-plot in the area defined by PC1 and PC2, four groups of genotypes were separated, based on their ShDM, RDM, and TDM. Group 1 is located on the left end of the PC1 and is composed of the genotypes with the lowest relative production of ShDM, RDM, and TDM (IAC-Iarama, BRS322, AG963, H251, and H360). Group 2, positioned on the bottom of the scores-plot and negatively correlated with both PC1 and PC2, included the genotypes AG960, Olisum 3, BRS323, EXP60050, Catisol, and BRS321. This group was characterized by having ShDM, RDM, and TDM values higher than those of

Group 1. Group 3, which is situated on the top of the scores-plot and correlated positively with PC1 and PC2, is formed by genotypes HLA860HO, EXP44-63, Olissum 5, TC8112, H250, EXP44-69, and EXP1126, which have high RDM values. Finally, Group 4 is found on the right end of the PC1 and gathered three sunflower genotypes (BRS-G27, AG975, and H863). This group was characterized by having the highest ShDM and TDM values. This group was characterized by higher values of RDM among all genotypes.

In agreement with PCA, the hierarchical cluster analysis (HCA) also separated the genotypes into four groups, estimated by the 'Ward' method based on Euclidian distance (Figure 1B). The first and second clusters (on the left) include all genotypes with simultaneously higher values of ShDM, RDM, and TDM in the Pb stress treatment (Table I). In contrast, the third and fourth clusters (on the right), grouped the genotypes with the lowest relative biomass yield. The BRS-G27 genotype (located on the right side of the PC1), and H251 and AG963 genotypes (found on the left side of PC1) were used in Experiment 2.

Experiment 2: Physiological, nutritional, and biochemical parameters and phytoextraction capacity in Pb-tolerant and Pb-sensitive sunflower genotypes

The presence of 0.65 mM Pb in the nutrient solution reduced the dry mass production of the genotypes BRS-G27, H251, and AG963 (Figure 2). This effect was noticeable in the H251 and AG963 genotypes. The genotypes H251 and AG963 showed, on average, reductions of 82% in LDM (Figure 2A), 90% in SDM (Figure 2B), 83% in RDM (Figure 2C), and 86% in TDM (Figure 2D) when the stress treatment was compared with the respective control. In the BRS-G27 genotype, Pb decreased SDM (23%) and TDM (18%), but did not affect LDM and RDM. Thus, the genotypes AG963 and H251 showed a pronounced reduction in dry mass production of all organs of plants, corroborating the results of Experiment 1, which showed that they are Pb-sensitive. In contrast, a relatively small effect of Pb in nutrient solution was observed on plant growth of BRS-G27, confirming that this genotype is Pb-tolerant.

The root apical meristem mitotic index is directly related to root growth (Adam & El-Ashry, 2010) since this meristematic zone is responsible for the production of new cells (Andrade et al., 2010) or the production of chromosomal abnormalities (Aslam et al., 2017). Alternatively, Pb stress may also have induced stomatal closure, reducing the fixation of atmospheric CO_2 in Pb-sensitive genotypes (Sharma & Dubey, 2005). Plants grown in environments contaminated by toxic metals show a series of physiological and nutritional disturbances, including reduced concentrations of chlorophylls and carotenoids when compared to those not stressed by metals (Yang et al., 2020).

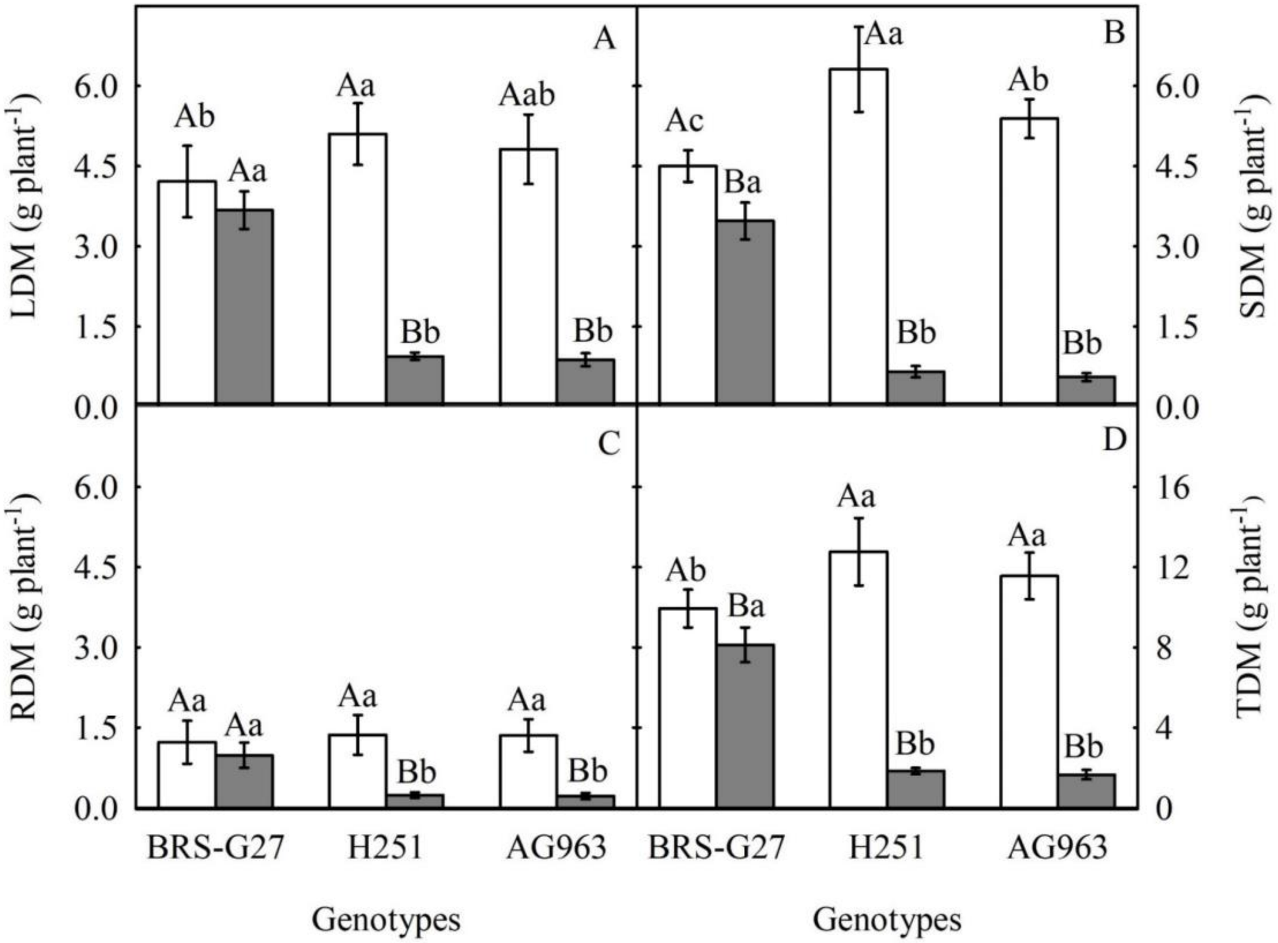

Figure 2. Leaf - LDM (A), stem - SDM (B), root - RDM (C), and total - TDM (D) dry mass production of sunflower genotypes after 10 days of cultivation in a greenhouse with nutrient solution (☐) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey test at p ≤ 0.05. Vertical bars represent the standard deviations (n=4).

Under control conditions, no significant differences were observed among the levels of Chl*a*, Chl*b*, Chlt, and Car in the studied genotypes (Figure 3). In the stress treatment, the level of pigments decreased in all genotypes; however, in BRS-G27, the reduction was less pronounced compared to H251 and AG963 genotypes. Thus, Chl*a* (Figure 3A), Chl*b* (Figure 3B), Chlt (Figure 3C), and Car (Figure 3D) decreased, respectively, by 13, 16, 11, and 14% in BRS-G27, whereas in H251 and AG963, Pb stress strongly decreased the levels of Chl*a* (51 and 42%), Chl*b* (51 and 51%), Car (44 and 29%) and Chlt (51 and 44%), respectively. The Chl*a*/Chl*b* (Figure 3E) and Chlt/Car (Figure 3F) ratios were not affected by Pb in BRS-G27, but Chl*a*/Chl*b* ratio increased by 19% in AG963 and Chlt/Car ratio decreased by 12 and 21% in H251 and AG963 genotypes, respectively, evidencing that the deleterious effect of Pb in these genotypes was more pronounced on chlorophylls than on carotenoids.

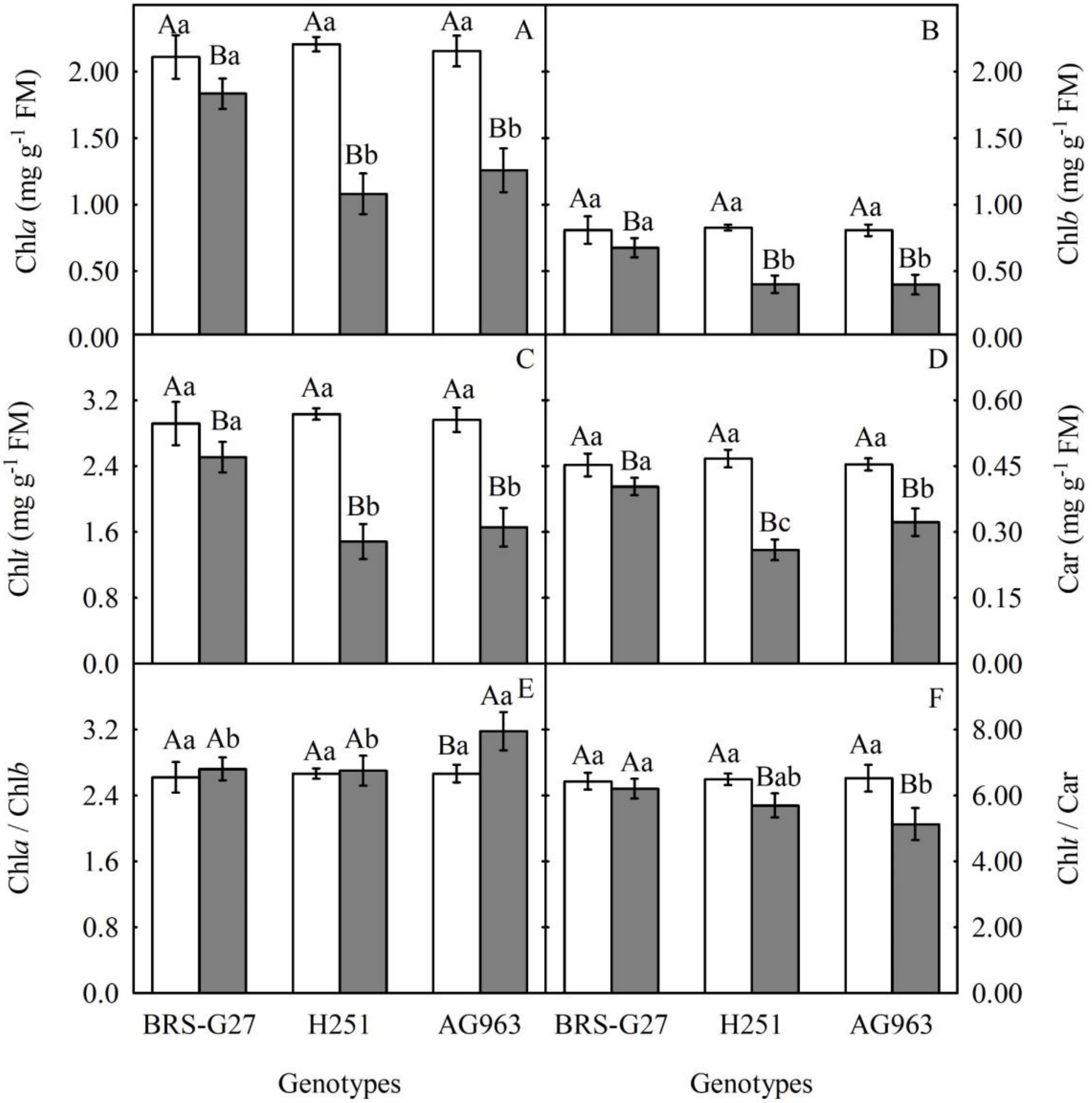

Figure 3. Chlorophyll *a* - Chl*a* (A), chlorophyll *b* - Chl*b* (B), total chlorophyll - Chl*t* (C) and carotenoids - Car (D) contents, chlorophyll *a* to chlorophyll *b* ratio - Chl*a*/Chl*b* (E) and total chlorophyll to carotenoid ratio - Chl*t*/Car (F) of sunflower genotypes after 10 days of cultivation in a greenhouse with nutrient solution (☐) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey test at p ≤ 0.05. Vertical bars represent the standard deviations (n=4).

While studying the growth, physiology, and cellular structure of *Ligustrum lucidum* subjected to Pb stress, Zhou et al. (2018) also found that Pb decreases chlorophyll concentrations. The Pb-induced reduction in the photosynthetic pigment content may be a consequence of thylakoid membranes peroxidation due to increased levels of reactive oxygen species generation (Malar et al., 2014). The reduction of pigment contents may also occur due to the inhibition of their biosynthesis (Houri et al., 2020; Dalyan et al., 2020) or an increase in degradation (Houri et al., 2020; Dalyan et al., 2020).

As a major pigment responsible for the uptake of light energy, chlorophyll is often used as an indicator of the environmental stress effect on plants (Cova et al., 2020). The chlorophyll and carotenoids concentrations in the leaves have been used to diagnose the integrity of photosynthetic apparatus in plants subjected to environmental stresses, such as heavy metal toxicity (Houri et al., 2020). The Pb-induced decline in photosynthesis may be the result of change in chloroplast ultrastructure, membrane content and permeability, reduced pigment concentration, decreased electron transport rate, or inhibition of Calvin cycle activity (Zhou et al., 2018; Dalyan et al., 2020).

The data obtained in this study indicate that, in sunflower, the determination of the pigment contents can also be used to diagnose the effect of Pb on the photosynthetic apparatus integrity. Therefore, the pigment content showed to be a good biochemical indicator of sunflower tolerance to Pb stress, and it may, at least in part, explain the greater tolerance of the BRS-G27 genotype when compared to H251 and AG963. The observation that the growth was only affected in the genotypes that showed a decrease in pigment contents supports this hypothesis.

The K, Ca, and Mg levels in leaves, stems, and roots of sunflower genotypes are shown in Figure 4. The presence of Pb in the nutrient solution increased K concentration in the leaves of all genotypes: 51% in BRS-G27, 30% in H251, and 12% in AG963 (Figure 4A). In the stems and roots, the Pb decreased the K content only in the genotype AG963 (23 and 45%, respectively) (Figures 4B and C).

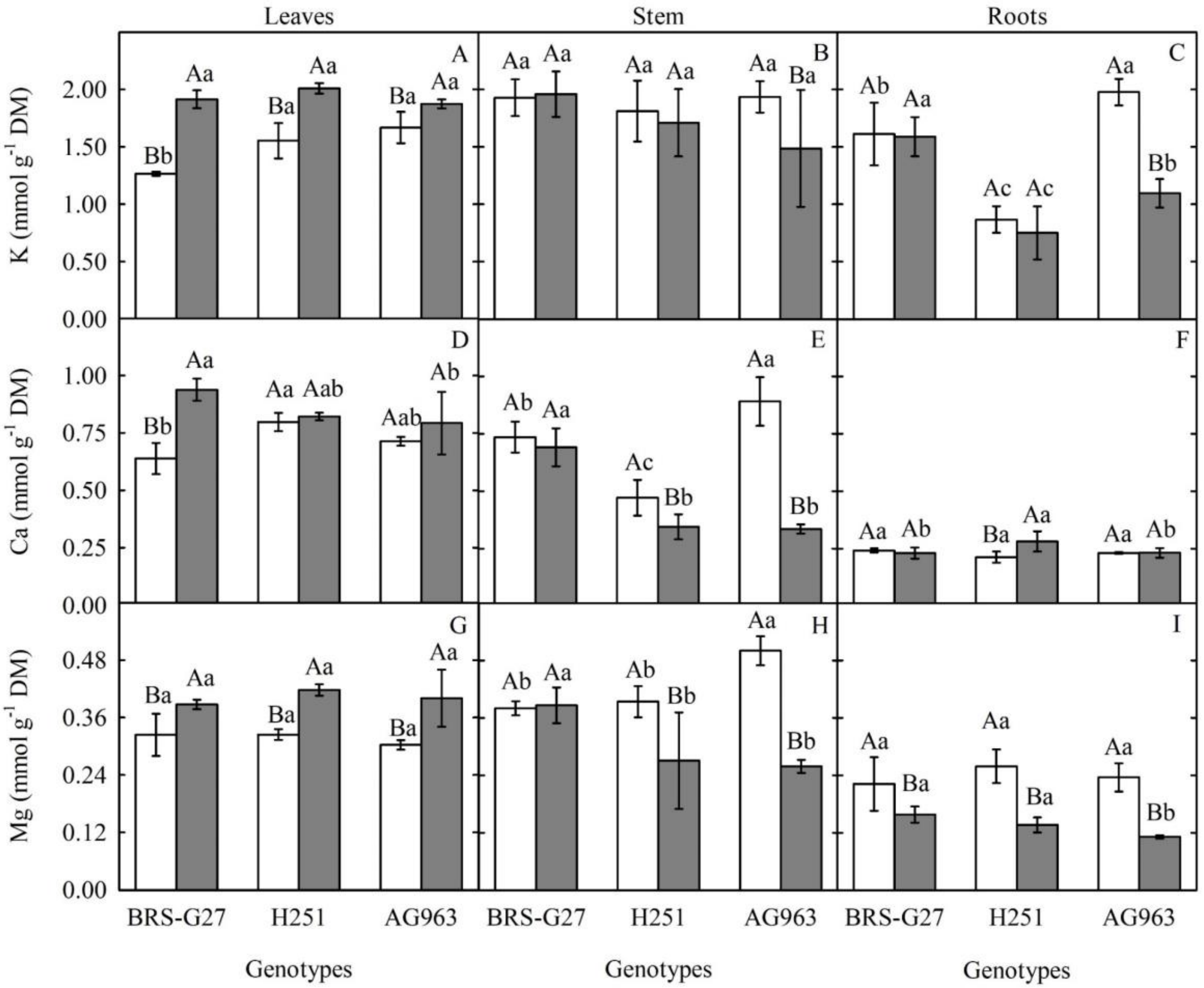

Figure 4. Potassium (K), calcium (Ca), and magnesium (Mg) contents in leaves (A, D, G), stem (B, E, H), and roots (C, F, I) of sunflower genotypes after 10 days of cultivation in a greenhouse with nutrient solution (□) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey's test at p ≤ 0.05. Vertical bars represent the standard deviations (n=4).

The Ca concentrations in leaves of BRS-G27 increased by 47% in the presence of Pb (Figure 4D). In the stem (Figure 4E), Pb stress decreased Ca levels in H251 (27%), and AG963 (62%) and, in the roots, the Ca content increased 32% only in the H251 genotype (Figure 4F). Lead stress increased the Mg levels in leaves of BRS-G27 (20%), H251 (29%) and AG963 (32%) genotypes. In the stem, Mg content decreased by 31 and 48% in H251 and AG963, respectively (Figure 4H). In the roots (Figure 4I), the Pb treatment decreased Mg concentration in all genotypes, but this reduction was less pronounced in BRS-G27 (29%) than in H251 (47%) and AG963 (53%).

The effects of Pb on the contents of micronutrients are presented in Figure 5. In leaves and stem of BRS-G27 genotype, Pb increased the contents of Mn (74 and 46%) (Figures 5D and 5E) and Fe (64 and 47%) (Figures 5G and 5H) and decreased those of Zn (23 and 32%) (Figures 5J and 5K). In roots of BRS-G27, Pb stress decreased by 29% the Mn content (Figure 5F) and increased by 30% the Zn content (Figure 5L).

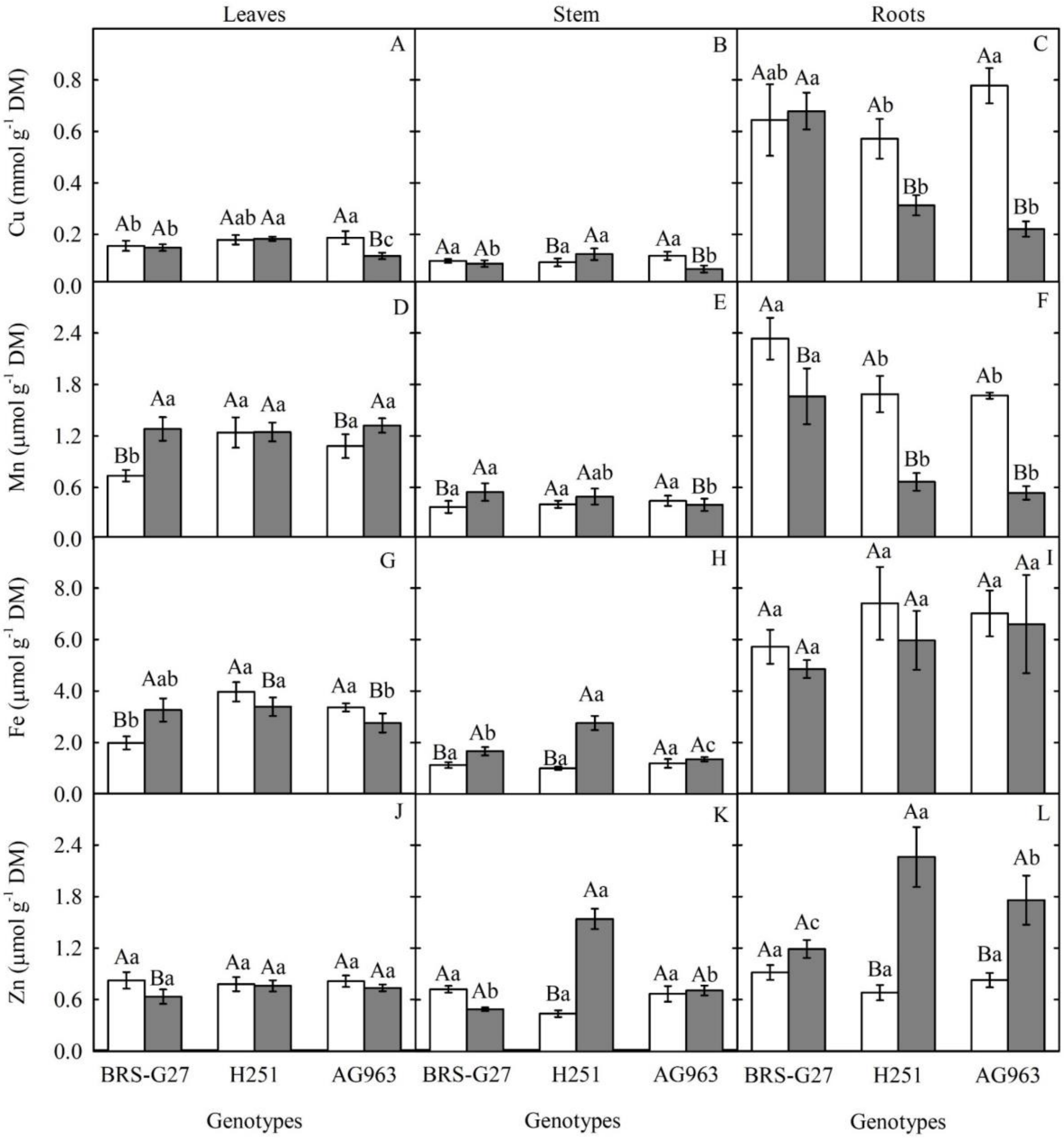

Figure 5. Copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) contents in leaves (A, D, G, J), stem (B, E, H, K), and roots (C, F, I, L) of sunflower genotypes after 10 days of cultivation in a greenhouse with nutrient solution (☐) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey test at p ≤ 0.05. Vertical bars represent the standard deviations (n=4).

In the leaves, Pb decreased the concentrations of Cu (38%) and Fe (18%) in the AG963 genotype (Figures 5A and 5G), and Fe in the H251 genotype (14%) (Figure 5G). In stem, there were increases in Cu (35%), Fe (176%), and Zn (121%) for H251 (Figures 5B, 5H, and 5K) and a decrease of 44% in Cu contents in AG963 (Figure 5B). In the roots of H251 and AG963, strong reductions in Cu (45 and 72%) and Mn (61 and 68%) and increases in Zn contents (232 and 113%) were observed, respectively. Lead can cause nutritional deficiencies or alterations in ion distribution in the plant, which may be caused by competition for transport systems across the plasma membrane or competition for binding sites on the cell (Malar et al., 2014).

K has important functions in plants, acting in the osmotic adjustment, xylem-phloem water content, activation of several enzymes, protein synthesis, photosynthesis, nutrient and metabolite transport, and responses to abiotic and biotic stress (Sardans & Peñuelas, 2021). Małkowski et al. (2005) observed a higher Ca uptake with increased accumulation of Pb at the tips of corn roots. On the other hand, Dalyan et al. (2020) reported that divalent cations like Pb can compete with other cations of the same charge, such as Ca, Mg, Zn, Mn and Fe, induce disturbances in absorption and transport, and inhibit the activity of some enzymes, which may result in an increase in reactive oxygen species.

Considering that Pb stress increased the K, Ca, and Mg contents in the leaves of genotypes, the data of this study does not indicate Pb-induced disturbances in the transport of these nutrients from the roots to the leaves of sunflower. Also, no differences were found in K, Ca and Mg contents of the studied genotypes that could be related to the Pb-tolerance or Pb-sensitivity of sunflower.

The differences between the micronutrient concentrations in leaves and stem of the stressed plants were not sufficient to explain the Pb-induced growth reductions in the genotypes studied. However, the concentrations of Cu and Mn in stressed roots of Pb-sensitive genotypes were much lower (61 and 64%, respectively) than that observed in the Pb-tolerant, which may have been the result of competitive inhibition caused by Pb (Sharma & Dubey, 2005).

Copper is a constituent of different plant proteins. It is involved in cell wall formation, oxidation and electron transport reactions, and root development (Fageria, 2009). Thus, Cu deficiency rapidly decreases the activity of several enzymes, and in most cases, such decreases are correlated with metabolic changes and plant growth inhibition (Marschner, 2012).

Like Cu, Mn is involved in several biochemical functions, acting as a cofactor of about 35 different enzymes, such as dehydrogenases, transferases, hydrolases, and decarboxylases involved in respiration, synthesis of amino acids and lignin, and concentrations of phytohormones, and its deficiency inhibits root growth (Marschner, 2012). Roots play a key role in controlling plant growth and development due to their importance in water and nutrient uptake. Roots also synthesize cytokinins, hormones responsible for cell division in all plant organs (Vissenberg et al., 2020). Thus, the data from this study suggests that the decrease in concentrations of Cu and Mn in the root may explain, at least in part, the greater sensitivity of the H251 and AG963 genotypes to Pb stress, and that these nutrients can be used as nutritional indicators for Pb tolerance in sunflower.

The variations in soluble carbohydrates, free amino acids, soluble proteins, and free proline in leaves and roots are presented in Figure 6. Lead stress decreased the soluble carbohydrates content in leaves of all genotypes (Figure 6A). In the root system, the carbohydrates decreased 35% in BRS-G27 and increased 42% in H251 and 68% in AG963 (Figure 6B). Free amino acids contents in leaves and roots of BRS-G27 genotype were not affected by Pb stress. On the other hand, Pb stress decreased the amino acids in leaves of genotypes H251 and AG963 by 35%, (Figure 6C) and increased in roots (76% and 134%, respectively) (Figure 6D). Lead decreased the content of soluble proteins in leaves of BRS-G27 (22%) and in roots of H251 (29%) and AG963 (36%) (Figures 6E and 6F). It can also be observed in Figures 5D and 5F that the increase in free amino acid concentration in roots of H251 and AG963 genotypes occurred simultaneously with the reduction of soluble proteins.

It has been shown that exposure to Pb reduces water uptake and transport in the roots, affecting the relative water content in plant tissue, thereby leading to the need to maintain cell turgor (Rucińska-Sobkowiak et al., 2013). Soluble carbohydrates and free amino acids are considered the main organic compounds involved in osmotic adjustment (Silva et al., 2020). As the increase in these compounds was observed only in the roots of Pb-sensitive genotypes, the results suggest that the energy cost for osmoregulation through the synthesis of the organic compounds was higher in the H251 and AG963 genotypes than in the BRS-G27. Changes in the concentrations of carbohydrates and amino acids also evidence Pb-induced disturbances in cell metabolism (Abreu et al., 2016).

The increase of free amino acids concentrations associated with reduction of soluble proteins, suggests that Pb-stress induced proteolysis in roots of Pb-sensitive genotypes. It is also well established that plant exposure to toxic metals triggers a range of physiological and metabolic changes (Hossain et al., 2012) as well as the formation of phytochelatins and metallothioneins, peptides rich in thiol groups that are synthesized from mechanisms that aim to restrict the excess of toxic metals (Hossain et al., 2012). Considering that the root is the first organ directly exposed to Pb and that the soluble proteins content in the root decreased only in H251 and AG963 genotypes, the results indicate that the Pb-sensitivity of these genotypes may, at least in part, be related to a smaller complexation of Pb by specific peptides.

Proline can be a defense system component, acting as a chelating agent and alleviating metal toxicity, stabilizing proteins (Lamhamdi et al., 2013), inhibiting lipidic peroxidation, and removing reactive oxygen species (White & Pongrac, 2017). In addition, proline and other amino acids are among the major compounds involved in protecting cell membranes from damage caused by stress (Silva et al., 2020).

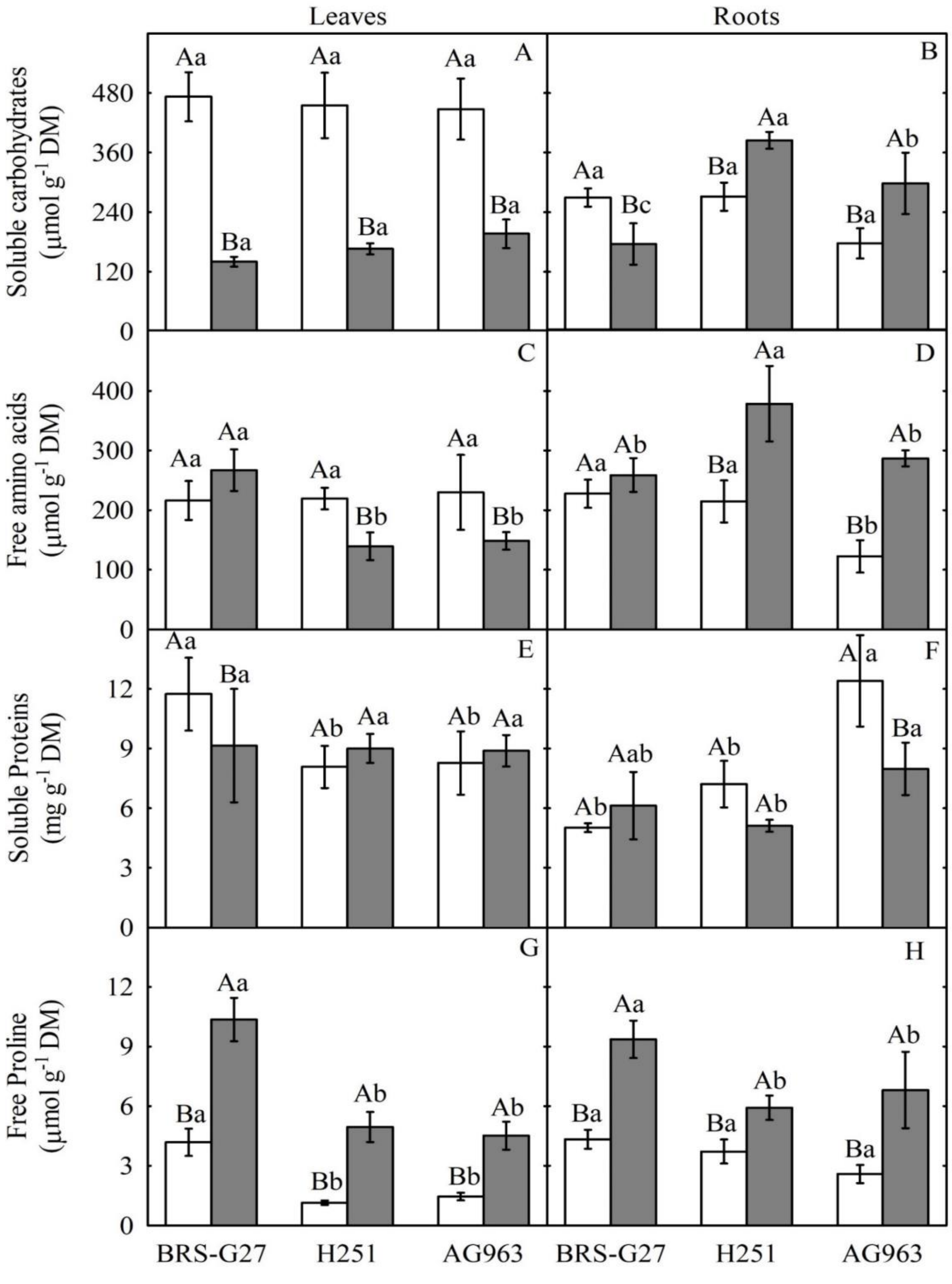

Figure 6. Soluble carbohydrates, free amino acids, soluble proteins, and proline free contents in leaves (A, C, E, G) and roots (B, D, F, H) of genotypes after 10 days of cultivation in greenhouse with nutrient solution (☐) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey test at p ≤ 0.05. Vertical bars represent the standard deviations (n=4).

According to Verbruggen and Hermans (2008), the proline content varies according to the species and stressed plants may have values 100-fold higher than those of the control plants. This accumulation has been correlated with stress tolerance and the concentration of this amino acid is generally higher in tolerant plants than in sensitive ones (Ashraf & Foolad, 2007). Thus, the results of this study suggest that the high proline content observed in BRS-G27 may be related to the greater Pb-tolerance of this genotype and that this amino acid may be a biochemical marker for Pb-tolerance in sunflower. Similar results regarding the increase of Pb contents (Figure 7) were reported by Abreu et al. (2016) in which the Pb contents increased significantly in all organs evaluated. Lamhamdi et al. (2013) also report an increase in proline content in spinach and wheat leaves with increasing doses of Pb up to 15 mM.

Lead increased proline content in leaves and roots of all genotypes studied (Figures 6G and 6H), but the values observed in the BRS-G27 genotype were higher than those in H251 and AG963. Thus, proline content in stressed leaves of BRS-G27 was 109 and 129% and in the roots was 58 and 37% higher than in H251 and AG963, respectively. The presence of Pb in the nutrient solution increased the content of this element in leaves, stem, and roots of the plants (Figure 7). Lead levels in H251 and AG963 were 132 and 375% higher than in BRS-G27 in leaves (Figure 7A), 125 and 62% in stem (Figure 7B), and 165 and 46% in roots (Figure 7C).

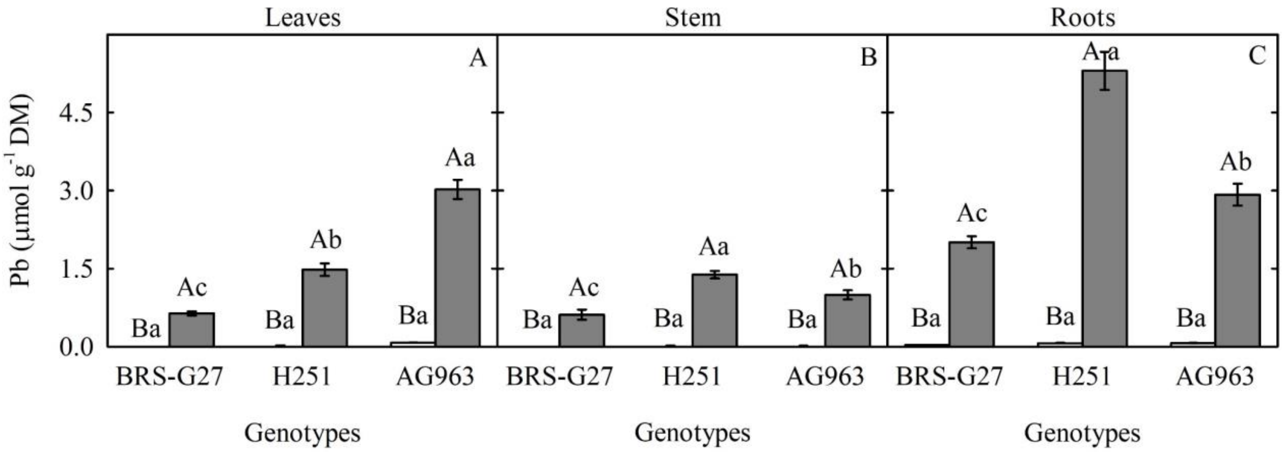

Figure 7. Lead (Pb) contents in leaves (A), stem (B), and roots (C) of sunflower genotypes after 10 days of cultivation in a greenhouse with nutrient solution (□) or nutrient solution containing 0.65 mM of Pb (■). Means followed by the same uppercase letters for the same genotype and lowercase letters for the same Pb level do not differ by Tukey test at $p \leq 0.05$. Vertical bars represent the standard deviations (n=4).

Lead uptake takes place passively through the root hair, where it is usually retained in the cell walls, so that its transport from the roots to the shoot is limited (Kabata-Pendias, 2010). Plants can exhibit different tolerance mechanisms in response to excess of toxic metals, including exclusion, reduction of membrane transport, adsorption, chelation, translocation, and detoxification (Revathi & Venugopal, 2013; Kumar et al., 2016). Considering that the Pb-tolerant genotype (BRS-G27) showed the lowest Pb contents in all plant parts, the data suggests that the exclusion and/or decrease in the transport across the membrane are the prevalent mechanisms in Pb-tolerance in sunflower.

When comparing plant parts of the three genotypes, the highest Pb accumulation occurred in the roots, followed by the leaves and stems. According to Dalyan et al. (2020), the accumulation of Pb in plants occurs mainly in the roots, causing a reduction in cell division and elongation. Studies have shown that, at root cell wall level, Pb can adhere especially in the form of pyrophosphate (Marschner, 2012) or undergo extracellular precipitation mainly in the form of carbonate (Almeida et al., 2007). Additionally, Pb retention in the root can also be attributed to its binding to negative charges on the cell wall (Liu et al., 2009). Regardless of the retention mechanism, the role of roots in Pb storage and, consequently, in the plant tolerance to this metal is indisputable (Azad et al., 2011). Like the results of Pb concentration, the TC and TF were higher in genotypes AG963 (3.12 and 9.68-fold) and H251 (1.84 and 2.89-fold) than in the BRS-G27 genotype, respectively (Figures 8A and 8B). In contrast, the TI of the BRS-G27 genotype was, on average, 5.67-fold higher than in the other genotypes (Figure 8C).

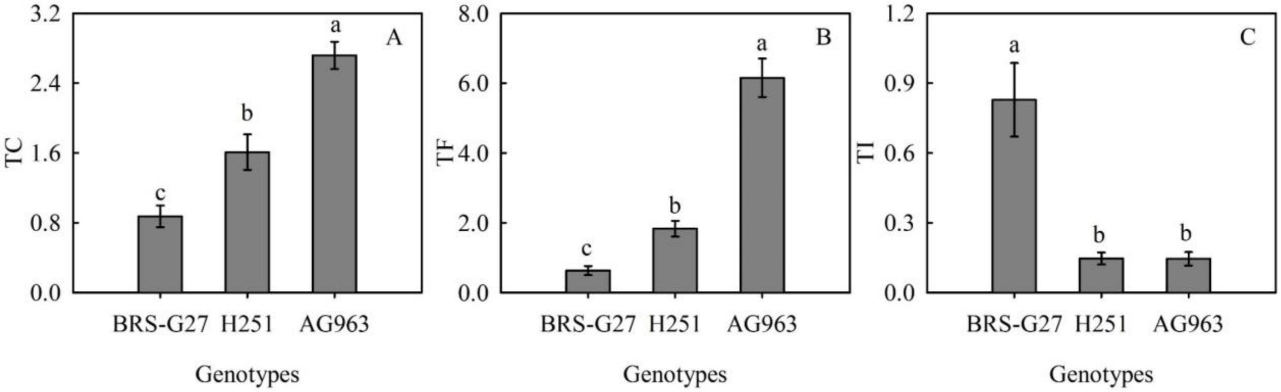

Figure 8. Transfer coefficient - TC (A), translocation factor - TF (B), and tolerance index - TI (C) of genotypes after 10 days of cultivation in a greenhouse with nutrient solution containing 0.65 mM of Pb (■). Means followed by the same letters do not differ by Tukey test at $p \leq 0.05$.

The TC represents the capacity of a plant species to absorb a given concentration of metal from the growing substrate (Bassegio et al., 2020) and can be used as a decisive factor for categorizing plant species for phytoremediation (Antoniadis et al., 2017). On the other hand, TF has been used to estimate metal translocation from the roots to the shoots. Both indicators (TC and TF) have proven to be suitable tools for identifying the ability of the growing plants to absorb metal ions (Alaboudi et al., 2018). In our study, TC values > 1.0 observed in AG963 and H251 indicate the potential ability of these genotypes for Pb accumulation. However, it is important to note that, in quantitative terms, the Pb accumulation in the shoot was more significant in the BRS-G27, mainly due to a greater amount of LDM and SDM of this genotype. Corroborating these results, TI values close to zero (observed in the AG963 and H251 genotypes) indicate plant sensitivity to Pb contamination (TI becomes zero when plants wither), while TI values close to 1.0 indicate greater tolerance to stress (Antoniadis et al., 2017).

Conclusion

Genotypes BRS322, AG963, H360, H251, and IAC-Iarama were characterized as Pb-sensitive, especially AG963 and H251, while BRS-G27, AG975, and H863 were characterized as Pb-tolerant, especially BRS-G27. The exclusion and/or reduction of transport across the membrane are the predominant mechanisms in Pb tolerance in sunflowers. At the whole-plant level, Pb stress induces nutritional changes in macro- and micronutrient contents; however, only the decrease in root concentrations of Cu and Mn may explain, at least in part, the greater sensitivity of the genotypes to Pb stress. The content of organic solutes in roots indicates that Pb-sensitive genotypes have higher energy costs for osmoregulation through the synthesis of carbohydrates and amino acids, and a lower Pb complexation by specific peptides. On the other hand, the high proline content in leaves and roots of the BRS-G27 genotype may be related to greater tolerance to Pb stress.

Considering the results of dry mass yield, transfer coefficient, translocation factor, and tolerance index, sunflower genotype BRS-G27 can be recommended for use in phytoremediation of Pb-contaminated soils.

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