International Journal of Mass Spectrometry 307 (2011) 55–60

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Evaluation of sunflower metabolism from zinc and selenium addition to the culture: A comparative metallomic study

Marcelo Anselmo Oseas da Silva[a], Sara Adrián López de Andrade[b], Paulo Mazzafera[b], Marco Aurélio Zezzi Arruda[a,*]

[a] Spectrometry, Sample Preparation and Mechanization Group (GEPAM) and National Institute of Science and Technology for Bioanalytics – Institute of Chemistry, University of Campinas – Unicamp, PO Box 6154, 13083-970, Campinas, SP, Brazil
[b] Institute of Biology, University of Campinas – Unicamp, PO Box 6109, 13083-970, Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 24 August 2010
Received in revised form
22 September 2010
Accepted 18 October 2010
Available online 23 October 2010

Keywords:
ICP-MS
Plant metabolism
Isobaric interference
Selenocysteine
Metal translocation

ABSTRACT

This work reports the evaluation of sunflower growth under different irrigation conditions and explores a metallomic approach for evaluation of metal and non-metal content in different plant tissues. Sunflowers were cultivated in the presence of zinc (acetate salt) and selenium (as sodium selenite) by adding *ca.* 230 or 430, and *ca.* 190 or 350 mg, respectively, during the cultivation period. These plants were compared with controls in terms of some monitored ions ($^{24}Mg^+$, $^{31}P^{16}O^+$, $^{32}S^{16}O^+$, $^{56}Fe^+$, $^{64}Zn^+$, $^{80}Se^+$) using ICP-MS. The results highlighted no apparent problem during the development period for those plants cultivated in the presence of zinc. However, higher selenium levels are present mainly in leaves, which can be due to its incorporation in synthesized amino acids such as selenocysteine and selenomethionine.

1. Introduction

The increase on the productiveness of a given culture has been searching not only through simple strategies such as artificial pollination, grafting or cutting, but also through more complex ones such as genetic modification [1]. Such strategies need an accurate description of mechanisms occurring at the molecular level inside a plant to understand the phenomena observed at the physiological level. This comprehension makes establishing the best condition for the development of a culture possible [2]. Target parameters (i.e., metal concentrations, light, temperature, etc.) have been used for comparing plants cultivated under the influence of these parameters against control plants [3–5].

Research involving metals and their interactions with cells or biomolecules is presented in a new area of "metallomics", which is shared between ionomics and metalloproteomics [6]. Interdisciplinary approaches are currently necessary for acting in this new field, creating a perfect atmosphere of integrated work [7]. As different expertise and techniques are necessary for carrying out a given study that clarifies different plant mechanisms, metallomics is available for the task. In this way, mass spectrometry is currently the key for elucidating organic structures, biochemical pathways, mechanisms, metals distribution, among others [8]. For metals, ICP-MS is an effective technique for metallomics studies [9], and examples can be found in the literature [10–12]. However, extracting accurate information about metals from a system, mainly for those complex ones involving plants, is not easy, owing to matrix interferences found (i.e., isobaric, polyatomic, matrix, etc.). In this work the influence of selenium and zinc in sunflower plants is systematically evaluated through metallomic approaches. Plants were grown at different irrigation conditions employing solutions containing different concentrations of selenium and zinc. The selenium correlation with phosphorous, magnesium, iron and sulfur present in roots, steams and leaves of the sunflower plants, as well as zinc concentrations were obtained after their quantification by ICP-MS. Strategies used for improving ICP-MS measurement selectivity for plant matrices are also described.

2. Experimental

2.1. Reagents and solutions

The irrigation of the plants was carried out using solutions prepared with analytical grade reagents (Sigma-Aldrich, Steinheim, Germany). All standard stock solutions, at 1000 mg L^{-1}, employed for ICP-MS determinations as well as reagents used in

* Corresponding author. Tel.: +55 19 3521 3089; fax: +55 19 3521 3023.
E-mail address: zezzi@iqm.unicamp.br (M.A.Z. Arruda).

1387-3806/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2010.10.023

the decompositions of vegetal materials were obtained from Merck (Darmstadt, Germany). All solutions were prepared with deionized water (≥18.2 MΩ cm) obtained through a Milli-Q water purification system (Millipore, Bedford, USA). Sub-boiling nitric acid was employed for both digestion procedures and standards/samples dilutions.

2.2. Sunflower cultivation

The sunflower seeds employed in this study (*Helianthus annuus* L.), obtained after plant breeding (variety IAC Iarama), were from the Campinas Agronomical Institute. When compared with plants from other varieties, some economical advantages such as fast growth (45–55 days for flowering) and high oil levels obtained from the seeds can be pointed out.

The soil used was autoclaved-sterilized at 120 °C for 1 h, just before planting to eliminate native arbuscular mycorrhiza fungi (AMF), which is mutualistic associated with the roots of the plant, enhancing its resistance to metal stress [13].

Sunflower seeds were germinated and grown in plastic pots (1 L capacity) containing *ca.* 400 g of soil. Once AMF was eliminated and its presence is also important to plant mineral nutrition [14], 300 mg of potassium hydrogen phosphate was added to each pot and homogenized with the soil to increase the availability of phosphate to the plant. Magnesium carbonate hydroxide pentahydrate was also added to the soil to ensure pH values greater than 5.5.

Before planting, seeds were surface-sterilized using a 1.0% sodium hypochlorite solution. Three seeds were sown per pot and after emergence (7 days after planting), the seedlings were thinned to one plant per pot. The plants were allowed to grow in a greenhouse for 44 days, and then harvested at the beginning of the flowering stage.

Five different irrigation conditions were established, as shown in Table 1, based on the amount of SeO_3^{2-} (as sodium selenite) or Zn^{2+} (as acetate) supplied weekly to the plants via irrigation procedure. Plants of the control group were irrigated with only deionized water. Zinc irrigation solutions were prepared using acetate as the counterion to avoid addition of nutrients, such as nitrate, that could stimulate the development of the plants. After harvest, roots, stems and leaves were immediately washed using deionized water.

Table 1
Elemental concentrations added to each group of sunflowers studied.

Treatment	SeO_3^{2-} or Zn^{2+} dose added per week (mg)	SeO_3^{2-} or Zn^{2+} added at the end of cultivation period (mg)
Control plants	–	–
Lower dose of zinc (Zn-lower)	46.6	233.2
Higher dose of zinc (Zn-higher)	86.6	433.2
Lower dose of selenium (Se-lower)	37.9	189.5
Higher dose of selenium (Se-higher)	70.4	352.0

2.3. Elemental determinations

Elemental determinations were performed in sunflower plant roots, shoots and seeds. The vegetal materials were ground manually in a mortar in the presence of liquid nitrogen, and dried to constant mass in an oven at 60 °C for 72 h. For the microwave-assisted sample decomposition (*ca.* 150 mg of vegetal material), a mixture composed by 6.0 mL of sub boiling concentrated nitric acid and 0.5 mL of hydrogen peroxide was employed. A microwave oven (DGT, Provecto Analítica, Jundiaí, Brazil) with a nominal power of 1200 W was used to perform the procedure [3], comprised of four steps: 5 min at 400 W; 8 min at 790 W; 4 min at 320 W; 3 min at 0 W. Then, the samples were gently heated to evaporate the excess of nitric acid, and the volumes were adjusted to 10 mL using 1.0% (v/v) nitric acid. Elemental determinations were performed with an ICP-MS (Elan DRC-e, PerkinElmer, Norwalk, CT, USA) using the operational conditions shown in Table 2. Depending on the analyte, different strategies for interferences removal were adopted, including two different reaction gases: oxygen and methane, and/or correction equations.

Table 2
ELAN DRC ICP-MS operating conditions.

ICP-MS operational parameters	
Spray chamber	Cyclonic
Nebulizer	Meinhard®
RF power (W)	1200
Nebulizer Ar flow (L min^{-1})	0.90–0.92 (optimized daily)
Auxiliary Ar flow (L min^{-1})	1.2

Measures	
Scan mode	Peak hoping
Dwell time (ns)	60
Sweeps/readings	20
Integration time (ms)	1000
Replicates	3
Monitored isotopes/species	$^{24}Mg^+$, $^{31}P^{16}O^+$, $^{32}S^{16}O^+$, $^{56}Fe^+$, $^{64}Zn^+$, $^{80}Se^+$

Dynamic reaction cell operational conditions

Element	Determined isotope/specie	Reaction gas	Gas flow (L min^{-1})	RPq (V)	RPa (V)
Selenium	$^{80}Se^+$	Oxygen	1.20	0.65	0
Sulfur	$^{32}S^{16}O^+$	Oxygen	0.75	0.40	0
Phosphorous	$^{31}P^{16}O^+$	Oxygen	0.75	0.40	0.01
Iron	$^{56}Fe^+$	Methane	1.20	0.75	0

Correction equation

$^{64}Zn^+$	$-0.035297 \times {}^{60}Ni^+$

3. Results and discussion

3.1. Analytical strategies adopted in the elemental determinations

The use of dynamic reaction cells (DRC) or collision cells consists of a general strategy adopted for removal of interferences when elemental determinations are carried out using a unit-resolution ICP-MS instrument. In this work, the application of DRC consisted of an alternative to promote adequate removal of several ionic species, since typical digests from biological materials may contain undesirable amounts of C and N.

Considering phosphorous determinations, the application of oxygen at 0.75 L min^{-1} flow rate results in the formation of PO^+, which can be measured at m/z 47 free of potential interferences, such as $^{47}Ti^+$ (which is not present in the sample) or NO_2H^+ (which formation is not thermodynamically favorable in DRC conditions) [15,16]. The employed conditions were adequate to work in a linear range between 10 and 250 mg L^{-1}. Similar to phosphorous, oxygen was also employed as reaction gas in the dynamic reaction cell at 0.75 L min^{-1} for sulfur determinations, allowing monitoring the formation of SO^+ at m/z 48, avoiding potential interferences such as O_3^+.

For selenium determinations, the application of oxygen at 1.2 L min^{-1} allowed blank signals close to the instrumental background owing to the efficient elimination of Ar_2^+ via a charge transfer reaction [17]. Additionally, the application of a narrow bandpass (RPq set at 0.65 V) minimizes the transmission or formation not only of undesired ions [18] but also of oxygen reaction products formed inside the reaction cell minimizing losses of $^{80}Se^+$.

For iron quantification, the strategy adopted consisted in applying a narrow bandpass (RPq set at 0.75 V) to contribute for the complete elimination of interfering polyatomic species and allow reproducible determinations. In addition, a high methane flow rate at 1.2 L min^{-1} was employed for reaction with polyatomic interfering compounds at m/z 56 that include $^{40}Ar^{16}O^+$ and $^{40}Ca^{16}O^+$. Methane is currently used as reaction gas for circumventing ICP-MS interferences, and in our laboratory showed better sensitivity and reproducibility for detection of $^{56}Fe^+$, being an alternative for equipments that do not use ammonia as reaction gas.

For zinc determinations, the ICP standard mode was employed, once that a semiquantitative determination of $^{60}Ni^+$ in roots, stems and leaves was carried out, reveling that samples contained small amounts (475 ng kg^{-1} as maximum value) of nickel. As the potential interfering specie to $^{64}Zn^+$ is $^{64}Ni^+$, which has a natural abundance of *ca.* 0.9%, then its level in the sample is lower than 5 ng kg^{-1}. In this way, the application of sample dilution and a correction equation was enough to eliminate these interferences adequately. The employed equation, shown in Table 2, follows the recommendations of the ICP-MS manufacturer.

The standard mode was also employed for magnesium determinations. In this case, the potential interfering specie $^{12}C_2^+$ was not present due to the low content of carbon after sample decomposition.

3.2. Plants development

After the cultivation period, the aerial parts of plants were measured just before the harvest procedure to identify possible differences in their development resulting from the addition of the elements. Up to now, little information is known about zinc and selenium phytotoxical levels for *Helianthus annuus*. Concentrations above 300 mg kg^{-1} of zinc can cause problems related to its hyper-accumulation in the plant, depending on weather or soil composition [3]. Sunflower is classified as a Se-indicator plant owing to the relative tolerance of such plants for growing in contaminated soils (containing up to 1 mg of Se per gram of dry matter) [19].

Table 3
Sunflower average heights (n = 7) obtained at the end of the cultivation period for each group of plant.

Irrigation condition	Average height (cm)
Control	81 ± 12
Lower dose of zinc (Zn-lower)	83 ± 7
Higher dose of zinc (Zn-higher)	84 ± 9
Lower dose of selenium (Se-lower)	75 ± 8
Higher dose of selenium (Se-higher)	59 ± 15

From data presented in Table 3 plants from irrigation of both Zn concentrations conditions presented similar growth when they are compared to the sunflowers of the control group. Thus, problems resulting from the addition of zinc were not evident. Moreover, a reduction in the average height of the Se-treated sunflowers (Table 3) indicates that the applied selenium dose drastically inhibited the growth of both groups of irrigated plants. The negative effects observed were not restricted to only the reduction on their heights, but also for problems related to necrosis in their leaves and at the bases of stems, as well as foliar chlorosis were observed.

3.3. Selenium absorption and translocation

The total concentrations of selenium and phosphorus were determined in sunflowers for each studied irrigation procedure to characterize the uptake mechanism of selenite ions by the plants. Although the literature reports a well-established mechanism for selenate uptake, in which ions are taken up by the plants via sulphate transporters located at their roots, few information is available about the mechanism involved when selenite ion is the predominant chemical specie in the soil. The literature suggests that selenite uptake can occur by passive diffusion or mediated by phosphate membrane transporters [20].

The concentrations of selenium and phosphorous in the leaves, stems and roots of the plants are shown in Fig. 1. The results indicate that selenite uptake process occurs effectively when these ions are present at high concentration in the soil. The selenium level in the roots of Se-higher group of plants was *ca.* 95 times higher than to those plants from other treatments. Additionally, data obtained for phosphorous concentrations do not provide apparent evidence for the participation of phosphate transporters on selenite uptake. The phosphorous concentration was kept at the same levels for all treatments, indicating that selenite uptake occurs by passive diffusion mainly as a result of the contamination caused by the addition of high contents of selenite ions. However, the data showed do not allow obtaining a conclusive answer about this question, because Se-treated plants may have been efficient enough to promote selenium absorption without hampering phosphorous uptake.

The translocation of selenium was efficiently carried out for both groups of selenium treated plants. A trend related to the selenium accumulation in leaves was observed, which was different from other species such as soybean (*Glycine max*), where the highest selenium concentration was found in roots, resulting from the direct contact of the plant with the soil containing high levels of selenite ions [21]. The tendency observed in sunflowers consists of an alternative found by the plant to minimize the negative effects caused by the high amounts of selenite ions in the soil. In this case, the plant transports the ions to their upper parts, and metabolizes volatile selenium species such as dimethylselenide or dimethyldiselenide, eliminating these compounds through foliar transpiration [22].

Such strategies consist in the main characteristic for differentiating Se-accumulator from the non-accumulator plants. Organisms that present a relative tolerance to selenium, like *Hellianthus annuus* exhibit the capacity to avoid their incorporation into pri-

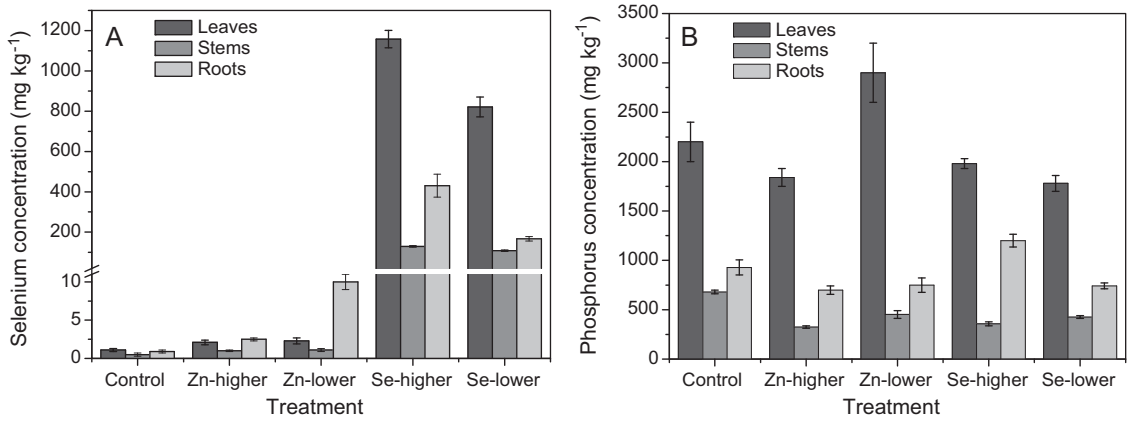

Fig. 1. Selenium (A) and phosphorus (B) concentrations in dry biomass (average ± standard deviation, $n = 3$) for leaves, steams and roots of sunflowers of the five different proposed treatments. Zn- or Se-higher and Zn- or Se-lower indicates plants treated with higher and lower doses of the zinc and selenium, respectively.

mary sequences of proteins [23], while those non-accumulators, such as *Glycine max* present no mechanisms to reduce the negative effects cause by the harmful ions.

3.4. Influence of selenium on the absorption of essential nutrients for the photosynthesis

One of the problems identified in the Se-treated plants during the cultivation period was the appearance of chlorosis in most of their leaves. When evaluated from a metallomic study point of view, it is plausible to suppose that selenium is involved in the inhibition of iron and magnesium uptake, leading to the inability of plants to start synthesis of pheophytin (the chlorophyll molecule lacking the central magnesium ion) and chlorophyll formation [24]. Absorption of high levels of selenium by the plants may cause reduction in the concentration of some metals in tissues, including iron [25]. Thus, iron and magnesium levels were evaluated in roots and shoots for the five different proposed irrigation procedures.

The results obtained for the concentrations of iron and magnesium in each part of the plants are shown in Fig. 2. As observed, iron is present in roots higher than in the aerial parts of the plants. These results are expected once that iron solubility is increased close to the roots owing to the exudation of the phenolic compounds, which increase the solubility of the metal and facilitate the absorption of the nutrient.

Moreover, no significant differences are found in the iron levels when Se-treated plants are compared with the plants from other irrigation procedures. In this case, the average Fe concentration in control plants and Zn-treated group is *ca.* $107 \pm 13\ mg\ kg^{-1}$, while the Fe concentration in Se-treated plants is $126 \pm 3\ mg\ kg^{-1}$. Thus, selenium contamination does not lead to problems related to iron uptake or pheophytin synthesis inhibition caused by the absence of iron atoms.

Magnesium concentrations in roots and shoots are maintained at the same level for all evaluated conditions (Fig. 2). Thus, magnesium ions are available to bind to pheophytin, forming the chlorophyll molecule. In this way, chlorosis in the Se-treated plants was not related to a disturbance in the uptake and distribution of mineral nutrients in the plant.

3.5. Selenium interference in sulfur metabolism

The sulfur concentration in different parts of the plants submitted to the irrigation treatments was evaluated to investigate possible changes in its distribution. Although sulfur concentration in roots and stems presented minor changes for all the irrigation procedures, its concentration in leaves was *ca.* 2.5 times higher in the Se-treated plants (see Fig. 3), indicating that selenium addition resulted in the increase on sulfur uptake, and its translocation to the region of the plant containing higher selenium amounts.

Sulfur uptake could be amended in the Se-treated plants by two factors. The first is related to the preferential capture of selenium via sulphate transporters. However, this hypothesis must not be regarded, once selenite ions were employed in the irrigation procedures and its uptake is not dependent on sulphate transporters, as discussed earlier.

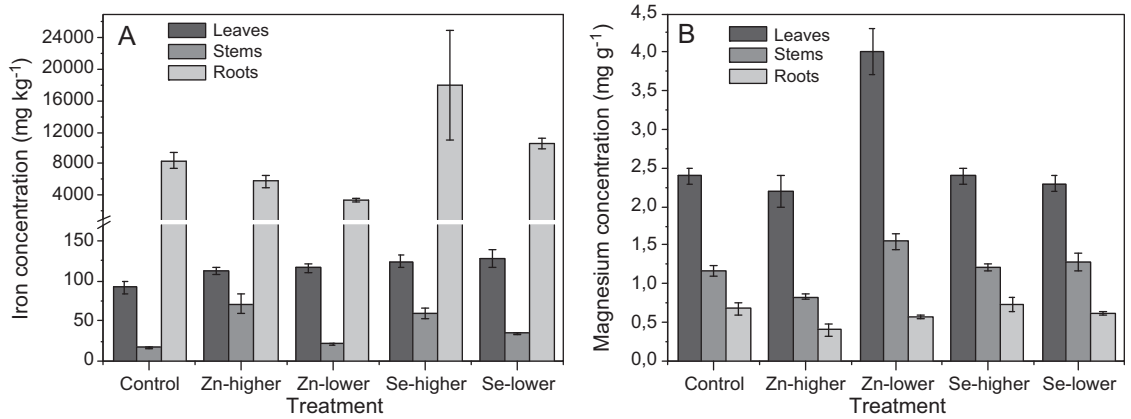

Fig. 2. Iron (A) and magnesium (B) concentrations in dry biomass (average ± standard deviation, $n = 3$) for leaves, steams and roots of sunflowers of the five different proposed treatments. Zn- or Se-higher and Zn- or Se-lower indicates plants treated with higher and lower doses of the zinc and selenium, respectively.

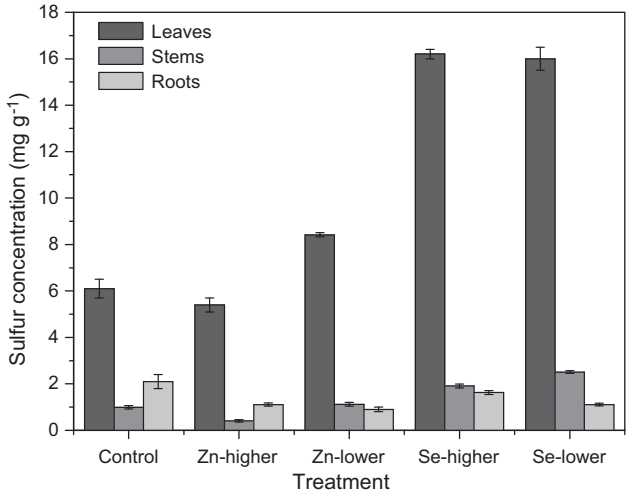

Fig. 3. Sulfur concentrations in dry biomass (average ± standard deviation, $n = 3$) for the different parts of the plants. Zn- or Se-higher and Zn- or Se-lower indicates plants treated with higher and lower doses of the zinc and selenium, respectively.

The second factor is related to the selenium interference on cysteine biosyntheses pathway caused by the similarities between the chemical proprieties of selenium and sulfur atoms [25]. Sulfur uptake is necessary owing to the biosynthesis of cysteine, glutathione and methionine (see Fig. 4) [26]. In the case of the Se-treated plants, selenium ions rather than sulfur are randomly employed for synthesis of these amino acids resulting from its higher concentration. Therefore, the excess of Se^{2-} activates the synthesis of selenocysteine as well as *o*-acetylserine (OAS), which consists in an intermediate in the biosynthesis of cysteine. When present at high concentrations OAS is an important molecule that up-regulates sulphate uptake at the gene level [25]. Thus, the obtained results provide evidence that the concentration of OAS is high enough to promote an increase in the sulfur uptake. The Se-treated plants tend to translocate the excess sulfur to regions of the plants where the selenium concentration is higher, i.e., the leaves.

The synthesis of Se-containing amino acids can rationalize the problems detected during cultivation of the Se-treated plants, owing to the possible incorporation of these synthesized molecules into the primary structure of proteins. The incorporation of selenium to proteins is the critical point that determines if an organism is tolerant or not to selenium. Generally, Se-tolerant organisms have the ability to transform Se-containing amino acids biologically, e.g., via methylation, avoiding their incorporation into proteins. Different Se-tolerant organisms contain selenocysteine derivates as major selenium constituents such as Se-methylselenocysteine in broccoli (*Brassica* family) [27] or γ-glutamyl-Se-methyl-selenocysteine present in garlic (*Allium* family) [28].

In this work, even with the translocation of selenium to plant leaves that promotes its elimination by foliar transpiration, the process is not efficient enough to avoid or at least minimize the unspecific incorporation of selenium to the cysteine pathway and, consequently, to the primary structure of proteins. Experiments regarding the Se incorporation to proteins are under development in our laboratory. This observation may also explain the problems related to the reduction in the chlorophyll synthesis, described in Section 3.4. The literature reports [29,30] that the presence of selenium can lead to a reduction on both protein synthesis and enzymes activities, especially if the Se-amino acids are located close to their active sites [31]. In this way, the synthesis of several molecules, including chlorophyll, may be affected.

3.6. Addition of zinc to the sunflower culture

The addition of zinc at two concentration levels to the plants resulted in changes in both zinc uptake and distribution when compared to control and Se-treated plants. According to Fig. 5, zinc concentrations in roots and stems from Zn-treated plants were *ca.* 7 and 13 times higher than to other irrigation conditions, demonstrating that the plants absorbed the added zinc.

The translocation of zinc to the leaves does not occur at high extension as already demonstrated for selenium. In this case, the concentration of this metal in Zn-treated plants was *ca.* 3 times higher when compared to the other irrigation conditions. However, the average concentration of zinc in the leaves of Zn-treated plants (around 100 mg kg^{-1}) cannot be considered ideal for the adequate sunflower development, since the literature reports a range of suitable zinc concentration between 29 and 43 mg kg^{-1} in sunflower leaves [32].

Additionally, the translocation of zinc from roots to shoots resulting from the binding capacity of fungal hyphae to the metals can be depressed [33]. However, even in the absence of AMF after sterilization of the soil, the translocation does not occur effectively, and the metal remains predominantly in the roots and stems of the sunflowers. The obtained metal distribution follows the behavior presented in a previous work [3] where sunflowers were cultivated without AMF removal.

Related to the stress caused by the addition of zinc, the literature reports the suppression of magnesium uptake in soils with high amounts of that metal. The similarity of the charges between both

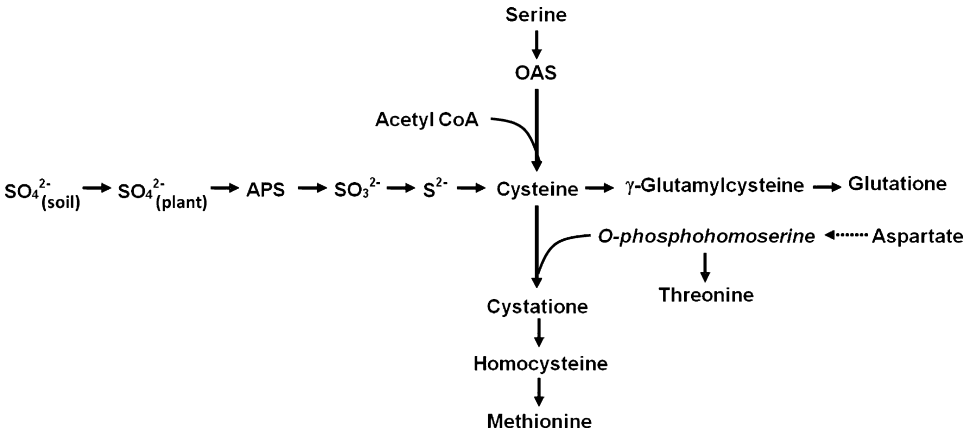

Fig. 4. Schematic diagram of the Cysteine biosynthesis pathway. OAS represents *o*-acetylserine and APS adenosine phosphosulphate. Enzymes involved in the reactions are not shown in the diagram. For more information see [26].

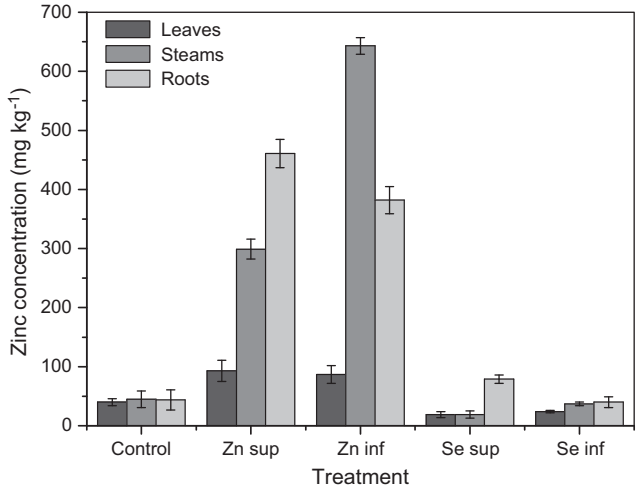

Fig. 5. Zinc concentrations in dry biomass (average ± standard deviation, $n = 3$) for leaves, steams and roots of sunflowers of the five different proposed treatments. Zn- or Se-higher and Zn- or Se-lower indicates plants treated with higher and lower doses of the zinc and selenium, respectively.

ions makes the absorption and translocation of magnesium to the aerial parts of the plants difficult, without apparent damage to their structures, as observed in leaves and fruits of tomato which grown in soil containing high amounts of zinc [34].

Thus, to obtain evidence of a possible stress caused by the addition of zinc, the magnesium concentration was evaluated in the plants. Comparing the data obtained for magnesium concentration in Zn-treated plants with those from control group, shown in Fig. 2, a reduction in the magnesium uptake as well as in the translocation of the metal to the aerial parts of the plant was not observed. Conversely, when the zinc concentration in leaves and roots is compared, an increase in the ratio C_{leaves}/C_{roots} from *ca.* 3.5 for the control plants to *ca.* 6.0 for the Zn-treated plants is observed, indicating that magnesium translocation was not impaired.

4. Conclusions

The application of an ICP-MS for elemental determinations in complex matrices, such as vegetal samples, requires adoption of specific strategies to promote adequate elimination of interfering species. The use of a collision/reaction cell pressurized with adequate gas proved to be efficient to perform the determinations of selenium and sulfur in complex matrices. The application of these strategies is fundamental in metallomics studies, allowing agility in the determinations, where the concentration of several elements in a matrix must be evaluated and, then, correlated with some biological function.

The proposed metallomic study contributed to characterization of some processes that occur at molecular level in sunflowers, which may be important to clarify some phenomena observed at physiological level, and to improve this culture.

The study showed that plants irrigated with selenium presented growth problems, as well as chlorosis and necrosis in leaves and stems. However, the presence of selenium did not impair the absorption and translocation of iron and magnesium ions, which are necessary for chlorophyll synthesis. These elements were available at the same levels as those found in the control and zinc treated plants. Additionally, there was no evident interference in phosphorous absorption caused by the excess of selenite in the soil.

Changes in sulfur uptake and in its distribution were observed in both groups of selenium treated plants. The up-regulation may be directly connected to the random synthesis of Se-containing amino-acids, such as selenocysteine and selenomethionine, activating the cysteine and methionine pathway. The next step in such direction is the development of analytical strategies for the measurement of selenoproteins, which is under investigation in our laboratory.

The study did not identify evidence of possible damages in the plants caused by the addition of zinc. The existence of mechanisms to avoid the translocation of metal to the leaves to minimize the negative effects caused by its excess can be inferred owing to the major concentration of zinc in roots and stems.

Acknowledgements

The authors thank the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil, grant numbers 2007/59184-2 and 2009/15449-8), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil).

References

[1] K.M. Davies, Mutat. Res.-Fund. Mol. M 622 (2007) 122.
[2] M.L. Guerinot, I. Baxter, D.E. Salt, From the ionome to the genome: identifying the gene networks that control the mineral content of plants, in: G. Coruzzi, R.A. Gutierrez, R. Gutiérrez (Eds.), Plant Systems Biology, John Wiley and Sons, Chichester, UK, 2009, pp. 298–303.
[3] J.S. Garcia, P.L. Gratão, R.A. Azevedo, M.A.Z. Arruda, J. Agric. Food Chem. 54 (2006) 8623.
[4] G. Oliveira, J. Peñuelas, Plant Ecol. 175 (2004) 179.
[5] J.A. Stavang, O. Junttila, R. Moe, J.E. Olsen, J. Exp. Bot. 58 (2007) 3061.
[6] S. Mounicou, J. Szpunar, R. Lobinski, Chem. Soc. Rev. 38 (2009) 1119.
[7] M.A.O. da Silva, A. Sussulini, M.A.Z. Arruda, Expert Rev. Proteomic 7 (2010) 387.
[8] M.A.Z. Arruda, R.A. Azevedo, Ann. Appl. Biol. 155 (2009) 301.
[9] R. Lobinski, J.S. Becker, H. Haraguchi, B. Sarkar, Pure Appl. Chem. 82 (2010) 493.
[10] A. Sussulini, H. Kratzin, O. Jahn, C.E.M. Banzato, M.A.Z. Arruda, J.S. Becker, Anal. Chem. 82 (2010) 5859.
[11] J.S. Becker, S. Niehren, A. Matusch, B. Wu, H.F. Hsieh, U. Kumtabtim, M. Hamester, A. Plaschke-Schlutter, D. Salber, Int. J. Mass Spectrom. 294 (2010) 1.
[12] J.S. Garcia, G.H.M.F. Souza, M.N. Eberlin, M.A.Z. Arruda, Metallomics 1 (2009) 107.
[13] S.A.L. Andrade, P.L. Gratão, M.A. Schiavinato, A.P.D. Silveira, R.A. Azevedo, P. Mazzafera, Chemosphere 75 (2009) 1363.
[14] M. Miransari, Plant Biol. 12 (2010) 563.
[15] D.R. Bandura, O.I. Ornatsky, L. Liao, J. Anal. At. Spectrom. 19 (2004) 96.
[16] D.R. Bandura, V.I. Baranov, S.D. Tanner, Anal. Chem. 74 (2002) 1497.
[17] B. Hattendorf, D. Günther, Spectrochim. Acta B 58 (2003) 1.
[18] D.R. Bandura, V.I. Baranov, A.E. Litherland, S.D. Tanner, Int. J. Mass Spectrom. 255–256 (2006) 312.
[19] P.J. White, H.C. Bowen, P. Parmaguru, M. Fritz, W.P. Spracklen, R.E. Spiby, M.C. Meacham, A. Mead, M. Harriman, L.J. Trueman, B.M. Smith, B. Thomas, M.R. Broadley, J. Exp. Bot. 55 (2004) 1927.
[20] H. Li, S.P. McGrath, F. Zhao, New Phytol. 178 (2008) 92.
[21] Q. Cham, S.E. Afton, J.A. Caruso, Metallomics 2 (2010) 147.
[22] P.K. Padmavathiamma, L.Y. Li, Water Air Soil Pollut. 184 (2007) 105.
[23] N. Terry, C. Carlson, T.K. Raab, A.M. Zayed, J. Environ. Qual. 21 (1992) 341.
[24] E. Schulze, E. Beck, K. Müller-Hohenstein, Plant Ecology, Springer, 2005, p. 181.
[25] M.J. Hawkesford, L.J. De Kok, Sulfur in Plants: An Ecological Perspective, Springer, 2007, p. 225.
[26] M.J. Hawkesford, L.J. Kok, Plant Cell Environ. 29 (2006) 382.
[27] G.S. Bañuelos, Z. Lin, Development and Uses of Biofortified Agricultural Products, CRC Press, 2009, p. 49.
[28] E.H. Larsen, R. Lobinski, K. Burger-Meÿer, M. Hansen, R. Ruzik, L. Mazurowska, P.H. Rasmussen, J.J. Sloth, O. Scholten, C. Kik, Anal. Bioanal. Chem. 385 (2006) 1098.
[29] D.C. Eustice, F.J. Kull, A. Shrift, Plant Physiol. 67 (1981) 1054.
[30] X. Yu, J. Gu, Environ. Sci. Pollut. Res. 14 (2007) 510.
[31] G.N. Schrauzer, J. Nutr. 130 (2000) 1653.
[32] R.M.V.B.C. Leite, A.M. Brighenti, C. Castro, Girassol no Brasil, Embrapa Soja, 2005, p. 332.
[33] P. Christie, X. Li, B. Chen, Plant Soil 261 (2004) 209.
[34] C. Kaya, M.A.S. Burton, D.E.B. Higgs, J. Plant Nutr. 24 (2001) 369.