# Comparative metallomics for transgenic and non-transgenic soybeans

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In this work, a comparative metallomics of transgenic and non-transgenic soybeans [*Glycine max* (L.) Merrill] was performed. Soybean proteins were extracted with a proper buffer and separated by two-dimensional polyacrylamide gel electrophoresis. Metal ions bound to a set of eight proteins randomly selected (ranging from 13.98 to 54.87 kDa), were characterized by matrix-assisted laser desorption-ionization quadrupole-time of flight mass spectrometry and mapped using synchrotron radiation X-ray spectrometry. The metal ions detected were: Ca(II), Cu(II), Fe(II), Mn(II), Ni(II) and Zn(II). Transgenic and non-transgenic soybeans proteins were found to display typical and random profiles for metal ions binding. To test the reliability of the qualitative metal ions profiles, quantification of Ca(II), Cu(II) and Fe(II) was performed *via* microwave-assisted decomposition in mini-vials followed by atomic absorption spectrometry determination. Qualitative and quantitative metallomics was found to be coherent and to match profiles expected from the known protein functions. The protein of spot 5, with molar mass of 37.62 kDa (amino acid sequence presented), was found to display the most characteristic change in metal ions content, with higher Ca(II), Cu(II) and Fe(II) concentrations for transgenic soybeans.



## Introduction

Soybeans culture has an expressive impact in the economy of many countries, as exemplified by the world production of *ca.* 200 million ton between 2005 and 2006.[1] Such large production can be explained by the exponential growth of soybeans by-products commercialization, which presents many benefits in terms of health and nutritional aspects. Additionally, biodiesel obtained from soybeans is being tested as a fuel alternative,[2] since soybeans contains 21% of oil. Soybeans also present a great amount of storage proteins, which correspond to *ca.* 41% in terms of their dry mass. The most abundant storage proteins in soybeans (*ca.* 80%) are glycinin and β-conglycinin, which are responsible for the main nutritional, physical–chemical and physiological properties of soybeans.[3] Owing to such characteristics and importance, transgenic soybeans cultures have been greatly developed, so that 60% of the cultivated world area (*ca.* 49 million hectares) is being occupied by soybeans cultured to be tolerant to herbicides.[4]

Transgenic organisms are those whose genome was modified from the introduction of hexogen DNA fragments (*i.e.* genes from organisms of different species than the target organism). Such hexogen genes are intended to confer enhanced characteristics to the target organism.[5] The introduction, elimination and/or replacement of genes can modify the protein production mechanism, with the main following consequences: (i) synthesis of new substances; (ii) absence of proteins, which were currently synthesized before the genetic modification; and (iii) synthesis of a greater amount of substances already present in the organism.[5] The effects of genetic modification are known to greatly change the proteome of an organism.[6] Based on such fact, it was rationalized that soybeans proteome changes after genetic modification should also affect soybeans metallomes. To test our hypothesis, non-transgenic and transgenic soybeans samples were used, the last being denominated as Roundup Ready® and obtained by a genetic modification process based on inserting the CP4 EPSPS gene[7] from *Agrobacterium* sp. strain CP4 that provides CP4 EPSPS (EC 2.5.1.19) protein, which confers soybeans tolerance to glyphosate.[6] A comparative metallomic study between transgenic and non-transgenic soybeans was performed, using four different powerful analytical techniques: two-dimensional polyacrilamide gel electrophoresis (2-D PAGE), matrix-assisted laser desorption-ionization quadrupole-time of flight mass spectrometry (MALDI-QTOF-MS), synchrotron radiation X-ray fluorescence (SR-XRF) and atomic absorption spectrometry.[8] In that metallomic study, transgenic and non-transgenic soybeans proteins were separated and a selected set of eight proteins was identified. Furthermore, the presence and quantity of metal species associated with the identified proteins in both soybeans samples were evaluated.

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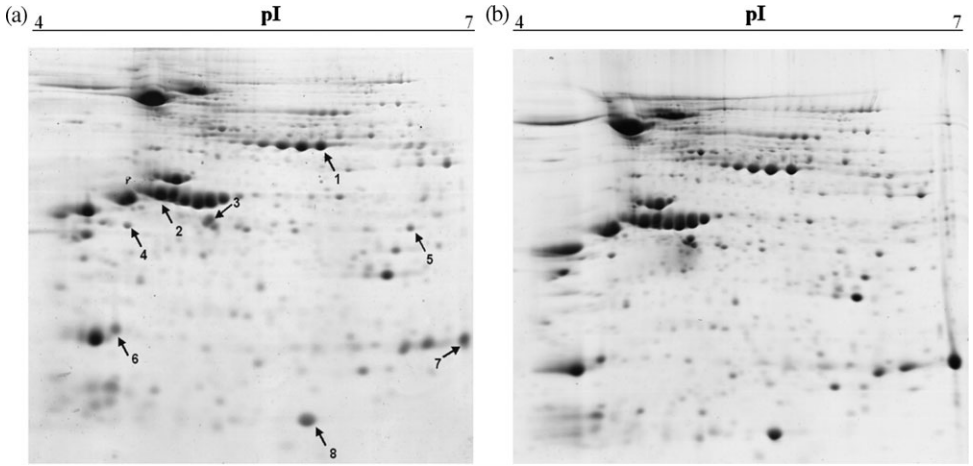


**Fig. 1** 2-D gel for 300 μg of proteins for (a) transgenic and (b) non-transgenic soybeans. Selected protein spots for analysis (1–8) are marked in (a).

## Results and discussion

### Two-dimensional polyacrylamide gel electrophoresis and protein characterization

Fig. 1 shows the results of two-dimensional polyacrylamide gel electrophoresis for the protein separation of transgenic and non-transgenic soybeans. These gels were obtained in an isoelectric point (pI) range between 4 and 7, since resolution was better than when using a larger isoelectric point range (3 to 10). The protein spot number calculated by the software ImageMaster 2-D Platinum version 6.0 (GeneBio, Geneva, Switzerland) for four gel replicates was $408 \pm 27$ for transgenic soybeans and $397 \pm 26$ for non-transgenic soybeans. A set of eight proteins, shown in Fig. 1(a), was randomly selected and then MALDI-QTOF-MS analyses were performed for protein identification (Table 1). In protein data banks,[9,10] the information about protein functions are generally available. Proteins identified in spots 1, 2, 7 and 8 are related to the plant seed storage and their function is to provide the major nitrogen source for plant development. Additionally, the protein identified in spot 3 has a legume lectin domain, and that one identified in spot 4 is a plant seed maturation protein. Finally, those proteins of spots 5 and 6 were still not identified, but their amino acid sequences are presented in Table 1.

### Metal ions identification by synchrotron radiation X-ray spectrometry in protein spots

To qualitatively evaluate the presence of metal ions associated with transgenic and non-transgenic soybeans proteins, the selected protein spots were analyzed by SR-XRF. Table 2 shows the results with the (+) sign beside some metal ions indicating a higher observed intensity. The metal ions detected in proteins spot mapping were Ca(II), Cu(II), Fe(II), Mn(II), Ni(II) and Zn(II). These metal ions are commonly found bounded to proteins or as an inherent part of their structure.[11] Ca(II) is considered to be a plant macronutrient and the other five metal ions are considered to be plant micronutrients.[12]

Characteristic and random changes in metal ions profiles were observed for the selected proteins. Protein from spot 1, from both non-transgenic and transgenic soybeans, is bounded to Fe(II), but the amount of this metal ion is higher for the transgenic counterpart. Ca(II) and Fe(II) were both detected in association with spot 2 non-transgenic soybeans protein, whereas no such metal ions were detected for the transgenic counterpart. Ca(II) and Cu(II) were both detected in spot 3 proteins, but higher intensity of their signals being observed for the transgenic counterpart. Ca(II) was detected for spot 4 transgenic protein, but none was found for the non-transgenic counterpart. The greatest diversity of metal ions—Ca(II), Cu(II), Fe(II) and Zn(II)—was detected for both transgenic and non-transgenic spot 5 proteins, but their intensities were consistently higher for the transgenic ones. In spot 6 non-transgenic protein, four metal ions were detected—Ca(II), Fe(II), Mn(II) and Ni(II)—whereas just two of those–Ca(II) and Fe(II)—with lower intensities were detected in the corresponding transgenic protein. Again for spot 7 protein, two metal ions were detected for the non-transgenic soybeans—Ca(II) and Fe(II)—with only lower Ca(II) intensity being observed for the transgenic counterpart. Finally, spot 8 non-transgenic protein was found to contain Fe(II), where no metal ion at all was detected in the transgenic counterpart.

Cu(II) and Zn(II) are metal ions that are commonly found in proteins related to oxidative stress (for example, superoxide dismutases, EC 1.15.1.1)[13] and the tendency of higher intensities of these metal ions in transgenic soybeans (such as for spot 3 and spot 5 proteins) appears to indicate some degree of plant stress induced by genetic modification.

### Calcium, copper and iron determination by atomic absorption spectrometry in protein spots

Since Ca(II), Cu(II) and Fe(II) were detected by SR-XRF in most of the selected proteins and owing to their importance in biochemical processes in plants, these metal ions were selected to be quantified in both transgenic and non-transgenic soybeans protein spots. For instance, Ca(II) functions as a structure stabilizer for plant membranes and cell walls. Ca(II) is found in proteins like calmodulins,[12] and it works as an important signalling ion, regulating plant nutrient transport. Cu(II) is an essential redox component required for many biological plant processes, such as electron transfer reactions

 

**Table 1** Characterization of protein spots in 2-D gels by MALDI-QTOF-MS

| Spot[a] | Protein | Accession or amino acid sequence | Molar mass/kDa |
|---|---|---|---|
| 1 | β-conglycinin | TA922_3847 | 50.55 |
| | β-chain (precursor) | | |
| 2 | Glycinin G2 (precursor) | T167_3847 | 54.39 |
| 3 | Soybean agglutinin | 1SBF | 25.53 |
| 4 | Seed maturation protein PM25 | Q9SEK9_SOYBN | 25.83 |
| 5 | Not identified | HAILLDIHQPRTDLEAKPKKMFMYQVKKNNLKMCFIKALLQLSRVWGPGLMLCSQNQLPSVFHP | 37.62 |
| | | YQSSNTHPERVLCSAMTGQETIPNRQQKKRSLIMFIFGQQCTYMYYCMMDDQIFQEKVTQHI | |
| | | PRSSSPQRDFVLFFLISFTNYLLVNNHRFSTIKEKAIEHRPKTFCASYYFVGFSLISTLVPNVSIR | |
| | | PSFSHSIRHMANKLEMSCQPKLIPSVNKNKNQFSITYKYFTREYSKTFIETKLRKNDEVSKLYF | |
| | | RTTXIFMYLEKKSTKAGKCNTRTFDFVCKRQQYEHFFINNNVSICYRSMKPEKKKSKNCNGD | |
| 6 | Not identified | RGEREKEERNPKKWNWIKKRKKKKLCNLRSSTGSKVWLFEEAKVGNFLIMYKCIPSWEKWIRRRPC | 22.51 |
| | | PPYSHRRTETPQVVFFYTRRTSASSAPADTSSSPEFCSPSTSEYGNSRRLRRHRRWHRRFRP | |
| | | RLVRRRRRRRCGRLGRRRGGRRRFRRRRFGALVARGGGASRWTWSRCWWGPSCRIRHE | |
| 7 | Glycinin chain A2B1a precursor | FWSYG1 | 54.87 |
| 8 | Glycinin G4 (precursor) | Q9SB11_S11004 | 13.98 |

[a] See Fig. 1(a) for spot location.

**Table 2** Identification of metal ions bound to soybeans proteins (transgenic and non-transgenic) by SR-XRF. Signals between brackets indicate higher signal intensities

| Spot | Transgenic soybeans | Non-transgenic soybeans |
|---|---|---|
| 1 | Fe (+) | Fe |
| 2 | ND[a] | Ca, Fe |
| 3 | Ca (+), Cu (+) | Ca, Cu |
| 4 | Ca | ND |
| 5 | Ca (+), Cu (+), Fe (+), Zn (+) | Ca, Cu, Fe, Zn |
| 6 | Ca, Fe | Ca (+), Fe (+), Mn, Ni |
| 7 | Ca | Ca (+), Fe |
| 8 | ND | Fe |

[a] Not detected.

of respiration (cytochrome c oxidase) and photosynthesis (plastocianine), the detoxification of superoxide radicals (Cu–Zn superoxide dismutase), and lignification of plant cell walls (laccase).[12] Fe(II) is an essential metal ion for photosynthesis and it is an enzyme cofactor in plants, related to electron transfer and reduction reactions as well as nitrogen fixation.[12] Therefore, to quantify such metal ions, the spots containing the proteins were subjected to microwave-assisted decomposition in mini-vials, and the analytes were determined by atomic absorption spectrometry. Since protein mass could not be estimated by the gel image analyses software, Table 3 summarizes the quantitative results expressing concentrations in terms of mass of metal ion per mass of protein spot.

The quantitative results (Table 3) corroborated properly with those obtained by qualitative analyses (Table 2). For instance, in spot 5 Ca(II), Cu(II) and Fe(II) were detected with higher intensity and also quantified in a higher concentration in transgenic soybeans, whereas in spot 6, higher intensities and concentrations were found for Ca(II) and Fe(II) but for the non-transgenic soybeans protein.

### Correlation between metal ions and proteins

Some comments can be made relating the protein identities of Table 1 and the results for metal ion quantification of Table 3. The proteins β-conglycinin β-chain (precursor) (spot 1), seed maturation protein PM25 (spot 4), glycinin chain A2B1a (precursor) (spot 7) and glycinin G4 (precursor) (spot 8) have no bounded metal ions and no information about metal ion binding is indeed mention in protein data banks.[10]

**Table 3** Ca(II), Cu(II) and Fe(II) concentrations in protein spots of transgenic (TS) and non-transgenic (NTS) soybeans, $n = 3$

| | $Ca^a$/mg $g^{-1}$ | | $Cu^b$/μg $g^{-1}$ | | $Fe^b$/μg $g^{-1}$ | |
|---|---|---|---|---|---|---|
| Spot | TS | NTS | TS | NTS | TS | NTS |
| 1 | ND[c] | ND | ND | ND | <LOQ[d] | <LOQ |
| 2 | <LOQ | <LOQ | ND | ND | 212 ± 31 | 348 ± 49 |
| 3 | 2.6 ± 0.2 | 2.3 ± 0.5 | 1.5 ± 0.2 | 2.8 ± 0.2 | ND | ND |
| 4 | <LOQ | <LOQ | ND | ND | ND | ND |
| 5 | 17 ± 2 | 3.6 ± 0.5 | 1.6 ± 0.1 | < LOQ | 691 ± 78 | 447 ± 54 |
| 6 | 3.5 ± 0.2 | 15 ± 2 | ND | ND | 663 ± 79 | 869 ± 93 |
| 7 | < LOQ | < LOQ | ND | ND | < LOQ | < LOQ |
| 8 | ND | ND | ND | ND | < LOQ | < LOQ |

[a] Determined by FAAS. [b] Determined by ETAAS. [c] Not determined. [d] Limit of quantification: 2750 μg $g^{-1}$ for Ca(II), 1.7 μg $g^{-1}$ for Cu(II) and 198 μg $g^{-1}$ for Fe(II).

In glycinin G2 (precursor) protein (spot 2), Fe(II) was mostly detected only in non-transgenic soybeans. As Fe(II) is a plant micronutrient[12] and this protein is involved in seed storage, our metalomic finding appears to indicate better nutrient preservation in non-transgenic soybeans.

For both transgenic and non-transgenic soybeans, soybean agglutinin protein (spot 3), the metal ions Ca(II) and Cu(II) were similarly detected. This protein is a legume lectin, which is involved in carbohydrates binding and in metal ion coordination. Legume lectins generally possess a single carbohydrate binding site per subunit as well as tightly bound Ca(II) and Mn(II) ions, or other transition metal ions such as Cu(II), which are required for their saccharide binding activity.[14]

In the spot 5 protein, the metal ions Ca(II), Cu(II), Fe(II) and Zn(II) were all highly detected, and Ca(II), Cu(II) and Fe(II) were quantified in a higher concentration in transgenic soybeans. However, for the spot 6 protein, Ca(II) and Fe(II) were highly detected as well as quantified in a higher concentration in non-transgenic soybeans. These proteins still do not have their identities well established (amino acid sequences are presented in Table 1), so once this is accomplished, it may become clear why these contrasting metal ion profiles between these transgenic and non-transgenic soybeans proteins were observed.

## Experimental

### Samples

Soybeans samples (both transgenic and non-transgenic) were provided by Monsanto do Brasil. Transgenic soybeans are those denominated Roundup Ready® (already defined in the Introduction section), and non-transgenic soybeans are those that have not underwent any genetic modification process.

### Protein extraction and separation by 2-D PAGE

Soybeans proteins were extracted according to the protocol proposed by Sussulini *et al.*,[15] based on the treatment of ground soybeans with petroleum ether and their extraction with a buffer containing 50 mmol $L^{-1}$ Tris-HCl pH 8.8 (such pH is commonly used for plant tissues total protein extraction and solubilization[16]), 10 mmol $L^{-1}$ dithiothreitol (DTT), 1.0 mmol $L^{-1}$ phenylmethanesulfonyl fluoride (PMSF), 0.1% (m/v) sodium dodecyl sulfate (SDS) and 1.5 mmol $L^{-1}$ KCl. After the extraction, the proteins were quantified by the Bradford method.[17] To precipitate the proteins for sample cleaning, a solution of 0.1 mol $L^{-1}$ ammonium acetate plus methanol (1 : 5 v/v) was added to the protein extract, keeping in contact for 1 h at −20 °C. The proteins were then collected after centrifuging at 4 °C and 5000 *g* for 10 min, washing twice with the ammonium acetate–methanol solution, twice with cold 80% (v/v) acetone and, finally, once with cold 70% (v/v) ethanol. 300 μg of precipitated protein were resolubilized in a rehydration buffer solution containing 7 mol $L^{-1}$ urea, 2 mol $L^{-1}$ thiourea, 2% (m/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.002% (m/v) bromophenol blue and 0.5% (v/v) carrier ampholytes, and loaded onto immobilized pH gradient strips in the 4–7 pH range. The protein separation by 2-D PAGE was performed according to manufacturer's (GE Healthcare, Uppsala, Sweden) recommendations.[18] For the 2-D SDS-PAGE, the final gel concentration was 12.5% (m/v) polyacrylamide and the buffer system consisted in a solution of 25 mmol $L^{-1}$ Tris-HCl pH 8.3, 192 mmol $L^{-1}$ glycine and 0.1% (m/v) SDS. Gels were stained with colloidal Coomassie brilliant blue,[19] scanned and then analyzed by ImageMaster 2-D Platinum 6.0 (GeneBio, Geneva, Switzerland) software.

### Protein spots in-gel digestion

In-gel digestion of protein spots was performed using a Montage In-Gel $Digest_{ZP}$ Kit (Millipore, Bedford, USA), containing a ZipPlate micro-SPE plate. The digestion and vacuum elution protocols were performed according to the manufacturer's recommendations. For vacuum elution, a Multiscreen® Vacuum Manifold (Millipore) was employed.

### Protein characterization by MALDI-QTOF-MS

All samples obtained by tryptic digestion were prepared for MALDI-QTOF-MS analyses using the dried droplet method. The sample was acidified by adding two sample volumes of 0.1% (v/v) trifluoroacetic acid (TFA) and was left at room temperature for a few minutes to reduce the droplet volume *via* evaporation. The matrix [1% (m/v) α-cyano-4-hydroxycinnamic acid (CHCA) in 1 : 1 (v/v) water : acetonitrile solution containing 0.1% (v/v) TFA] was added and the sample was allowed to dry at room temperature. Mass spectrometry data was collected in the positive ion mode. Mass spectra were acquired in a MALDI-QTOF Premier™ mass spectrometer (Waters–Micromass, Manchester, UK). Mass spectra were obtained in positive mode (LDI+) with a fixed nitrogen ion source using the following main parameters: laser firing rate of 20 Hz, mass range of 880.0 to 3000.0 Da, peak detection threshold for MS-MS of 1500.0, mass threshold of 200.0 Da, scan time of 2 s, Np multiplier set to 0.70, resolution of 10 000 with "V" mode, trigger threshold of 700, signal threshold of 80, and microchannel plate (MCP) set to 2100 V. Each spectrum was collected over 2 s scans, and the spectra was accumulated over 2 min approximately. Argon gas was used as the collision gas and the typical collision energy used was in the range of 34–161 eV depending on the size of the ions. The instrument was controlled by MassLynx 4.1 software. All mass spectra were processed into peak list files with a *.pkl extension using ProteinLynxGlobalServer version 2.2.5 (Waters, Manchester, UK) and MASCOT Distiller with MASCOT Daemon "in-house" databank server searching (Matrix Science, London, UK). Protein identification was achieved by performing a database search using the peptide peak list masses and intensities files generated by MALDI-QTOF pos-processing mass spectra through ProteinLynxGlobalServer and MASCOT. The expressed sequence tag (EST) databank was obtained through The Institute of Genomic Research (TIGR) file transfer protocol download (ftp://ftp.tigr.org/pub/data/plantta/Glycine_max)[20] and was added into ProteinLynxGlobalServer to generate concise open reading-frames (ORF) of protein aminoacids. Monoisotopic peak lists were processed with the following search parameters: GLYCINEMAX-1.0 databank field input file, one

 

missed cleavage, tryptic digestion, carbamidomethylation as a cysteine modification. The search error tolerance was set at 10 ppm with a $[M + H]^+$ charge state.

### Mapping of metal ions in protein spots by SR-XRF

The experiments using SR-XRF were carried out at the X-ray fluorescence beam line of the Brazilian Synchrotron Light Laboratory (LNLS) in Campinas, São Paulo, Brazil. A computer-controlled set of slits was used to collimate the white beam so as to deliver a 200 μm × 200 μm microbeam to the experimental station. An aluminum filter was placed in front of the microbeam and before the sample, in order to reduce the intensity of the high-energy components of the spectrum. An HPGe energy dispersive detector was used to collect the fluorescence as well as the scattered radiation coming from the samples. Before irradiation, the protein spots were cut out from the gel, dried in an oven at 40 °C to constant mass and then fixed with sticky tape on the sample holder. The spots were irradiated for 200 s in the central point. This procedure was done in triplicate for each sample. The obtained spectra were processed with AXIL software[21] and were normalized to the incident intensity to correct for the variation of the incident photon flux on the sample during collecting time. The analytical blank for SR-XRF analysis was a gel piece containing no protein spot.

### Microwave-assisted decomposition of protein spots

For gel spot decomposition, a microwave-assisted system using mini-vials was employed as proposed by Sussulini *et al.*[22] The protein spots were cut out from the gel and dried in an oven at 40 °C to constant mass. The dry spot was weighed using a Perkin–Elmer AD6 autobalance (Norwalk, USA) and placed in a 1.8 mL Cryovial® polypropylene (PP) mini-vial (Simporte, Beloeil, Canada). 200 μL of concentrated sub-boiling nitric acid and 150 μL of 30% (v/v) hydrogen peroxide were added to the mini-vial. The pre-reaction time was 20 min. The mini-vials were capped and placed in a polytetrafluoroethylene (PTFE) holder (four mini-vials per holder). The holder was placed into the Teflon microwave closed vessel, containing 15 mL of distilled–deionized water to keep the equilibrium pressure, as previously reported.[23] Then, the entire set (mini-vials and Teflon vessels) was heated in a DGT100 Plus microwave oven (Provecto Analítica, Jundiaí, Brazil). The heating decomposition program was composed by seven steps, executed twice: (i) 60 s @ 300 W; (ii) 30 s @ 500 W; (iii) 60 s @ 500 W; (iv) 30 s @ 800 W; (v) 30 s @ 800 W; (vi) 30 s @ 800 W; (vii) 60 s @ 500 W. For cooling, a time of 2 min after each step and a time of 10 min after each microwave run were employed. After decomposition, the mini-vial volumes were made up to 1.0 mL with deionized water.

### Metal ions determination by atomic absorption spectrometry

A Perkin–Elmer AAnalyst 300 atomic absorption spectrometer (Norwalk, USA), equipped with a deuterium lamp background correction system was used for Ca(II) determination, which was performed in an air–acetylene flame. A Perkin–Elmer Ca(II) hollow cathode lamp ($\lambda$ = 422 nm, slit 0.7 nm) was employed as the primary radiation source. Before the determinations, a 1% (m/v) lanthanum concentration was added to both the standard and sample solutions. The analytical curve ranged from 0.5 to 5.0 mg $L^{-1}$. A Perkin-Elmer AAnalyst 600 electrothermal atomic absorption spectrometer (Norwalk, USA), equipped with a transverse heated graphite atomizer graphite furnace, as well as an AS-800 auto sampling device and a longitudinal Zeeman effect background corrector, was also used for Cu(II) and Fe(II) quantification. A Perkin–Elmer Cu(II) hollow cathode lamp ($\lambda$ = 324.8 nm, slit 0.7 nm) and the matrix modifier containing 0.015% (m/v) magnesium nitrate were used for Cu(II) determinations. A Perkin–Elmer Fe(II) hollow cathode lamp ($\lambda$ = 248.3 nm, slit 0.2 nm) was used for Fe(II) determinations. The analytical curves ranged from 5 to 20 μg $L^{-1}$ and from 5 to 25 μg $L^{-1}$ for Cu(II) and Fe(II), respectively. The heating program for both analytes can be seen in Table 4. The analytical blank for atomic absorption spectrometry analysis was obtained decomposing a gel piece containing no protein spot.

**Table 4** Heating programs for Cu(II) and Fe(II) determinations by electrothermal atomic absorption spectrometry

| | Temperature/°C | | Ramp/s | | Hold/s | | Ar flow rate/mL $min^{-1}$ | |
|---|---|---|---|---|---|---|---|---|
| Step | Cu(II) | Fe(II) | Cu(II) | Fe(II) | Cu(II) | Fe(II) | Cu(II) | Fe(II) |
| 1 | 110 | 110 | 1 | 1 | 30 | 30 | 250 | 250 |
| 2 | 130 | 130 | 15 | 15 | 30 | 30 | 250 | 250 |
| 3 | 1200 | 1400 | 10 | 10 | 20 | 20 | 250 | 250 |
| 4 | 2000 | 2100 | 0 | 0 | 5 | 5 | 0 | 0 |
| 5 | 2400 | 2450 | 1 | 1 | 3 | 3 | 250 | 250 |

## Conclusion

Comparative metallomics of transgenic and non-transgenic soybeans was successfully performed, inside of the used experimental domain. Using 2-D PAGE, *ca.* 400 protein spots were well resolved in the 4–7 pH range. From these, eight proteins ranging from 13.98 to 54.87 kDa were (up to now) analyzed through mass spectrometry. Six important metal ions Ca(II), Cu(II), Fe(II), Mn(II), Ni(II) and Zn(II) were detected in association with these proteins, and the amount of Ca(II), Cu(II) and Fe(II) were determined for non-transgenic and transgenic soybeans samples. Qualitative and quantitative metallomic profiles were found to be consistent, validating the characteristic metal ion profiles found for the whole set of selected proteins for non-transgenic and transgenic soybeans.

Until now, the information available for both soybeans samples (as well as for any comparative study) was frequently disconnected in regard to metal ions and protein contents. Accurate metallomics, as reported in this work, can greatly increase the knowledge about the role of metalloproteins. The case of the protein of spot 5 described herein is illustrative. This protein, among the selected set of soybeans proteins, was found to have the greatest ability to bind different metal ions. As non-transgenic and transgenic soybeans may also be differentiated *via* chemotaxonomic small molecule markers,[24] the referred protein could therefore be used as a target protein for studies involving metabolomic biomarkers for non-transgenic *versus* transgenic soybeans or other related organisms.

Such useful applications are under investigation in our laboratory, as well as a more complete metallomic study for soybeans. Finally, this work put in evidence the importance of this new analytical chemistry research field for protein/metal ion control in biological samples.

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