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FITOCHELATINE RESEARCH IN ENVIRONMENTAL STRESS CONDITIONS BY HEAVY METALS: A REPORT FOR CANCER PREVENTION

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Abstract – *Objective:* *Phytochelatins (PCs) are cytoplasmic plants proteins, glutathione (GSH) derived, capable of chelating heavy metals present in culture environments. This mechanism allows plants to reduce the toxicity due to these metals and preserves its organoleptic qualities, with positive consequences also for the man who feeds on them. The qualitative-quantitative synthesis of Phytochelatins in response to the presence of metals depends on the plant's characteristics so that different plants or different "cultivars" show distinctive tolerance to them.*

Materials and Methods: *In the present study we compared three plant cell lines, which differ in heavy metals tolerance, grown with two types of soils with divergent concentrations of nutritional and toxic metals elements.*

Results: *By analyzing the concentration of component amino acids of the biopeptides in the three-plant cell line we could better understand the mechanisms responsible for tolerance to toxic metals. Moreover, our results suggest that the metals in the environment when present, without exceeding the levels of acute toxicity, stimulate the plant cells to synthesize GSH and/or PCs in stressful conditions.*

Conclusions: *Taken together, these preliminary data would be conceivable to think that PCs should be involved in the assessment of the environmental impact and adaptive capacity of vegetables for food use. Furthermore, that could be useful to plan a specific project of nutritional diet for people who lived in High Environmental Risk area.*

KEYWORDS: *Heavy metals, Chelants, Phytochelatine.*

INTRODUCTION

Heavy metals are non-essential elements, toxic to all living organisms, if present at a sufficiently high concentration in the environment (air, water, soil). Plants and plant cells that grow in culture, respond to the presence of metals by synthesizing molecules belonging to a family of peptides, classified in recent decades as "cadistine" and "poly-cysteine". We consider Erwin Grill, Ernst-L, to be the most appropriate definition, postuled in 1985: Phytochelatines are substances with general structure y-GluCys-based molecules. These molecules are ligands (chelants) of metals[1] induced by the environment (esposoma) in which these plants

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are grown or grow spontaneously. The qualitative-quantitative synthesis of Phytochelatines (PCs) in response to the presence of metals also depends on the taxonomic class (family, kingdom and domain) of the plant, the protein transcription rate and the genotype (hybridized or not) of the plant. Therefore, the typical cultivars of the area should be favored, varieties selected during millennia with better genetic characteristics in terms of resistance to diseases and climatic adversities.

These bio-complexes are believed to be fundamental for plant tolerance to metals to protect the product intended for human consumption, with a mechanism similar to a chela (phytochelatine); this phenomenon limits the damage to the vegetable and preserves its organoleptic qualities.

In this regard, chelatins are functionally analogous to metals – thioneins[2] with the difference that the latter are produced by the translation of mRNA on ribosomes, while PCs are enzymatically synthesized by Pc synthase[3] from the GSH[4-6]. We have selected three cell lines (Table 1) and two types of soils with different concentrations of nutritional and toxic metal elements (Table 2). We studied the present concentration of component amino acids of the biopeptides responsible for tolerance to toxic metals.

MATERIALS AND METHODS

The samples (plants and soil) were harvested in the theatre of study and collected in the "Sialab" laboratory. Each sample is homogenized and dried; solid residues are measured. An aliquot 0.5000 ± 0.0010 g of homogenized sample is weighed into a PTFE vessel on an analytical balance and 9 ml of 14 mmol/L 68% V/V nitric acid are added + 3 ml of conc. 37% V/V chloridric acid, the mixture is carefully shaken and the vessel is tightly closed with the special closing device equipped with a safety valve. Then the sample is mineralized via microwave radiation.

The mineralization process has a total duration of 35 minutes. Firstly, in the mineralization step, the sample is subjected to a temperature ramp increasing up to 175 ± 5°C at 1000 W for 5 ± 0.25 minutes. Secondly, the temperature is kept constant for 10 minutes at 175 ± 5°C. The final step is its cooling which is set at 20 minutes even if it is necessary to wait longer times to guarantee complete cooling.

The mineralized sample is then analyzed by ICP OES (inductively coupled plasma optical emission spectrometry). It is an analysis technique used to determine the qualitative and quantitative elemental composition of samples generally in liquid state.

Analytical information is obtained through the introduction of the sample into the nebulization chamber where it is converted into an aerosol of droplets through a process called nebulization. The aerosol is then transported to the plasma where the atoms and ions undergo an excitation process followed by the emission of characteristic radiation. The radiation is selected, detected and converted into an electrical signal which is used to trace the type

TABLE 1. Heavy metal values in soils and plants.

Heavy metal	Pachino city (mg/kg s.s.)	Pachino tomato (mg/kg)	Priolo city (mg/kg s.s.)	Priolo city olives (mg/kg)	Priolo city prickly pear cactus (mg/kg)
As	<0,50	<0,05	2,3	0,113	0,180
Ba	64	0,135	168	0,353	0,101
Be	<0,50	<0,05	1,2	<0,050	<0,050
Cd	<0,50	<0,05	0,5	<0,050	<0,050
Co	29	<0,05	12,2	<0,050	<0,050
Cr	116	0,018	49,7	0,046	0,041
Cu	29	0,843	43,7	3,218	0,387
Fe	34325	4,350	35401	4,535	1,705
Mn	505	1,213	888	1,464	2,835
Mo	<0,50	<0,05	<0,50	<0,050	<0,050
Ni	152	0,05	28,5	0,094	0,884
Pb	17	0,05	17,6	<0,050	<0,050
Sb	3	0,478	2,6	0,329	0,320
Se	<0,50	<0,05	<0,50	<0,050	<0,050
V	62	<0,05	93,6	<0,050	<0,050
Zn	58	2,055	51,8	3,702	1,391
Al	14212	0,683	17342	3,121	0,640
Hg	<0,50	<0,05	<0,50	<0,050	<0,050
Tl	<0,50	<0,05	<0,50	<0,050	<0,050

TABLE 2. Aminoacids values in plants.

Amino acids	*Pachino tomato (mg/kg)*	*Priolo city olives (mg/kg)*	*Priolo city prickly pear cactus (mg/kg)*
Alanina (ALA)	51	360	nr
Glycine (GLY)	47	302	nr
Valine (VAL)	55	712	121
Leucine (LEU)	76	1058	167
Isoleucine (ILE)	50	632	137
Threonine (THR)	46	146	194
Serine (SER)	152	365	289
Proline (PRO)	56	480	639
Asparagine (ASN)	213	53	319
Methionine (MET)	236	nr	308
4-Hydroxyproline (HYP)	4972	999	1472
Glutamic Acid (GLU)	47	755	121
Phenylalanine (PHE)	52	848	121
Lysine (LYS)	117	508	110
Histidine (HIS)	53	536	287
Hydroxylysine (HLY)	59	412	362
Tyrosine (TYR)	31	929	172
Tryptophan (TRP)	nr	nr	nr
Cystine (C-C)	23	124	127

of analyte that emitted this radiation and after the construction of an appropriate calibration curve at its concentration. The instrument used is ICP OES AVIO 200 PerkinElmer (Waltham, MA, USA), the reference material at appropriate Ultra scientific concentrations.

Once the measured values have been obtained, the analyst proceeds to convert the result into mg/kg (on a dry matter) according to the weight, dilution and moisture content carried out in soil subsamples.

For foods the procedure followed is similar, the mineralization conditions and the volumes of acids used are different, since they are of organic matrix.

Weigh 1.00,00 ± 0.1000 g of sample into 100 ml microwave vessels. 3.0 ± 0.1 ml of 68% Ultra-pure HNO3 are added for trace metal analysis and 1 ml ± 0.1 ml of H2O2. The mineralization process has a total duration of 20 minutes, plus the time required for cooling (about 40 minutes for total ventilation and cooling). Once the reading values have been obtained from the instrument, the technician proceeds to convert the result into mg/kg according to the weight and dilution carried out on the sample.

RESULTS

The selected plant cell lines maintain various degrees of tolerance with a different expression of the synthesis of antioxidants and PCs. Synthetase activity was about two times higher in plants with a habitat that was richer in toxic metals. In fact, the exposure of plants to toxic substances (metals) involves an increase in the activity of enzymes involved in the biosynthesis of GSH and assimilation to sulphates (data not shown)[7-9].

Further, we can observe (Table 1) how the levels of heavy metals are substantially fewer in plants compared to the soil where they were grown (Figure 1). In general, the quantity of toxic metals in the soil and their respective decrease in plants is highly variable. In some cases, we find almost a total absence of particular metals in plants respecting the soil. Moreover, because of the differences in tolerance to heavy metals, we can observe in the three plants taken into consideration (Table 2) peculiar patterns about the presence and quantity of specific amino acids.

DISCUSSION

In this study we compared three plant cell lines that differ in heavy metal tolerance. We can clearly distinguish the different concentration of amino acids and the different presence of methyl groups, as an adaptive response of plants and plants to the presence of metals (increase in substrates for PCs)[10,11]. Note: the synthesis of both GSH and PCs is severely inhibited if the concentration of toxic metals in the environment exceeds the tolerance levels (toxicity) of plants and in this case, we detect the proportional presence of metals in vegetables[12].

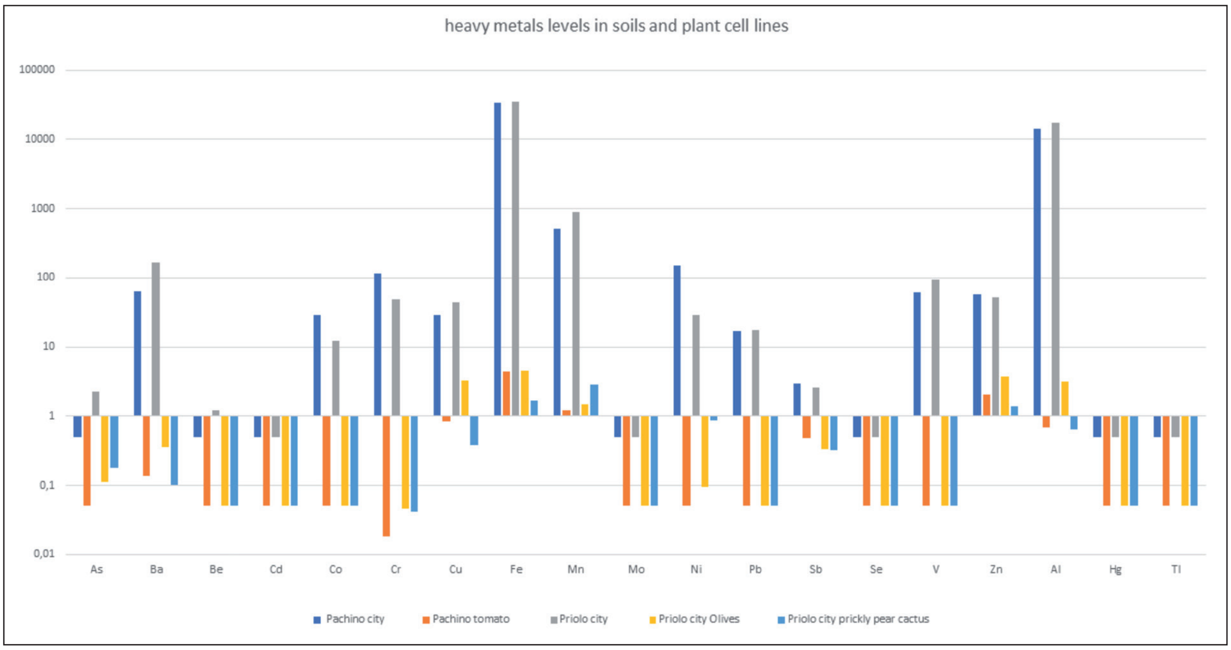

Fig. 1. Difference in heavy metals levels between soil and plant cell lines. the values are expressed on a logarithmic scale.

The observations indicate that PCs synthesis is a mechanism used by vegetables to adapt to metal-induced oxidative stress[13]. This include the induction of enzymes for PC synthesis in high concentrations of PC-Metal complexes in tolerant plant lines; when the level of environmental toxicants exceeds this compensation, we obtain a reduced synthesis due to the blockade of PC synthase[14].

CONCLUSIONS

In light of these observations, it would be conceivable to think that PCs were involved in the assessment of the environmental impact and adaptive capacity of vegetables for food use.

Our results suggest that the metals in the environment, when present without exceeding the levels of acute toxicity, stimulate the plant cells (such as those of the tomato and the olive) to synthesize GSH and/or PC in stressful conditions [15]. It would be useful to test variants of cultivars on areas contaminated by metals to select vegetables that develop more the ability to adapt.

Promise in next future, these preliminary results could be related to environmental issue in order to plan a specific project of nutritional diet [16,17] for people who live in High Environmental Risk area[18].

Conflict of interest:

The Authors declare that they have no conflict of interests.

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