Enhancement of Proline and Hydroxyproline Metabolism and Heavy Metals Toxicity in Plant Life: A Study

Mohd. Iqbal[1] and Mohd. Tariq[2]
[1]Research Scholar, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[2]Professor, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

[1]Corresponding Author: iqbalhgr44@gmail.com

Received: 26-06-2023 Revised: 10-07-2023 Accepted: 28-07-2023

ABSTRACT
A significant amount of heavy metals have been added to the soil and water as a result of more anthropogenic and natural activity. Both humans and plants are much disturbed by these. As a result, there is a growing interest in the scientific study of the toxicity of heavy metals in plants. In this way, osmolytes, such as proline, are organic substances that typically control the sustenance and growth of plants that are commonly grown in the areas. Therefore, increasing the activity of various enzymes and metabolic pathways by adding proline or/by introducing gene(s) is a very effective strategy to deal with the toxicity of heavy metals. The specific function of proline in protecting against the toxicity of heavy metals has been briefly reviewed in this review.

Keywords: proline, heavy metals, toxicity, growth and nourishment of plants

I. INTRODUCTION

World population is increasing day by day on an alarming situation in which urbanization, industrialization, weathering of rocks, mineralization have added massive amount of waste water into the soil. This situation is not decreasing but increasing heavily which deteriorates the environment. The released air is also dangerous for environment as it causes addition of heavy metals to the environment. So, heavy metals are present in the polluted soil, water and air which cause their addition into the plants. Heavy metals are harmful to plants as well as humans. In humans, they cause the problems of bones, kidneys, liver, and nerves. In plants, we are well-known to disorders caused by heavy metals. As heavy metals are those metals which have toxic effects to living organisms, they cause serious effects in plants. Due to non-bio-degradable and persistent in nature, they cause various effects in plant metabolic pathways in which there are blockage of photosynthetic pathways, disruption of Xylem tissues, stunted plant growth, chlorosis of plant tissues, disruption in Cell biology and etc. A number of heavy metals such as Cu, Zn, Pb, Mn, Ni, As, Cd and others are present in the environment that causes serious harms to food web. Up to a certain limit, heavy metals are useful for plants as essential elements but out of this limit they are harmful for plant growth. In order to handle such problems, different agronomical and cultural practices have been introduced. In chemical practices; by applying exogenous osmolytes such as Proline, Polyamines, glycine betaeins etc, we can make plants defensive against heavy metals toxicity.

Proline is a multifunctional amino acid, acting like a signaling molecule and starts the waves of signaling processes. It regulates the osmotic pressure inside the cell, restricts denaturation of proteins, membrane consistency, stabilization of enzymes and reducing the toxic ROS. Different research works showed that by applying exogenous Proline may improve the health of plants against selenium and cadmium. Proline plays an important role in increasing the plant stress tolerance and may be useful in reducing adverse effects caused by different heavy metals toxicities. Therefore, heavy metals have a negative pressure from molecular to whole plant level and harm the plant very seriously by reducing its growth and yield. So, the investigation of heavy metals toxicities is remaining an area of scientific research and further it requires understanding their toxicities and harmful effects to plants and how to minimize the effects of heavy metals toxicities in plants. In this short review, we will discuss adaptation strategies of plants with specific role of proline in reducing their toxicities. We will also discuss the role of proline in fluctuating environment of plants by adjusting plant mechanisms to cope with heavy metals toxicity.

II. SOURCES AND DIFFERENT LEVELS OF HEAVY METALS ACCUMULATION IN THE ENVIRONMENT

An enormous amount of heavy metals have been deposited into the environment through both natural as well as anthropogenic activities like geogenic process, weathering of rocks, numerous irrigation , improper agricultural practices, industrial wastes, organic wastes, combustion of petroleum, and power generation. This contaminates the environment and is a global problem. Anthropogenic activities are regularly increasing the heavy metals contamination into the biosphere and causing enormous pollution. Municipal solid waste is also adding heavy metals to the biosphere due to improper disposal processes .So, such situation causes the addition of huge amount of heavy metals to the aquatic as well as terrestrial ecosystems which leads to destruction of biosphere of the world

III. FACTORS AFFECTING HEAVY METALS ASSIMILATION

Anthropogenic activities can seriously affect the presence of heavy metals in the environment. Heavy metals can suddenly break the major physiological processes of the cellular system including the gaseous exchange, CO2 fixation, respiration absorption potential and nutrient utilization prospective. Other biotic as well as abiotic factors such as soil pH, temperature, moisture ,soil aeration, type of plants and their root shoot system highly affect the metals uptake tariff in plants.

IV. UPTAKE OF HEAVY METALS BY PLANTS

Due to acidity near root zone and leeching out of carboxylates are understood as a potential cause of accumulation of heavy metals inside the plants. In the way of heavy metals utilization, primarily a metal gets its way into the root cells and sticks to the outer cell wall. Secondarily, many transporters proteins with their high attraction to different binding sites aid the metal uptake through the cell membrane. Moreover, the secondary transporters i.e. channel proteins speed up the metal uptake and their addition. These secondary transporters obtain their energy from the fluctuations of membrane potential of the cell membrane and root epidermal cells. All at once, transporters proteins can also be involved in metal detoxification through their regulation of metals utilization from root cells and their transformation from cytosol to the vacuole. However, these proteins also increase the antioxidant system, genetic homeostasis and osmolytes concentration. Heavy metals such as copper, manganese and zinc can act as essential micro nutrients for different physiological processes of plant cells like Mn, Cu and Zn dependent enzymes. So, plants have evolved some mechanisms of controlling and responding to the utilization and addition of both essential and non-essential heavy metals inside the plant cells.

V. CONSEQUENCES OF HEAVY METALS ON PLANTS GROWTH

The toxicity present due to heavy metals is considered as one of the major abiotic stress which leads towards the reduced plant biomass and crop yield. When there is high concentration of heavy metals in cells, it leads to the changing in physiological, biochemical, and cellular level and leads towards abnormality. Panda et al. have reported Cr induced chlorosis in young leaves of wheat leads towards destruction of root cells, impaired photosynthesis, abnormal protein function, dwarfing of plants and in the end plant dies. In another finding, Yadav et.al2 observed that with replacement of central atom Mg of chlorophyll molecule with Arsenic leading to the instability of chlorophyll molecule resulting in the breakdown of photosynthesis and stunted growth of the Sunflower seedlings under Arsenic stress.

The fate of cell functioning depends on the production of reactive oxygen species (ROS). Over production of ROS in different compartment of cells may direct damage to the many vital macromolecules including peroxidation of proteins and lipids, DNA breakdown and many other parts of the plant cells. Evidences have shown that ROS formed by Haber-Weiss reaction results in oxidative suppression during heavy metals stress. The most producing sites of ROS are the Chloroplast, Mitochondrion and cell membrane which are linked with electron transport system. Thus, ROS are the byproducts produced during Oxygen metabolism. By focusing on our point, heavy metals are classified into two groups which are redox active and redox inactive element. Redox active elements directly participate in production of ROS while redox inactive elements indirectly produce the ROS by disrupting the electron transport chain and boosting the production of ROS in the Cell.

VI. ROLE OF PROLINE

Proline has been understood as a vital osmoticum present in cellular system exposed to water stress, saline stress and heavy metals stress. In the current years, the role of proline has also been observed as scavenger of ROS, produced during the stress condition. Proline production takes place with glutamate involving two consecutive reductions catalyzed by pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), respectively. Another alternative producer of proline has been considered to ornithine (Orn) that can be transferred to P5C by Orn-d-aminotransferase (OAT), the helper enzymes are present in the mitochondrion. However, proline can be synthesized from either glutamate or arginine in animals. The relative contribution of these two precursors to proline synthesis in many tissues is not searched more and requires more research. In addition, proline also plays a role as molecular chaperon stabilizing the structure of protein and it maintains the cytosolic pH which aids to balance the redox reactions status of the cell. Proline has also another role in the prevention of disrupting of proteins and membranes by producing the clusters with H2O molecules and stabilizes their structures. Moreover, in Arabidopsis, the overexpression of an antisense proline dehydrogenase cDNA has been observed to speed up the proline addition which improves the resistance potential of plant against the hyper salinity and freezing condition. Likewise, Su and Wu showed that over expression of P5CS gene from Moth bean increased the addition of P5CS mRNA and proline level in transgenic rice which increases the tolerance capability of plant against salt and drought stress. In case of human cells, proline metabolism is concerned with mitochondria-dependent signaling that regulates the apoptosis and programmed cell death. Different research works showed that by the use of proline we can easily remove the toxic ROS in yeast and fungi, so stopping the apoptosis and programmed cell death. This also saves the human cells from carcinogenicoxidative pressure.

VII. ROLE OF PLANT DEFENSE SYSTEM AGAINST HEAVY METALS TOXICITY

Proline can be accumulated in cytosol under numerous biotic and abiotic stresses. Hayat et.al experimented that by exogenous application of proline can increase the endogenous proline level under heavy metals stress situation that helps to regulate the intracellular redox homeostasis potential. It protects the enzymes structures, 3-D structures of proteins and cell organelles including the plasma membranes and also preventing the chance of peroxidation of lipids and proteins. Proline is also helpful in increasing the tolerance potential of plants by detoxifying the heavy metal effects in the cytoplasm. It regulates the water potential that regulates osmotic adjustment by cellular homeostasis and reduce metal uptake. Wu et al. observed that minimum efflux of K ions in Anacystis nidulans after spraying of proline under Cu stress showing as protective role of proline which saves the cell membrane from the toxic effects of Cu.

Specific Role of genes in metals assimilation

Numerous genes can be expressed under the heavy metals stress which activates the particular enzymes to cope with negative response. It is investigated that genetically modified tobacco callus showed more resistance to methyl mercury (CH_3Hg^+) as compared to wild type. The merB gene that encodes the MerB enzyme which dissects the CH_3Hg^+ to Hg^{2+} which is less toxic and is accumulated in the form of Hg-polyP complex in tobacco cells. In addition to this, the over expression of CePCS and AtPCS1 also increased the detoxification potential under As and Cd stress in tobacco plant with the enhancement in phytochelatins (PC) level. In another investigation, Clemens found that Cd ions are apparently entered into the plant cells by Zn^{2+}/ Fe^{2+} transporters of ZIP family and may be possible from Ca^{2+} transporters or channels. There are also numerous genes which are expressed in the hyper accumulator phenotypes such as HMA4 gene under heavy metals pressure. Therefore, some genes, at the same time, called orthologous genes consistently are over-expressed in Cd and Zn hyper tolerant accessions in non-hyper accumulator metallophyptes i.e. SvHMA4 and SpHMA4 in Silene vulgaris and S. paradoxa (according to Arnetoli and Schat unpublished data). MT2b-orthologous genes are overexpressed in hyper accumulators, particularly in metallicolous accessions. In this way, proline has observed to enhance the tolerance capability of a transgenic alga Chlamydomonos reinhardtii under much dose of Cadmium. In this research, a gene encoding moth bean D1-pyrroline-5-carboxylate synthetase (P5CS) starts the proline production and it is introduced into the nuclear genome of green micro alga Chlamydomonas reinhardtii. This transgenic alga yielded 80% more proline levels than the wild type cells and it flourished more rapidly under more concentrations of Cd heavy metal.

VIII. PLANT DEFENSE SYSTEM THROUGH ANTIOXIDANTS

Plants have evolved numerous strategies to cope with the adverse effects caused by the heavy metals. In plants, heavy metals toxicity leads to the over production of ROS which results in peroxidation of many vital constituents of the cell. To cope with such an adverse situation, plants have a well efficient defense system consisting of a set of enzymatic as well as non-enzymatic antioxidants. Numerous types of enzymatic antioxidants consisting of superoxide dismutase (SOD), peroxidase (POD), glutathione-s-transferase (GST) and catalase (CAT) which have the ability to convert the superoxide radicals into hydrogen peroxide and then water and oxygen while low molecular weight non-enzymatic antioxidants comprising of proline, ascorbic acid and glutathione can neutralize the toxic effects of ROS. These two groups of antioxidants can knock out a wide variety of toxic oxygen derivatives and saves the cellular structure from oxidative stress. Depending on the locality of these antioxidants in the cells, their knock out system also varies. In this way, SODs are a group of metalo-enzymes that speed up the conversion of superoxide radical (SOR, $O_2{}^{.-}$) into hydrogen peroxide (H_2O_2). However, CAT, guaiacol peroxidase (GPX) and a variety of general PODs catalyze the breakdown of H_2O_2. Similarly, GST is able to catalyze the conjugation of different electrophilic substrates with reduced glutathione. Several studies report that under stress condition proline has a role like osmolytes and may enhance antioxidant enzyme to minimize the adverse effects of oxidative stress. In another research work, Islam et al. reported that proline acts like a plant growth regulators and maintains the osmotic adjustments, saves the cells against ROS addition under Cd stress. In the same way, exogenous application of proline minimizes the phenotypic effects of selenium by reducing oxidative stress and improves the growth in Phaseolus vulgaris seedlings. Moreover, Hayat et al. showed that the exogenous application of proline enhances the alarming effects of Cd in plants and thereby boosts the growth and photosynthetic rate. At the same time, heavy metals stress leads in a down regulating of leaf water potential from the other side when gets the exogenous application of proline and it enhances the leaf water potential by protecting the membranes from injuries due to heavy metals toxicity.

IX. CONCLUSION

According to the study mentioned above, ROS created as waste products during a terrestrial organism's normal metabolic system is engaged in the maintenance of a number of physiological, physical, biochemical, and cellular activities, but excessive ROS production causes a number of issues in plants. With the rise of metal pollution, the problem only becomes worse. The macromolecules necessary for plants' biosynthetic machinery can be harmed directly by heavy metals. Finding a method to eliminate the damaging effects of heavy metals is necessary to deal with this problem. Therefore, these elements—which include proteins, metabolites, and gene transcription factors—can be exploited to increase the tolerance of plants to the toxicity of heavy metals. The use of Proline as Osmolyte is best in such a case to combat with heavy metals toxicity in plants.

REFERENCES

1. Yamamoto F, & Kozlowski TT. (1987). Effect of flooding, tilting of stem, and ethrel application on growth, stem anatomy, and ethylene production of Acer platanoides seedlings. *Scand J For Res, 2*, 141-156.
2. Yadav G., Srivastava P.K., Singh V.P. & Prasad S.M. (2014). Light intensity alters the extent of arsenic toxicity in Helianthus annuus L. seedlings. *Biol Trace Elem Res, 158*, 410-421.
3. Wu J.T., Hsieh M.T., & Kow L.C. (1998). Role of proline accumulation in response to toxic copper in Chlorella sp. (Chlorophyceae) cells. *J Phycol, 34*, 113-117.
4. Wu J.T., Chang S.C. & Chen K.S. (1995). Enhancement of intracellular proline level in cells of Anacystis nidulans (Cyanobacteria) exposed to deleterious concentrations of copper. *J Phycol, 31*, 376-379.
5. Wu G., Bazer F.W., Burghardt R.C., Johnson G.A., & Kim S.W. (2011). Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids, 40*, 1053-1063.
6. Wojas S., Clemens S., SkÅ‚odowska A.Y., & Maria Antosiewicz D. (2010). Arsenicresponse of AtPCS1- and CePCSexpressing plants - effects of external As(V) concentration on as-accumulation pattern and npt metabolism. *J Plant Physiol, 167*, 169-175.
7. Wojas S., Clemens S., Hennig J, Sklodowska A., & Kopera E. (2008). Overexpression of phytochelatin synthase in tobacco: distinctive effects of AtPCS1 and CePCS genes on plant response to cadmium. *J Exp Bot, 59*, 2205-2219.
8. Vinodhini R., & Narayanan M. (2009). Heavy metal induced histopathological alterations in selected organs of the Cyprinus carpio L (Common Carp). *Int J Environ Res, 1*, 95-100.
9. Verbruggen N., Hermans C., & Schat H. (2009). Mechanisms to cope with arsenic or cadmium excess in plants. *Curr Opin Plant Biol, 12*, 364-372.
10. Szabados L., & Savouré A. Proline: a multifunctional amino acid. *Trends Plant, Sci, 15*, 89-97.