IJSAR, 4(9), 2017; 01-06

International Journal of Sciences & Applied Research

www.ijsar.in

Proline accumulation under Zinc stress in *Sorghum bicolor* (L.) Plants

P. Usha Shri*

St. Ann's college for women, Mehdipatnam, Hyderabad – 28, Telangana state, India.

Corresponding author: *P. Usha Shri, St. Ann's college for women, Mehdipatnam, Hyderabad – 28, Telangana state, India.

Abstract

Zinc a micronutrient is found in the earth's crust naturally. But due to excessive use and exploitation by anthropogenic sources it has now become a major contaminant. The extent of zinc contamination due to various sources is very high as compared to any other heavy metal, posing a great risk to the environment. In the present investigation a detailed study on the effect of excess zinc on relative water content and proline in the leaves of *Sorghum bicolor* was carried out at different stages of plant growth. The seven day old plants were treated with five different concentrations of zinc solution viz: 1.5, 3.5, 5.5, 7.5 and 9.5mM of zinc taken as zinc sulphate. The plants were sampled at 15, 30 and 50 days of plant growth. Relative water content (RWC) in *Sorghum bicolor* plants decreased at all stages of plant growth however marked increase in proline content was observed with increase in externally applied soil zinc. The increase in proline could be a result of reduced RWC causing zinc induced water stress. Elevated levels of proline may perhaps be a measure helping in ameliorating oxidative damage under excess zinc.

Keywords: Zinc, Contamination, relative water content, proline

Introduction

Agricultural ecosystems are contaminated by chemical fertilizers, pesticide and irrigation with waste water of low quality or addition of sewage sludge which are loaded with heavy metals. Among all the heavy metals zinc is the most common element found in major concentration in all these contaminants and over a period of time has led to elevated levels of zinc in the soils. Such Higher concentration of heavy metals in the soils can lead to a number of direct and indirect effects on growth and development of plants. Zinc is an essential micronutrient involved in a wide variety of physiological processes (Reeves and Baker, 2000; Doncheva *et al*., 2001; Stoyanova and Doncheva, 2002; Di Baccio *et al*., 2005; Broadley *et al*., 2007). Though zinc is a very crucial heavy metal with a number of positive role in several metabolic processes it can cause oxidative stress and cellular damage in plants when present in higher concentrations in the soil. This oxidative stress inturn stimulate the antioxidant system comprising of numerous enzymes and compounds of low molecular weights in the plants. These antioxidants provide protection against oxidative damage (Noctor and Foyer, 1998).

Proline is one such non enzymatic antioxidant which plays an important role in osmoregulation (Ahmed and Hellebust, 1988), scavenging free radicals (Smirnoff and Cumbes, 1989), and quencher of singlet oxygen (Alia et al., 2001). A number of reports are available on accumulation of proline under salinity and drought stress. However, accumulation under heavy metal stress is not clearly understood. The present investigation is to understand the accumulation and role of proline in ameliorating oxidative damage caused by zinc stress. The study is carried out in *Sorghum bicolor* plants, one of the major crops of Arid and Semi- arid regions of the world,

Materials and methods

Seeds of *Sorghum bicolor* Moench (CSH 14) were surface sterilized with 0.001M mercuric chloride for two minutes and thoroughly washed with water several times. Ten sterilized seeds were sown in each pot. All the pots were watered to field capacity daily. Plants were thinned to a maximum of three seedlings per pot after a week of germination. The seven day old plants were treated with five different concentrations of zinc solution viz: 1.5, 3.5, 5.5, 7.5 and 9.5mM of zinc taken as zinc sulphate. Zinc solution (300 ml) was given once in two days to the field capacity, total of ten such doses were given during the experimental period. Plants treated with water served as control. Two doses of soil application of NPK in the ratio of (100:109:137 ppm) was given to the plants on 25^{th} and 35^{th} day of plant growth. The plants were grown under natural photoperiod. Each treatment including the control was replicated six times.

Sample collection

The plant samples were collected at fifteen day intervals approximately viz: 15, 30 and 50 days. The plants were first removed from the soil, the entire plant with roots and shoots were put under constant flow of water to remove the soil particles and exogenous contaminants adhered to the plants. The water droplets were blotted dry with help of blotting paper. Sampling was done in the early hours for the measurement of various morphological, growth and biochemical parameters.

Relative water content (RWC)

RWC was calculated according to the formula of Liu and Ding, (2008) with a slight modification

RWC (%) = [(FM - DM)/ (TM - DM)] X100, Where FM = Fresh mass, DM = dry mass and TM = turgid mass. Fresh weight was obtained by weighing the fresh plants. The plants were then immersed in water over night, blotted dry and weighed to get turgid weight. These plants were dried at 80˚C in a hot air oven for 48 hours to obtain constant dry weight.

Estimation of Proline

Proline was extracted with 3% sulphosalicylic acid, and determined according to Bates *et al*., (1973).

Assay

Proline was extracted from 0.2 g of leaf in 5 ml of 3% sulfosalicylic acid. One ml of extract was reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid for 7.5 min at 100 °C. The reaction was terminated by keeping the tubes in an ice bath. The reaction mixture was extracted with 4 ml of toluene and vortexed. The absorbance of toluene layer was spectrophotometrically determined at a wavelength of 520 nm. Concentration was determined from a standard curve and calculated on a fresh weight basis (µM proline/g.fr.wt). Proline content is expressed on fresh weight basis as follows:

µ Moles/g. tissue = µg proline/ml × ml toluene /115.5 × 5/g. sample. Where, 115.5 is the molecular weight of proline.

Results

The effect of Zn on the relative water content and proline in the shoots of *Sorghum bicolor* at three different stages of plant growth is represented in Fig.1, 2 and 3 respectively.

The RWC was determined on 15, 30 and 50 days of plant growth. RWC of the shoots showed significant decrease ($P < 0.05$) both with increase in zinc treatment and age of the plants. The percent reduction in RWC in 30 days old plants was more as compared to other stages of growth.

Significant ($P < 0.05$) increase in the proline content was noted with increase in zinc treatment and age of the plant. In fifteen day old plants, the proline content recorded lower values compared to control plants. However, in thirty and fifty day old plants proline content showed progressively increased accumulation in the leaves of plants treated with different concentrations of zinc. Proline content was significantly enhanced in the stressed plants over control plants in both thirty and fifty days of plant growth.

Discussion

Relative water content is the ability of plant to maintain high water in the leaves under moisture stress conditions and has been used as an index to determine drought tolerance in crop plants. Flower and Ludlow, (1986) stated that RWC was considered an alternative measure of plant water status, reflecting the metabolic activity in tissues and lethal leaf water status.

In the present investigation the relative water content in the leaves of *Sorghum bicolor* decreased with increasing zinc treatments at all stages of plant growth. The decrease might be attributed to the inhibitory effect of metals on water uptake by roots. These results are in accord with those obtained by Haroun *et al*, (2003) in *Sorghum* plants under cadmium stress, Vassilev, (2004) in barley plants treated with Cd. Alsokari and Aldosuquy, (2011) in wheat plants treated with Cd, (Najafi *et al*., 2011) in sunflower under nickel treatment. Excess concentrations of Pb, Cd, Cu and Zn are reported to significantly affect the plant water status (Kastori *et al*., 1992). Within plants Zn seems to affect the capacity for water uptake and transport (Kasim, 2007; Disante *et al*., 2010).

Proline, however increased with increase in zinc treatment in 30 and 50 day old plants. The increase was three times higher than the control at 9.5 mM zinc treatment in 30 day old plants. It was observed that proline accumulation was dependent on the relative water content and increased with decrease in relative water content. One of the consequences of exposure to heavy metal is the deterioration of plants water status causing dehydration or deficit. This disturbance in plant water relation due to high zinc concentration for prolonged periods could have triggered proline accumulation. Schats *et al*., 1997 established the dependence of metal imposed proline induction on the development of water deficit. Accumulation of proline is either due to increase in denovo synthesis or decrease in its degradation or both (Kasai *et al*., 1998).

Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and V*igna radiate* in response to Cd^{2+} toxicity has been demonstrated by Dhir *et al*., (2004), in Sunflower by Zengin and Munzuroglu, (2006), in *Lemna polyrrihza* under Pb stress by John *et al*., (2008) and in *Pistia stratiotes* under Ni stress by Singh and Pandey, (2011).

Enhanced levels of proline might be playing an important role in mitigating metal stress due to its antioxidant properties (Matysik *et al*., 2002; Siripornodulsil *et al*., 2002).

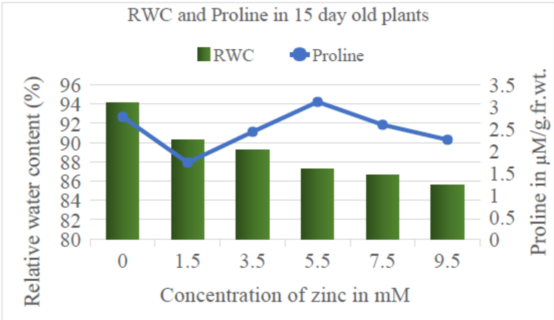

Figure 1: Effect of zinc on Relative water content and Proline content (µM / g.fr.wt) in the leaves of 15 day old *Sorghum bicolor* (L.) Moench (CSH 14) plants.

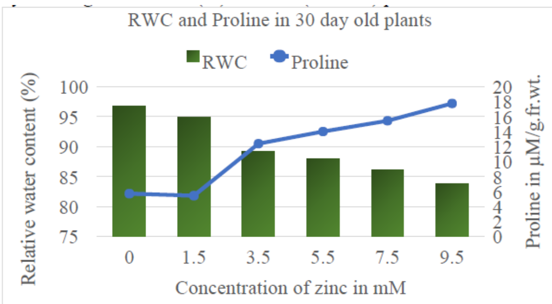

Figure 2: Effect of zinc on Relative water content and Proline content (µM / g.fr.wt) in the leaves of 30 day old *Sorghum bicolor* (L.) Moench (CSH 14) plants.

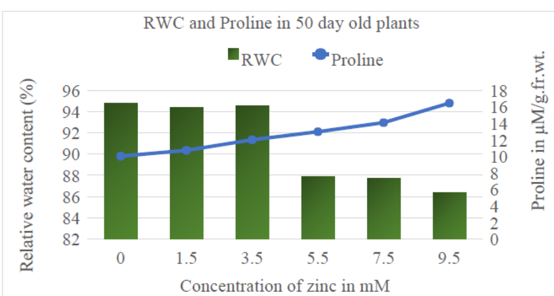

Figure 3: Effect of zinc on Relative water content and Proline content (µM / g.fr.wt) in the leaves of 50 day old *Sorghum bicolor* (L.) Moench (CSH 14) plants.

Conclusion

Reactive oxygen species are unavoidable byproducts of normal cell metabolism. However, prolonged environmental stress disrupts the cellular homeostasis and enhances the production of ROS causing intense damage to cellular components. In the present study excess soil zinc decreased relative water content causing water stress. Proline an indicator of stress increased in the leaves of *Sorghum bicolor* plants at later stages of plant growth. With decrease in RWC an increase in proline was observed. Proline accumulation could be due to its activated biosynthesis or repressed catabolism during dehydration. The accumulation of proline was probably due to zinc induced water stress. Increase in proline content could be a protection mechanism adopted by the plants against water stress.

References

Ahmad, F., Ahmad, I., and Khan, MS., 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Research*. 168: 173-181.

Ahmad, I. and Hellebust, J.A., 1988. The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory responses of two euryhaline microalgae. *Plant Physiology*. 88:348–354.

Ahmad, P. and Jhon R., 2005. Effect of salt stress on growth and biochemical parameters of *Pisum sativum* L. *Archives Agronomy* Soil *Science*. 51: 665-672.

Alia, MP. and Matysik, J., 2001. Effect of proline on the production of singlet oxygen. *Journal of Amino Acids*. 21: 191–203.

Alsokari. and Aldesuquy, HS., 2011. Synergistic Effect of Polyamines and Waste Water on Leaf Turgidity, Heavy Metals Accumulation in Relation to Grain Yield. Journal of Applied Sciences Research. 7(3): 376-384.

Barcelo, J. and Poschenrieder, CH. 1990. Plant water relations as affected by heavy metal stress: a review. *J. Plant Nutrition*. 13(1): 1-37.

Barrs, H.D. and Weatherley, PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*. 15: 413 - 428,

Bassi, R. and Sharma, S.S. 1993 b. Proline accumulation in wheat seedlings exposed to zinc and copper. *Phytochemistry*. 33: 1339-1342.

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205-207.

Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and Lux, A. 2007. Zinc in plants. *New Phytologist*. 173: 677–702.

Costa, G. and Morel, J.L. 1994. Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiology and Biochemistry*. 32 (4): 561-570.

David, E.S., Rogger, C.P., Longrid, J.P. and Ilya, R. 1995. Mechanism of cadmium mobility and accumulation in Indian mustard. Plant physiology. 109: 1427-1433.

Dhir, B., Sharmila, P. and Saradhi, P.P. 2004. Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. *Aquatic Toxicology*. 66: 141-147.

Di Baccio, D., Kopriva, S., Sebastiani, L. and Rennenberg, H. 2005. Does glutathione metabolism have a role in the defence of poplar against zinc excess? *New Phytologist*. 167: 73-80.

Disante, K.B., Fuentes, D. and Cortina, J. 2010. Response to drought of Zn-stressed *Quercus suber* L. Seedlings. *Environmental and Experimental Botany*. 70: 96-103.

Doncheva, S., Stoyanova, Z. and Velikova, V. 2001. Influence of succinate on zinc toxicity of pea plants. *Journal of Plant Nutrition*. 24(6): 789-804.

Flower, D.J. and Ludlow, M.M. 1986. Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus cajan* (L.) millsp.) leaves. *Plant Cell Environment*. 9: 33–40.

Haroun, S.A., Aldesuquy, H.S., Abo-Hamed, S.A. and El-Said, A.A. 2003. Kinetin-induced modification in growth criteria, ion contents and water relations of *Sorghum* plants treated with cadmium chloride. *Acta Botanica Hungarica*. 45: 113-126.

John, R., Ahmad, P., Gadgil, K. and Sharma, S. 2008. Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environvironment*. 54: 262-270.

Kasai, Y., Kato, M., Aoyama, J. and Hyodo, H., 1998. Ethylene production and increase in 1aminocyclopropane-1-carboxylate oxidase activity during senescence of broccoli florets. Acta Horticulturae. 464: 153-157.

Kasim WA., 2007. Physiological consequences of structural and ultra-structural changes induced by Zn stress in *Phaseolus vulgaris*. I. Growth and Photosynthetic apparatus. Int. J. Bot. 3(1), 15-22.

Kastori R, Petrovic M and Petrovic N., 1992. Effect of excess lead, cadmium, copper and zinc on water relations in sunflowers, J. PlantNutr. 15(11), 2427-2439.

Kavi Kishor PB, Sangam S, Amruth RN, Sri Laxmi P, Naidu KR and Rao K.R.S.S., 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Current Science. 88, 424-438.

Liu Y and Ding H., 2008. Variation in air pollution tolerance index of plants near a steel factory: Implications for landscape-plant species selection for industrial areas. WSEAS Trans. Environ. Develop. 4, 24-32.

Matysik J, Alai Bhalu B and Mohanty P., 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Science. 82, 525-532.

Najafi Farzaneh, Ramazan Ali Khavari-Nejad and Fatemeh Hasanjanzadeh. 2011. The Physiological responses of sunflower (*Helianthus annuus* L.) to $NiSO_4$. African Journal of Plant Science 5(3), 201–206.

Nalini P and Chandra PS. 2002. Effect of heavy metals Co, Ni and Cd on growth and metabolism of cabbage. Plant Sci. 163, 753-758.

Noctor G and Foyer CH., 1998. Ascorbate and glutathione: keeping active oxygen under control. A. Rev. Plant Physiol. Plant Mol. Biol. 49, 249-279.

Poschenrieder C, Gunse B and Barcelo J., 1989. Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiology. 90, 1365–1371.

Rauser WE and Dumbroff E.B., 1981. "Effect of excess cobalt, nickel and zinc on the water relations of *Phaseolus vulgaris*". Environmental and Experimental Botany. 21, 249 - 255.

Reeves RD and Baker AJM., 2000. "Metal accumulating plants," in Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment, I. Raskin and B.D. Ensley, Eds., John Wiley andSons, New York, NY, USA, pp. 193–230.

Saradhi A and Saradhi PP., 1991. Proline accumulation under heavy metal stress. J. Plant Physiol., 138, 554-558.

Saradhi P, Alia and Vani B., 1993. Inhibition of mitochondrial electron transport is the prime cause behind proline accumulation during mineral deficiency in *Oryza sativa*. Plant Soil. 155/156 (1), 465–468.

Schat H, Sharma SS and Vooijs R., 1997. Heavy metal-induced accumulation of free proline in metal tolerant and a non-tolerant ecotype of *Silene vulgaris*. Physiol. Plant. 101, 477–482.

Smirnoff N. and Cumbes QJ., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 28, 1057–1060.

Singh K and Pandey SN., 2011. Effect of nickel-stresses on uptake, pigments and antioxidative responses ofwater lettuce, *Pistia stratiotes* L. J. Environ. Biol. 32, 391-394.

Siripornadulsil S, Traina S, Verma DPS and Sayre RT., 2002. Molecular mechanisms of proline-mediated tolerance to toxic heavymetals in transgenic microalgae. Plant Cell. 14, 2837-2847.

Stoyanova Z and S. Doncheva,, 2002. The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plants. Braz. J. Plant Physiol. 14(2), 1-9.

Valeria S, Rita C, Bruno T, Valeriana M and Anna S., 2006. Uptake and toxicity of Cr (III) in celery seedlings. Chemosphere. 64, 1695-1703.

Vassilev A., 2004. Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. Biologia Plantarum. 48, 153-156.

Zengin F and Munzuroglu O., 2005. Effect of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris L.*) seedlings. Acta Biol Crac. 47(2), 157–164.