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EFFECT OF ZINC TOXICITY ON PROTEIN CONTENTS OF CHICKPEA

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ABSTRACT

The effect of heavy metal on Chickpea protein content was observed. Low amount of metal is essential for the growth of plant, but their high amount in soil causes stress to plants. Many metals like Cd, Co, Cr, Hg, and Pb are toxic in their all forms. Human activities such as industrialization release toxic elements like heavy metals in natural resource which affect the entire ecosystem. Heavy metals affect the protein content as well as growth of chickpea plant. Seed germination and growth in the presence of 50ppm and 100ppm of Zn was observed. The effect of metal was found to be dependent on the concentration used; more concentration resulted in more proteolysis. The results indicated that although low level of Zn is essential for plant growth but its higher concentration leads to stress.

Key words: *Germination, SDS-PAGE, Chick Pea, Zinc*

I. INTRODUCTION

Low concentration of metal is essential for the growth of plant but their high concentration leads to toxicity. Industrial wastes (contain heavy metal) release in natural sources which affect the plant as well animal. Heavy metal retards the seed germination and reduce root & shoot elongation. It is also decreases the level of total soluble protein of seed and it may change the dry weight (Wang, 2003)). Heavy metal may cause oxidative damage and membrane alteration in plants (Ahmad MS et al., 2011). Heavy metal can decelerate the altered sugar level and protein metabolisms (Pourrut B et al 2011). Higher level of heavy metals is toxic for plants and their toxicity retard the plant growth, reduce nutrient uptake and may cause many disorders in plants. In leguminous plants, nitrogen fixation ability is affected by heavy metal.

Chickpea is important pulse it has well nutritive. It's containing carbohydrate and protein, together composition about the 80% of the total dry weight of seed (Chibbar RN et al., 2010). Monosaccharide, disaccharide and oligosaccharide are present in Chickpea. Protein quality is considered to be better than other pulse so it is a best source of protein for those individual who cannot afford the animal protein. Except sulphur containing amino acid, all amino acids are present in chickpea. It is a good source of vitamin, minerals and dietary fiber because is free from cholesterol (Wood JA & Grusak MA, 2007). Chickpea is not only providing protein and energy but also provide some minerals such as Ca, Mg, Fe, P and, especially, K. (Chibbar RN et al., 2010)

II. MATERIALS AND METHODS

Chemicals and Reagents

$HgCl_2$, Sodium azide, [Zinc oxide, Calcium chloride, Cobalt chloride (MERCK)], Lead acetate (RANBAXY), Sodium carbonate, Sodium hydroxide, Copper sulphate, Potassium Sodium tartrate, Folin Ciocotteant Reagent, Bovine Serum Albumin (BSA) ,Sodium chloride, Tris HCl buffer, Stock acrylamide solution, Separating gel buffer, Ammonium per sulphate, TEMED, Running Tris buffer, SDS, Coomassie brilliant blue R 250, Methanol, Acetic acid.

Equipments

Autoclave, Incubator, Vortex mixture, Centrifuge, pH meter, PAGE assembly, Spectrophotometer.

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Seed Germination

The seeds of chickpea were obtained from **RVS KRISHI VISHWA VIDYLAYA ALL INDIA COORDINATED RESEARCH PROJECT ON CHICKPEA (ICAR) COLLEGE OF AGRICULTURE, INDORE (MP)**. Seeds were disinfected with 0.1% HgCl2 solution to remove surface contamination and then thoroughly washed & finally rinsed with autoclaved deionised water. Seeds were then transferred to Petri-dish lined with wet filter paper and germinated in an incubator for 24,48,72,96 and 120hrs at 25°C. Sodium azide at a concentration of 0.01% was added to autoclaved distilled water. During germination deionised water (containing sodium azide) was sprinkled on seeds every 12hours.

Treatment with Heavy Metals of Experimental Group

Seeds of experimental group were treated with sprinkling 5ml each of 50ppm, 100ppm and 150ppm salt solution of Zn^{2+} at interval of 24hours. Distilled water was used for control group. Samples were taken from each group at interval of 24hours till 120hours. The dried samples were stored at room temperature for further analysis.

Albumin and Globulin Extraction

The seeds coats were removed and 50gm of the powdered sample was defaulted using chilled water.100mg (0.1gm) of the sample was taken in 2.5ml of eppendorf tube. 1ml of chilled water was added to it and mixed properly. The contents were soaked for 12hrs at 4˚C with regular shaking in vortex mixture. The contents were then after centrifuge at 10,000rpm for 10min in cold. The supernatant was collected to serve as a source of albumin (Sauvaire et. al., 1984). The pellet was collected and soaked in 1.5ml of chilled NaCl which was maintained at 4˚C for 6hrs to collect globulin fraction. The content was than centrifuge at 12,000rpm for 20min in cold. The supernatant was designed as globulin fraction.

Total Protein Extraction

Seeds coats were removed and seed storage protein were extracted. The seed material was homogenised with using 0.1M Tris HCl buffer (pH 7.5) in ratio 1:10w/v (0.5gm seed sample in 5ml Tris buffer). Vortex for 5-10 min and freeze thaw for several times. Total protein was extracted after centrifugation at 10,000rpm for 30min at 4˚C and clear supernatant were used for analysis.

SDS-PAGE of Protein

Prepared a sufficient volume of separating gel (for 10% gel) in mixture by mixing the Stock acrylamide solution 13.3mL, Tris-HCl buffer 8mL (pH 8.8), water 18.1ml,ammonium persulphate solution 0.2mL, 10% SDS 0.4mL and TEMED 20µL. Mixed gently and carefully, pour the gel solution in the solution in the chamber between the glass plate. Samples were prepared by treating15µl protein extract with 3µl SDS-PAGE gel loading dye in boiling water bath for 5min to ensure complete denaturation of proteins. Samples were loaded and allowed to run at 50volt till completion. Gel was stained with Coomassie brilliant blue. Protein bands were observed after distaining. (Laemmeli 1970)

III. RESULTS AND DISCUSSION

Fig1 shows variations in total protein of control after 24h, 48, 72h, 96h and 120h, Fig 2 shows variations in total protein of seed treated with Zn^{2+} 50ppm and Fig 3 shows variations in total protein of seed treated with Zn^{2+} 100ppm. Zinc is less toxic at low concentration but their high concentration cause toxicity. When compared, bands of samples treated with 100ppm are more distorted than 50 ppm. This is a clear indication of toxicity effects of the metal which increase with the concentration. After 96h &120h protein band diffuse in treated seed as compared with control. This diffusion can be a result of breakdown of protein molecules into small peptides. At 100 ppm after 72 hours it can be seen that two protein bands of higher molecular weight disappear as compared with Control. This can be attributed to very high proteolytic activity due to metal stress.

In similar study, same variations observed by Neves, V.A. et al., 2001, higher drop in chickpea major globulin between the fourth and sixth day of germination.

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SDS PAGE of total protein of Control

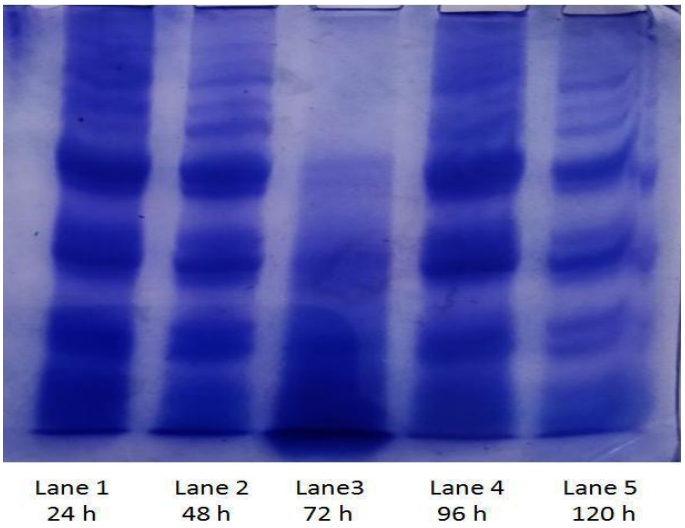

Fig 1

SDS PAGE of total protein of Zinc (50ppm)

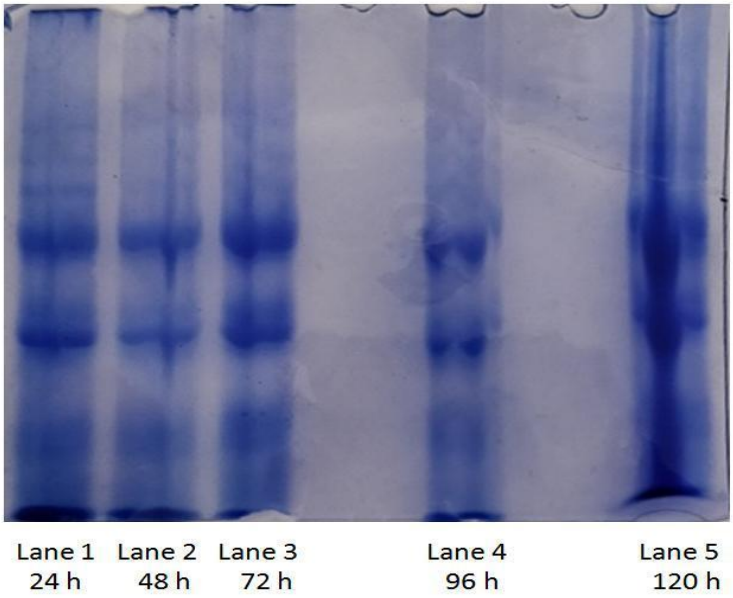

Fig 2

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SDS PAGE of total protein of Zinc (100ppm)

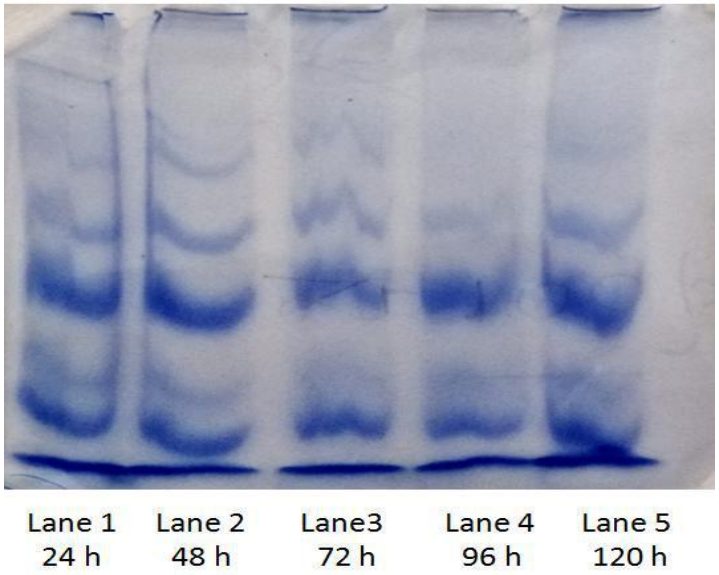

Fig 3

IV. CONCLUSION

There is correlation of protein content and Zinc levels. The effect of metal was found to be dependent on the concentration used; more concentration resulted in more proteolysis. The results indicated that although low level of Zn is essential for plant growth but its higher concentration leads to stress. Further analysis targeting specific protein fractions can reveal whether albumin or globulin content is affected.

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