ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.59073-59076, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CHANGES IN PLANT GROWTH AND PROTEIN CONTENT OF SOME LEGUME CROPS GROWN ON URANIUM MILL TAILINGS

Vijayakumar, A. S., Anthony Johnson, A. M., Vijayalakshmi, T. and *Chinta Sudhakar

Plant Molecular Biology Laboratory, Department of Botany, Sri Krishnadevaraya University, Anantapur -515003 A.P. India

ARTICLE INFO

Article History:
Received 12th July, 2017
Received in revised form
08th August, 2017
Accepted 17th September, 2017
Published online 31st October, 2017

Key words:

Legume plants, Uranium mill tailings,
Germination & Growth,
SDS-PAGE profiles.

ABSTRACT

Uranium (U) tolerance potential of locally cultivated six legume crop species namely groundnut, greengram, bengalgram, redgram, cowpea and horsegram grown on U mill tailings was assessed to understand the impact of uranium on plant germination. Percent germination of seeds, plant growth parameters and protein profiles were analyzed using biometry and Electrophoresis methods. Plants were grown on U mill tailings for 30 days under controlled environmental conditions before the analysis. The U mill tailings showed negative impact on morphological parameters such as percent germination of seeds, plant growth, etc. in all the six legume plants grown on U mill tailings. During the study, greengram, showed best tolerance, redgram showed moderate to leranceand horsegram showed least tolerance under U stress among the six legume crop species studied. Protein profiles of the three legume species (greengram, redgram and horsegram) grown on U mill tailings were studied by SDS-PAGE.

Citation: Vijayakumar, A. S., Anthony Johnson, A. M., Vijayalakshmi, T. and Chinta Sudhakar, 2017. "Changes in plant growth and protein content of some legume crops grown on uranium mill tailings", *International Journal of Current Research*, 9, (10), 59073-59076.

INTRODUCTION

Heavy metal miningleads to accumulation of toxic materialmostly extracted wastes in the neighbourhood of mining industries. Heavy metal extraction left overs such as such as copper, zinc, lead, Uranium tailings have higher impact on the flora and fauna and could be be toxic to the organisms (Antonovics *et al.,* 1971). The reclamation of toxic heavy metal wastes is by utilising heavy metal tolerance of plants (Wu, 1990). Various tolerant species ofgrasses and legumes are used as herbaceous plants for initial coverage on disturbed land (Day and Ludeke, 1981). Exploration of legume species that are able to colonize in metal enriched soils for use in land reclamation has been on interest in research (Day and Ludeke, 1981). Besides legumes are well known to enhance the nitrogen status of the soil through atmospheric nitrogen fixation. Evolution of heavy metal tolerance is known to be a complex mechanism than in non-legumes (Wu and Lin, 1990). Since various plant species exhibit various levels of tolerance to heavy metals, the current study was undertaken to identify the U tolerance levels of six legume species on exposure to U mill tailings and examination of germination, growth ability and protein profiles of the selected legume species based on their performance on U mill tailings. Uraniumis a naturally occurring chemotoxicradionuclideand a heavy metal presentin mineral form. Active anthropogenicactivities such as miningenhanced the uranium levels in domestic areas and cultivated lands (Vandenhove, 2002).Uranium mills that process the excavated uranium ores leave a bulk of radioactive wastes such as U mill tailing dumplings. These result in short and long term pollution hazards due to presence of traces of uranium isotopes, its radioactive successors and stable toxic elements.Published Studies of Vandenhove *et al.,* 2006; Vanhoudt *et al.,* 2008have shown that alterations of growth development and oxidative stress is an important response mechanism under uranium stress.

******Corresponding author:* **Chinta Sudhakar,**
Plant Molecular Biology Laboratory, Department of Botany, Sri Krishnadevaraya University, Anantapur -515003 A.P. India

MATERIALS AND METHODS

Maintenance of Plant species and induction of heavy metal stress

U mill tailings were collected from Uraniumextraction plant (UCIL), Thummalapalle, sited 15 km from Pulivendla in YSR Kadapa district, Andhra Pradesh, India. Legume crop species, namely Groundnut (*Arachis hypogea* L.), bengalgram (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* (L.) Wilczek), horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and redgram (Cajanus cajan L.) seeds were procured from Regional Agricultural Research Station, ANGRAU, Rekulakunta, Anantapur dist., A.P., India. Seeds were sown in

earthen pots containing air dried red soil(garden soil), Uranium mining area (UCIL-Tummalapalli,Y.S.R Kadapa Dist.) surface soil and Uranium mill tailings amended in 1:1 proportion with garden soil. The pots were maintained under natural photoperiod (12-14 h at temperature 28 ± 4°C) in the mesh house and were irrigated once a day with tap water. After germination, seedlings were thinned to three per pot and maintained for 30 days. 30-day-old plants were uprooted carefully, the leaves and roots were separated and were used freshly for experiments or flash frozen in liquid nitrogen, stored at -80°C until further use.

Germination studies

After a week of sowing the seeds, the germinated seed number was counted in every pot of all the crop species. Later the percentage germination is calculated by using the formula. Percent germination of seeds sown = (No. of seeds germinated/No. of seeds sown) X 100

Growth parameters

The 30-days-old plants of all the six crop species were carefully uprooted from pots and washed thoroughly under running tap water, later, further with R.O. water (Millipore, USA). Plant growth parameters were determined by measuring the length of the root and shoot system. Percent of plant growth was determined by assuming the control plant growth as 100%.

SDS PAGE analysis of total plant proteins under U stress
Extraction of plant proteins

Plant proteins were prepared by extraction with Trichloroacetic acid (TCA)/acetone method (Damerval *et al.,* 1986). 1gm of 30 days old plant leaves were dry-crushed in a liquid nitrogen-cooled mortar. To this, 5 ml of extraction buffer (0.1MTris pH 7.4, 0.05M EDTA, 10% Glycerol and 5% β-mercaptoethanol) was added and centrifuged at 5000g for 10 min in a refrigerated centrifuge at 4°C. Supernatant was collected into a fresh tube and pellet was discarded. Centrifuge the supernatant at 10,000g for 30 min and obtained a clear supernatant. This is used as crude extract for isolation of proteins by SDS-PAGE.

SDS polyacrylamide gel electrophoresis

Total protein separation and analysis was done on SDS-polyacrylamide gels (10 x 14 x 0.15 cm) containing 0.1 % SDS (Laemmli, 1970) with a 1.5 cm long 5 % stacking gel on top of an 8.5 cm long 11 % separating gel. Proteins were mixed in sample buffer (50 mM Tris-HCI, pH 6.8,2 % SDS, 10 % glycerol, 3 % β-mercaptoethanol) in 1:1 ratio, heated for 3 min at 95°C and subjected to electrophoresis. Gels were subsequently stained for overnight using Comassie brilliant blue R-250 and destained adequately before analysis in a mixture of methanol, acetic acid and water.

RESULTS

U impact on Seed germination

Seed germination percentageof the six legume species was counted, calculated and represented in Fig 1. Seed germination percentageof greengram was more compared to all other legume species. The percent of reduction in seed germination ability of the plants was 10% in greengram, 12% in groundnut, 18% in redgram, 27% in bengalgram, 35% in cowpea and 48% in Horsegram compared to that of their respective controls (Fig. 1).

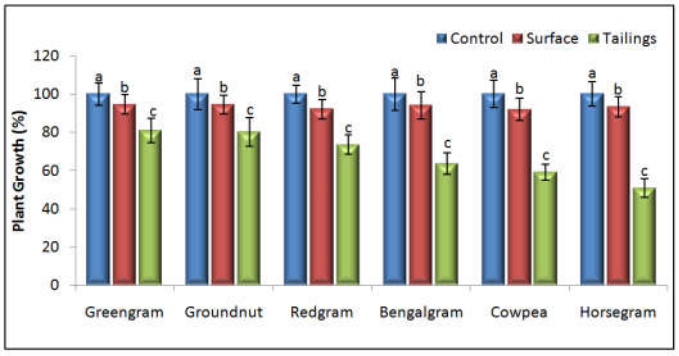

Figure 1. Percent of plant growth of legumes grown on control, surface soil and uranium mill tailing soils. The mean values (n=5) in a row followed by a different letter for each plant species significantly different (p ≤ 0.05) according to Duncan's multiple range (DMR) test

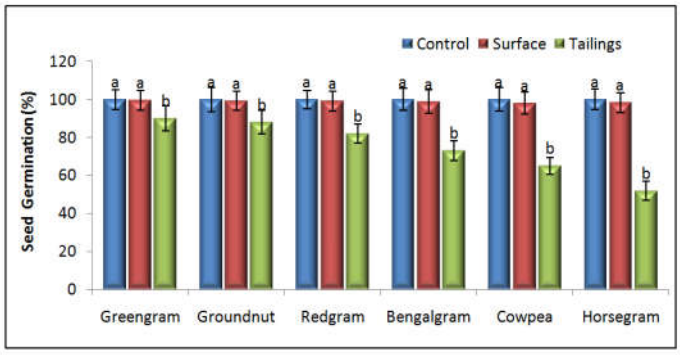

Figure 2. Percent of seed germination of legumes grown on control, surface soil and uranium mill tailing soils. The mean values (n=5) in a row followed by a different letter for each plant species significantly different (p ≤ 0.05) according to Duncan's multiple range (DMR) test

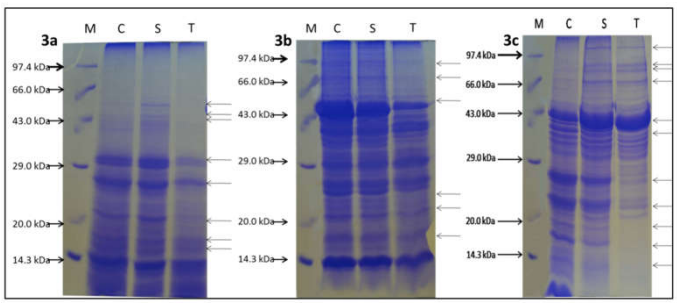

Figure 3. SDS PAGE Profiles of Greengram (3a), Redgram (3b) and Horsegram (3c). Lane M; Low Molecular Weight Protein Marker (kDa) 18.4- Lysozyme; 20.0 Soybean trypsin inhibitor; 29.0-Carbonic anhydrase; 43.0-Ovalbumin; 66.0- Bovine serum albumin; 97.4-Phosphorylase B C – Control (Plants grown on garden soil), S – Surface (Plants grown on Surface soil in the affected area), T- Uranium mill tailings (Plants grown on garden soil blended with Uranium mill tailings in 1:1 ratio)

U impact on Plant growth

Plant growth of the six legume species was measured and represented in Fig 2. Growth of greengram was less affected

compared to all other legume species. Plants grown onUranium mill tailings showed reduction in the growth among all the plant species and the percent of reduction was 19% in greengram, 20% in groundnut, 27% in redgram, 36.4% in bengalgram, 40.9% in cowpea and 51% in Horsegram than that of their respective controls (Fig 2).

Proteome analysis

Based on the results obtained from SDS-PAGE protein profiling of the three legume species grown on U mill tailings, significant difference in banding pattern and band intensitywere observed compared to their controls (Fig 3a, 3b and 3c). Greengram grown on tailings, showed variance inbanding pattern compared to its control plant as the protein bands with molecular weight 15, 16, 20, 25 and 32 (kDa) are down regulated and 46, 49 and 58 are absent (figure 3a). Redgram grown on tailings showed variance in banding pattern compared to its control plant as the protein bands with molecular weight 17, 22, 24, 50, 70 and 97 (kDa) are down regulated (Figure 3b). Horsegram grown on tailings variance in banding pattern compared to its control plant as the protein bands with molecular weight 10, 16 and 19 are absent, 23, 27, 38, 64, 78, 82 and 95 are down regulated and 43 (kDa) is up regulated (Figure 3c).

DISCUSSION

Heavy metals in high concentration inhibit seed germination, plant growth anddevelopment by disturbing many biochemical and physiological processes. Although seed germination is one of the most complex physiological processes in the plants, germination and subsequent emergence of young roots are less sensitive to metal toxicity than are established seedlings (Ma *et al.*, 1997; Pandey *et al.*, 2008). During seed germination, embryonic cells switch from the quiescent state to a metabolically active state in which complex biochemical and physiological changes occur including protein biosynthesis, chlorophyll synthesis, photosynthesis (Munzuroglu and Geckil, 2002). During seed germination so many hydrolyzing enzymes are synthesized and activated to hydrolyse polysaccharides, proteins and lipids into their monomers. Reduction in germination percentage by heavy metals could be attributed to their inhibitory effect on the activity of hydrolytic enzymes during germination (Bhardwaj *et al.*, 2009). Growth inhibition is a common response to heavy metal stress and is also one of the most important indices of heavy metal tolerance of plants (Fontes *et al.,* 1998; Doncheva *et al.,* 2005; Sundaramoorthy *et al.,* 2010; Hossain *et al.,* 2012; Thounaojam *et al.,* 2012). Green gram (*Vigna radiata* L.) is tested for heavy metal tolerance by Singh *et al.,* 2005. Morphological changes such as root and shoot growth in response to U stress has been studied by various researchers (Schutzendubel *et al.,* 2002; Vandenhove *et al.,* 2006; Cuypers *et al.,* 2011; Vanhoudt *et al.,* 2011). Similarly, in the present study, the root and shoot elongation of Greengram, Redgram and Horsegram were inhibited by U stress (Fig.1). However, the percent decrease in plant growth was less in Greengram than in other legumes, which indicates the better adaptation of Greengram to U stress due to the inhibition in cell elongation process or due to reduced mitotic activity which is evident from the proteome analysis.Total soluble protein content upregulation or down regulation under heavy metal stress may be related to the induced synthesis of stress proteins predominantly enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins (Mishra *et al.,* 2006). Based on the results of the study, tolerance oflegume plants to stress of Uranium, it is recommended that greengram may be used for cultivation in the affected area of study.

REFERENCES

Antonovics, J., Bradshaw, A.D. and Turner, R.G., 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res*, 7, pp.2-85.

Bhardwaj P., Chaturvedi A.K. and Prasad P. 2009. Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaselous vulgaris* L. *Nature and Science*. 7(8): 63-75.

Cuypers A, Karen S, Jos R, Kelly O, Els K, Tony R, Nele H, Nathalie V, Yves G, Jan C, Jaco V. 2011.The cellular redox state as a modulator in cadmium and copper responses in *Arabidopsis thaliana* seedlings. *Journal of plant physiology* 168(4):309-16.

Damerval, C., De Vienne, D., Zivy, M. and Thiellement, H., 1986. Technical improvements in two□dimensional electrophoresis increase the level of genetic variation detected in wheat□seedling proteins. *Electrophoresis*, 7(1), pp.52-54.

Day, A.D. and Ludeke, K.L., 1981. The use of legumes for reclaiming copper mine wastes in the southwestern USA. *Minerals and the Environment*, 3(1), pp.21-23.

Doncheva S, Amenós M, Poschenrieder C, Barceló J. 2005. Root cell patterning: a primary target for aluminium toxicity in maize. *Journal of experimental botany*. 56(414):1213-20.

Fontes RL, Cox FR. 1998. Zinc toxicity in soybean grown at high iron concentration in nutrient solution. *Journal of Plant Nutrition*. 21(8):1723-30.

Hossain, M.A., Piyatida, P., da Silva, J.A.T. and Fujita, M., 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany*, 2012. Article ID 872875, 37 pages, 2012. doi:10.1155/2012/872875.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), pp.680-685.

Ma, J.F., Sasaki, M. and Matsumoto, H., 1997. Al-induced inhibition of root elongation in corn, Zea mays L. is overcome by Si addition. *Plant and Soil*, 188(2), pp.171-176.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. and Gupta, D.K., 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, 65(6), pp.1027-1039.

Munzuroglu, O. and Geckil, H., 2002. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Archives of Environmental Contamination and Toxicology*, 43(2), pp.203-213.

Pandey, S.N., Nautiyal, B.D. and Sharma, C.P., 2008. Pollution level in distillery effluent and its phytotoxic effect on seed germination and early growth of maize and rice. *Journal of Environmental Biology*, 29(2), p.267.

Schutzendubel A, Polle A. 2002. Plant responses to abiotic stresses: heavy metal□induced oxidative stress and protection by mycorrhization. *Journal of experimental botany*. 53(372): 1351-65.

Singh, S., Malhotra, R. and Bajwa, B.S., 2005. Uranium uptake studies in some plants. *Radiation Measurements*, 40(2), pp.666-669.

Sundaramoorthy P, Chidambaram A, Ganesh KS, Unnikannan P, Baskaran L. 2010. Chromium stress in paddy:(i) nutrient status of paddy under chromium stress;(ii) phytoremediation of chromium by aquatic and terrestrial weeds. *Comptes rendus biologies.* 333(8):597-607.

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma GD, Sahoo L, Sanjib P. 2012. Excess copper induced oxidative stress and response of antioxidants in rice. *Plant Physiology and Biochemistry* 53:33-9.

Vandenhove, H., 2002, February. European sites contaminated by residues from the ore-extracting and-processing industries. *In International Congress Series* (Vol. 1225, pp. 307-315). Elsevier.

Vandenhove, H., Cuypers, A., Van Hees, M., Koppen, G. and Wannijn, J., 2006. Oxidative stress reactions induced in beans (*Phaseolus vulgaris*) following exposure to uranium. *Plant Physiology and Biochemistry*, 44(11), pp.795-805.

Vanhoudt, N., Vandenhove, H., Horemans, N., Remans, T., Opdenakker, K., Smeets, K., Bello, D.M., Wannijn, J., Van Hees, M., Vangronsveld, J. and Cuypers, A., 2011. Unraveling uranium induced oxidative stress related responses in *Arabidopsis thaliana* seedlings. Part I: responses in the roots. *Journal of environmental radioactivity*, 102(6), pp.630-637.

Vanhoudt, N., Vandenhove, H., Smeets, K., Remans, T., Van Hees, M., Wannijn, J., Vangronsveld, J. and Cuypers, A., 2008. Effects of uranium and phosphate concentrations on oxidative stress related responses induced in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 46(11), pp.987-996.

Wu, L. and LIN, S.L., 1990. Copper tolerance and copper uptake of *Lotus purshianus* (Benth.) Clem. & Clem. and its symbiotic Rhizobium loti derived from a copper mine waste population. *New phytologist*, 116(3), pp.531-539.

Wu, L., 1990. Colonization and establishment of plants in contaminated sites. *Heavy metal tolerance in plants:evolutionary aspects*, pp.269-284.

****