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LIGNIN ESTIMATION IN SORGHUM LEAVES GROWN UNDER HAZARDOUS WASTE SITE

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Abstract

The diminished substance of all-out lignin [%] shows the noteworthy unsafe impacts of cadmium nitrate (70 and 150ppm) and oxidative damage. The present investigation distinguishes the fittingness of polyamines and mycorrhiza to relieve the incited lethal impact of cadmium at DAS 30, 60 and 90 more established of sorghum assortment CSV15 by the turn around reactions were seen by the outside use of putrescine (2.5 and 5.0 mM) and mycorrhiza (Glomus; 150 inoculants for every kg of soil). Polyamine levels are adjustable in stressed plants due to their involvement in regulating the cell ionic atmosphere, maintaining membrane integrity, pigment loss interference and supermolecule stimulation, and protecting alkaloids. Abiotic injury response and mineral nutrient deficiency are associated with plant-based conjugated PAs. In stress conditions, the interaction of polyamines with membrane phospholipids involves membrane stability. Heavy metal contamination threatens the critical limit of alarm in most of the cultivated and periurban areas around us.
Keywords : Applied, Bio, Crop, DAS, Efficiency, Foliar, Lignin, Sorghum:

Introduction

Especially in the vascular, and support tissues: xylem tracheids, vesselling elements, and scleride cells, lignins fill cell wall spaces between the components of cellulose, hemicellulose, and pectin. It is connected with hemicellulose and thus links several plant polysaccharides together, giving mechanical strength to the cell wall and the entire plant by extension. Polyamine like Putrescine content is altered in response to heavy metal exposure (Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i, Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n). In the water supply in plant stems, lignin plays a decisive role. The components of polysaccharides of plant cell walls are highly hydrophilic and therefore water-durable, while lignin is hydrophobic more (Kumar, P. 2013o, Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q.

Kumar, P., 2014r, Kumar, P., Dwivedi, P., Singh, P., 2012s, Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t). The interconnection of polysaccharides with lignin impedes water absorption into the cell wall. This enables lignin to efficiently conduct water in the vascular tissue of the plant (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima, *et al.*, 2018e). The idea that lignin's original function was restricted to transport by water, however, is supported in all vascular plants but not in bryophytes. However, it is present in red algae, which seems to suggest that the common ancestor of plants and red algae also synthesized lignin (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima, *et al.*, 2018e). This would suggest that its original function was structural; it plays this role in the red alga Calliarthron, where it supports joints between calcified segments (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima, *et al.*, 2018e). Another possibility is that the lignins in red algae and plants are a result of convergent evolution and not of a common origin (Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t, Kumar, P., Mandal, B., Dwivedi, P. 2011u. Kumar, P., Mandal, B., Dwivedi, P. 2011v, Kumar, P., Pathak, S. 2016w, Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x). Polyamines such as Putrescine also protect the membrane against oxidative damage by acting as free radical scavengers (Kumar, P., Harsavardhn, M. *et al.*, 2018y. Kumar, P., Yumnam, J. *et al.*, 2018z, Kumar, P., Pandey, A.K., *et al.*, 2018aa, Kumar, P., Kumar, S. *et al.*, 2018bb, Kumar, P., Krishna, V., *et al.*, 2018cc). *Sorghum vulgare* L. is one of the tested plants is a lot of custom - made to grow on contaminated sites about alternative plants and ready to mitigate the significant metal toxicity from venturous waste sites or cultivated sites (Singh *et al* 2020a., Singh *et al.*, 2020b., Sood, *et al.*, 2020., Bhadrecha *et al* 2020, Singh *et al.*, 2020c, Sharma *et al.*, 2020, Singh *et al.*, 2020d, Bhati *et al.*, 2020, Singh *et al.*, 2019, Sharma *et al.*, 2019). Polyamine levels are adjustable in stressed plants due to their involvement in regulating the cell ionic atmosphere, maintaining membrane integrity, pigment loss interference and supermolecule stimulation, and protecting alkaloids [A. Prakash and P Kumar 2017k, P Kumar *et al.*, 2014L, P Kumar 2014m]. Abiotic injury response and mineral nutrient deficiency are associated with plant-based conjugated PAs. In stress conditions, the interaction of polyamines with membrane phospholipids involves membrane stability (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g). Heavy metal contamination threatens the critical limit of alarm in most of the cultivated and periurban areas around us. Sorghum is one of the largest staple foodstuffs of the semi-arid tropics for the poorest and unsafe people in the world. That's why it's regarded as India and abroad's biggest concern. A metallic element (Cd) has been the seventh hierarchical of the twenty highest toxins (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al*. (2018b), Kumar, P., Misao, L., *et al*., 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima, *et al*., 2018e). Current scenario of alternative arrangements of power generation energy resources and reserve in India is discussed (Kumar *et al.,* 2019).

The metallic element can undoubtedly be death metal and therefore its transfer from plants to humans is of great concern.

Material and Methods

This was the pot for the experiment with a 30 cm diameter and a 25 cm height and ten kilograms of soil each with a small hole underneath it. Under the work plan, targeted pots with Endomycorrhiza have been inoculated. The *Sorghum vulgar* L seeds inoculate pots containing combined soil (Soil+ FYM 3:1). The exogenous use of cadmium 0.07 and 0.15 % per 10 kilos of soil on the plant creates heavy metal stresses. Seven days interval application with Putrescine 2.5 and 5 mM. Three phases such as 30 DAS, 60 DAS, and 90 DAS were measured in the respective pots. The leaf sample will be extracted in a NaOH solution with a pH of 7.0 and 12.3 settings in the aliquot samples. The difference of A245 (pH7.0) and A350 (pH12.3) is calculated for lignin amount

1. 0.1 M Sodium phosphate buffer pH7.0
2. 0.1 N and 0.5 N NaOH
3. 2N HCl
4. Stock Standard (100 mg Lignin in 100 ml of water).

The working standard was prepared by dilution of Stock Standard 10 times.

100 mg of oven-dried plant material was moistened in the mortar with the help of water. This ground with ether until it is free from chlorophyll. This homogenate was centrifuged at 2000g for 5 min. The residue was washed, centrifuged and the supernatant was discarded. This washing was repeated twice. 2 ml of NaOH was added into the residue and kept it for 25 h. After 24 h, 0.45 ml of 2N HCl was added. pH was adjusted at 7 with the help of NaOH. The final volume was made 2 ml with the help of water. This was centrifuged again and the supernatant was collected. The 0.8 ml of supernatant was added with 0.8 ml of 0.1M sodium phosphate buffer. The pH maintained at 7.0. Similarly, another aliquot of 0.8 ml was taken. 0.8 ml of 0.1N NaOH was added and pH maintained at 12.3. At 245 and 350 nm, the absorption was measured. The amount of lignin was expressed as ΔE_{350}/sample. Lignin (100 mg) was taken and was dissolved in 0.5N NaOH solution. The stock solution was diluted to 1:10 for the working standard. The 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the diluted working standard were taken into different test tubes. The final volume was raised to 1ml with the help of 0.5N NaOH. The lignin concentration was measured from the difference between A245 and A350 on pH 7.0 and 12.3 working standard diluted with buffer and NaOH, respectively. The amount of lignin was expressed as ΔE_{350}/sample. The graph was prepared. From this graph, lignin was calculated.

Results and Discussion

In sorghum CSV15 the effect of polyamine (putrescine), mycorrhiza, and its combination on lignin (percent) was analyzed under cadmium stress. 30-60-90 days after sowing (DAS) data were recorded (Fig. 1a, b & c). The average lignin content has clearly not fallen significantly in comparison to control (T0) at 30 DAS, 60 DAS, and 90 DAS at the interval, by 18.01 percent, 14.55 percent, and 12.22 percent, respectively. Similarly, the lignin content of plants exposed to higher heavy metal doses (T12) decreased by 28,81%, 22,89% and 19,53% in comparison to the control (T0) at the dates for the interval suggested. Endomycorrhiza exogenous application in soil (T7) revealed an effect of soil mitigation by increasing the lignin content by 2.64%, 2.13 and 1.79% as compared to 30, 60 and 90 DAS, in comparative with T6. When compared with T12, T13 increased its lignin content by 0.48%, 0.38% and 0.32% for the DAS. Compared to T6, the exogenous use of putrescine (T8) showed a mitigating effect by an increase of 5.28%, 4.26% and 3.58% on proposed DAS. Compared to T6, the average lignin content in treatment with higher dose putrescent (T9) for T8 was improved considerably by 7.20%, 5.82%, and 3.88%. Similarly, the T14 treatment was compared to T12, with 5.28%, 4.26% and 3.58% improved in the lignin content at the proposed DAS not significantly. When treated with a higher dose of putrescine (T15) compared to T14, the average lignin content was not significantly increased by 8.64 percent, by 6.98 percent, and by 5.85 percent. In T10, the combination of putrescine and mycorrhiza has been best mitigated by 10.56%, 8.53%, and 7.16%, when compared to a T6 therapy with the suggested DAS. When compared to T6, the non-significant lignin content was 11.28%, 9.11%, and 7.64% respectively, when compared to T11. The treatment (T16) had a similar effect on T12 treatment. The lignin contents of this treatment (T16) were found to rise significantly by 10.56%, 8.53% and 7.16% at the proposed DAS respectively. The T17 therapy showed improvement in results. In comparison with T12, it was noticed an insignificant growth in lignin levels of 12.0%, 9.70%, and 8.13%. The combination of putrescine and mycorrhiza has therefore proved to be the best way to mitigate lignin content cadmium toxicity. In the various realms of plants, such as conifers [P. Kumar2018I, P.Kumar *et al*., 2018aa, P.Kumar *et al*., 2018bb], poplars [S. Pathak *et al*., 2016x, P. Kumar *et al*., 2018y], and herbaceous species, activation of secondary metabolism, and lignification have common responses to Cd. The relationship between lignin formation and auxin has been demonstrated in early studies [P Kumar *et al*., 2018cc]. For example, incubation of wheat internodes with auxin inhibited lignin formation [29, 30]. Stimulation of auxin catabolism by Cd resulted in increased lignification in the stems of pea [25, 26, 27]. Copper stress in Arabidopsis lowered the auxin, increased lignification and lowered the length of the root [P Kumar *et al* 2018aa]

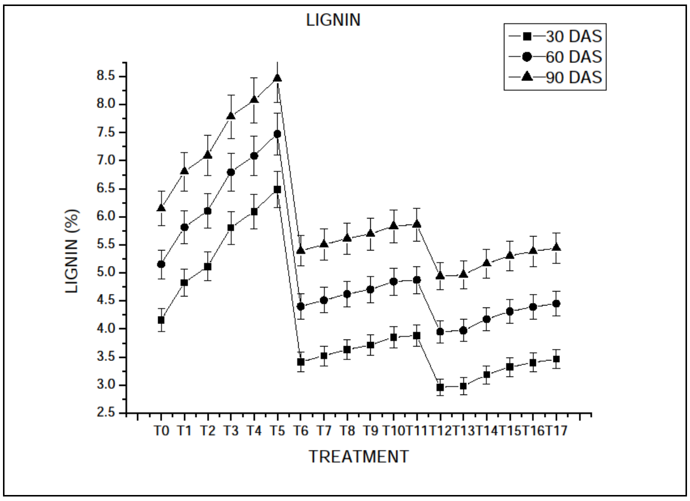

Fig. 1a : Lignin content (%) of sorghum during *Kharif* season of two subsequent years

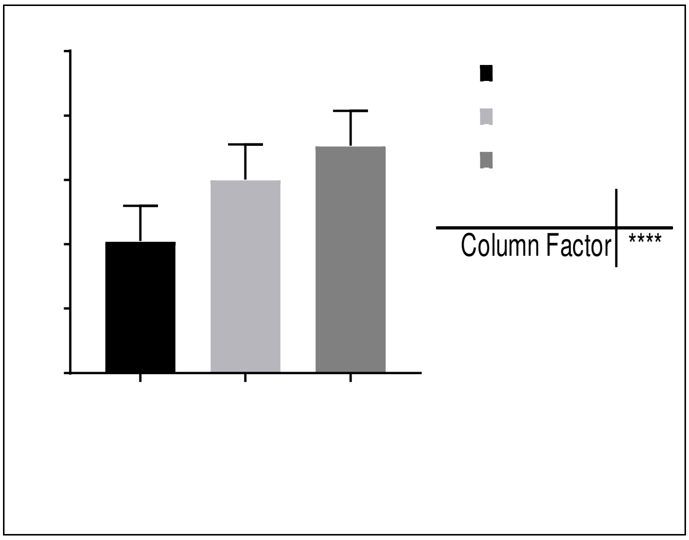

Fig. 1b: Column bar mean graph mean SD

where DAS=Days after sowing. Data are in the form of Mean ± SEM. S=Significance at P≤0.05 and P≤0.01, NS= Non-Significant at P≤0.05 and P≤0.01 using Origin 6.1.

Conclusion

Polyamines like putrescine and mycorrhiza Glomus significantly mitigate cadmium-induced toxicity to sorghum, as their defensive role in plants reduces lignin in sorghum leaves. Heavy metal contamination threatens the critical limit of alarm in most of the cultivated and periurban areas around us. Sorghum is one of the largest staple foodstuffs of the semi-arid tropics for the poorest and unsafe people in the world. That's why it's regarded as India and abroad's biggest concern. A metallic element (Cd) has been the seventh hierarchical of the twenty highest toxins. The metallic element can undoubtedly be death metal and therefore its transfer from plants to humans is of great concern.

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Author Contributions

P.K designed the study, established the biochemical protocols, performed the experiments and collected the data analyzed and interpreted the data. P.K, along with P.D. wrote the paper.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Kumar, P.P. *et al.* (2018e). Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity, International Journal of Current Microbiology and Applied Sciences, 7(10): 1635-1639.

Kumar, P. and Pathak, S. (2019f). Responsiveness index of sorghum (*Sorghum bicolor* (L.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza" Bangladesh J. Bot. 48 (1).

Kumar, P. and Siddique, A. *et al.* (2019g). Role of Polyamines and Endo-mycorrhiza on Leaf Morphology of Sorghum Grown under Cadmium Toxicity, Biological Forum – An International Journal. 11(1): 01-05.

Siddique, A. and Kumar, P. (2018h). Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview, Plant Archive, 18(2): 1933-1937.

Siddique, A.; Kandpal, G.; Kumar P. (2018i). "Proline accumulation and its defensive role under Diverse Stress condition in Plants: An Overview" Journal of Pure and Applied Microbiology, vol.12 (3) pp.1655-1659.

Pathak, S.; Kumar, P.; P.K Mishra, M. Kumar, (2017j). "Mycorrhiza assisted approach for bioremediation with special reference to biosorption", Pollution Research, Vol. 36(2).

Prakash, A. and Kumar, P. (2017k). "Evaluation of heavy metal scavenging competence by in-vivo grown Ricinus communis L. using atomic absorption spectrophotometer" Pollution Research, 37(2): 148-151.

Kumar, P. and Mandal, B. (2014L). Dwivedi, "Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants" vegetos, 26(2): 416-425.

Kumar, P.; Mandal, B.; Dwivedi P.; (2014m). "Phytoremediation for defending heavy metal stress in weed flora" International Journal of Agriculture, Environment & Biotechnology, 6(4): 587-595.

Kumar, P.; Kumar, P.K.; Singh, S. (2014n). "Heavy metal analysis in the root, shoot and a leaf of psidium guajava l. by using atomic absorption spectrophotometer" Pollution Research, 33(4): 135-138.

Kumar, P. (2013o). Cultivation of traditional crops: an overlooked answer. Agriculture Update, 8(3): 504-508.

Kumar, P. and Dwivedi, P. (2015p). Role of polyamines for mitigation of cadmium toxicity in sorghum crop, Journal of Scientific Research, B.H.U.; 59: 121-148.

Gogia, N.; Kumar, P.; Singh, J.; Rani, A. Sirohi, Kumar, P. (2014q). Cloning and molecular characterization of an active gene from garlic (*Allium sativum* L.), International Journal of Agriculture, Environment and Biotechnology, 7(1): 1-10.

Kumar, P. (2014r). Studies on cadmium, lead, chromium, and nickel scavenging capacity by in-vivo grown Musa paradisiacal. using atomic absorption spectroscopy, Journal of Functional and Environmental Botany, 4(1): pp.22-25.

Kumar, P.; Dwivedi, P.; Singh, P. (2012s). "Role of polyamine in combating heavy metal stress in stevia rebaudiana Bertoni plants under in vitro condition" International Journal of Agriculture, Environment and Biotechnology, 5(3): 185-187.

Mishra, P.K.; Maurya, B.R.; Kumar, Pp. (2012t). Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season, Journal of Functional and Environmental Botany, 2(2): 1-6.

Kumar, P.; Mandal, B.; Dwivedi, P. (2011u). Heavy metal scavenging capacity of *Mentha spicata* and *Allium cepa*, Medicinal Plant-International Journal of Phytomedicines and Related Industries. 3(4): 315-318.

Kumar, P.; Mandal, B.; Dwivedi, P. (2011v). "Screening plant species for their capacity of scavenging heavy metals from soils and sludges. Journal of Applied Horticulture, 13 (2): 144-146.

Kumar, P.; Pathak, S. (2016w). "Heavy metal contagion in seed: its delivery, distribution, and uptake" Journal of the Kalash Sciences, An International Journal, 4(2): 65-66.

Pathak, S.; Kumar, P.; Mishra, P.K.; Kumar, M. (2016x). "Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for a cure". Journal of the Kalash Sciences, An International Journal, 4(2): 61-65.

Kumar, P.; Harsavardhn, M. *et al*.; (2018y). "Effect of Chlorophyll a/b ratio in Cadmium Contaminated Maize Leaves Treated with Putrescine and mycorrhiza" Annals of Biology 34(3)-281-283.

Kumar, P.; Yumnam, J. *et al*. (2018z). "Cadmium Induced Changes in Germnation of Maize Seed Treated with Mycorrhiza" Annals of Agri-Bio Research, 23(2); 169-170.

Kumar, P.; Pandey, A.K.; *et al*.; (2018aa). "Phytoextraction of Lead, Chromium, Cadmium, and Nickel by Tagetes Plant Grown at Hazardous Waste site" Annals of Biology, 34(3): 287-289.

Kumar, P.; Kumar, S. *et al*.; (2018bb). "Evaluation of Plant Height and Leaf Length of Sorghum Grown Under Different Sources of Nutrition" Annals of Biology, 34(3): 284-286.

Kumar, P.; Krishna, V.; *et al*.; (2018cc). "Assessment of Scavenging Competence for Cadmium, Lead, Chromium and Nickel Metals by in vivo Grown *Zea mays* L. using Atomic Absorption Spectrophotometer, Annals of Ari-Bio Research, 23(2): 166-168.

Kumar, R.; K. Ojha, M. H. Ahmadi, R. Raj, M. Aliehyaei, A. Ahmadi and N. Nabipour, (2019). A review status on alternative arrangements of power generation energy resources and reserve in India. International Journal of Low-Carbon technologies.; ctz066, p. 1-17.

Singh, S.; Kumar, V.; Datta, S.; Wani, A.B.; Dhanjal, D.S.; Romero, R. and Singh, J. (2020). Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity, and degradation: a review. Environmental Chemistry

Singh, S.; Kumar, V.; Singla, S.; Sharma, M.; Singh, D.P.; Prasad, R.; Thakur, V.K. and Singh, J. (2020). Kinetic Study of the Biodegradation of Acephate by Indigenous Soil Bacterial Isolates in the Presence of Humic Acid and Metal Ions. Biomolecules, 10: 433.

Sood, M.; Sharma, S.S.; Singh, J, Prasad, R.; and Kapoor, D. (2020). Stress Ameliorative Effects of Indole Acetic Acid on Hordeum vulgare L. Seedlings Subjected to Zinc Toxicity. Phyton – International Journal of Experimental Botany, 89(1): 71-86.

Bhadrecha, P.; Bala, M.; Khasa, Y.P.; Arshi, A.; Singh, J. and Kumar, M. (2020). *Hippophae rhamnoides* L. rhizobacteria exhibit diversified cellulase and pectinase activities. Physiology and Molecular Biology of Plants.

Singh, S.; Kumar, V.; Datta, S.; Dhanjal, D.S.; Sharma, K.; Samuel, J. and Singh, J. (2020). Current advancement and future prospect of biosorbents for bioremediation. Science of the Total Environment, 709: 135895.

Sharma, R.; Jasrotia, K.; Singh, N.; Ghosh, P.; Sharma, N.R.; Singh, J.; Kanwar, R. and Kumar, A. (2020). A Comprehensive Review on Hydrothermal Carbonization of Biomass and its Applications. Chemistry Africa, 3(1):1-19

Singh, S.; Kumar, V.; Kapoor, D.; Kumar, S.; Singh, S.; Dhanjal, D.S.; Datta, S.; Samuel, J.; Dey, P.; Wang, S.; Prasad, R. and Singh, J. (2020). Revealing on hydrogen sulfide and nitric oxide signals co-ordination for plant growth under stress conditions. Physiologia Plantarum, 168(2): 301-317.

Bhati, S.; Kumar, V.; Singh, S. and Singh, J. (2020). Synthesis, Characterization, Antimicrobial, Anti-tubercular, Antioxidant Activities and Docking Simulations of Derivatives of 2-(pyridine-3-yl)-1H-benzo[d]imidazole and 1,3,4-Oxadiazole Analogy. Letters in Drug Design & Discovery.

Singh, S.; Kumar, V. and Singh, J. (2019). The effects of Fe(II): Cu(II) and Humic Acid on biodegradation of atrazine. Journal of Environmental Chemical Engineering, 8: 103539.

Sharma, M.; Singh, J.; Chinnappan, P.; and Kumar, A. (2019). A comprehensive review of renewable energy production from biomass-derived bio-oil. Biotechnologia 100(2):179-194.