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## Effect of putrescine and glomus on total reducing sugar in cadmium treated sorghum crop

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**Abstract**

The present study was carried out to evaluate the ameliorative effect of polyamines and mycorrhiza in the induced toxic effect of cadmium at 30, 60 and 90 DAS older of sorghum variety CSV15. The significant hazardous effects and oxidative damage of cadmium nitrate (70 ppm and 150ppm) were evidenced by decreased content of total reducing sugar content ( $\text{mg g}^{-1}$  fresh weight). The reverse responses were observed by the external application of putrescine (2.5 and 5.0 mM) and mycorrhiza (Glomus; 150 inoculants per kg of soil).

**Keywords:** abiotic, biotic, crop, dose, economy, foliar, gap, higher

**Introduction**

Sorghum is one of the most important staple foods for the world's poorest and most food-unsafe people across the semi-arid tropics (Kumar *et al.*, 2016a) [11]. Heavy metal contamination threatens the critical limit of alarm in most of the cultivated and periurban area around us. That's why it is considered as the major concern in India and abroad. Polyamine like Putrescine contents are altered in response to the exposure of heavy metals (Kumar *et al.*, 2011a, b, Kumar *et al.*, 2016a, b) [11, 12]. Polyamines level in stressed plants have adjustive importance thanks to their involvement in regulation of cellular ionic atmosphere, maintenance of membrane integrity, interference of pigment loss and stimulation of super molecule and protecting alkaloids (Kumar and Dwivedi, 2018 a, b, c, Kumar *et al.*, 2018b) [12-14]. Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions. Polyamines like Putrescine also protect membrane from oxidative damage as they act as free radical scavengers (Kumar *et al.*, 2018a, Pathak *et al.*, 2017) [13, 16]. Response to abiotic injury and mineral nutrient deficiency is associated with the production of conjugated PAs in plants. We have tested many plant species for his or her capability of scavenging significant metals from soil and sludge and eventually we tend to reach on the conclusion that among the tested plants, *Sorghum vulgare* L is a lot of custom-made to grow on contaminated places with relation to alternative plant and ready to mitigate the significant metal toxicity from venturous waste site or cultivated site (Kumar and Dwivedi, 2018, Kumar *et al.*, 2012, Kumar *et al.*, 2013, Siddique *et al.*, 2018) [11, 12, 15, 15, 17]. Metallic element (Cd) may be an extremely deadly element and has been hierarchal seventh among the highest twenty toxins (Kumar and Dwivedi, 2018a, b, c) [13-15]. Metallic element may be a doubtless deadly metal and so its transfer from plants to humans is of major concern.

**Materials and Methods**

The pot experiment was conducted within the poly house of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, with one genotype of sorghum CSV 15. Sorghum seeds were taken from board of directors of Sorghum Research Hyderabad, India. The pot size for the experiment was within the diameter of thirty cm and twenty five cm tall and every with capability of ten kilo soil, with a tiny low hole at the underside. Pots containing soil combine (Soil + FYM in 3:1) are inoculated with seeds of *Sorghum vulgare* L. in step with arrange of labor, targeted pots were inoculated with Endomycorrhiza Glomus sp. and at that time significant metal stress was created in plant by the exogenous application of metallic element cadmium nitrate in soil. Two best concentrations of significant metals on the idea of initial screening were selected i.e., 0.07 % per 10 kilo and 0.15 % per 10 kilo of soil. Putrescine was applied at the rate of 2.5 mM and 5.0 millimetre through foliar spray at the seven days of interval. The experiment was ordered go in CRD design. There have been eighteen treatments. Every treatment was replicated 5 times. All the numerical knowledge

obtained were analyzed through applied stat package of Origin 6.1-advance scientific graphing and knowledge analysis [Origin Lab Corporation, One Round House Plaza, Northhampton, MA 01060]. Multivariate analysis was performed for interaction between mycorrhiza and metallic element treatments. One way multivariate analysis was performed.

### Estimation of total reducing sugar

The total reducing sugar content in the plant sample was estimated by following the method proposed by Somogyi, (1952). The reducing sugar when heated with alkaline copper tartrate reduces the copper from the cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdic acid the reduction of molybdic acid to molybdenum blue takes place.

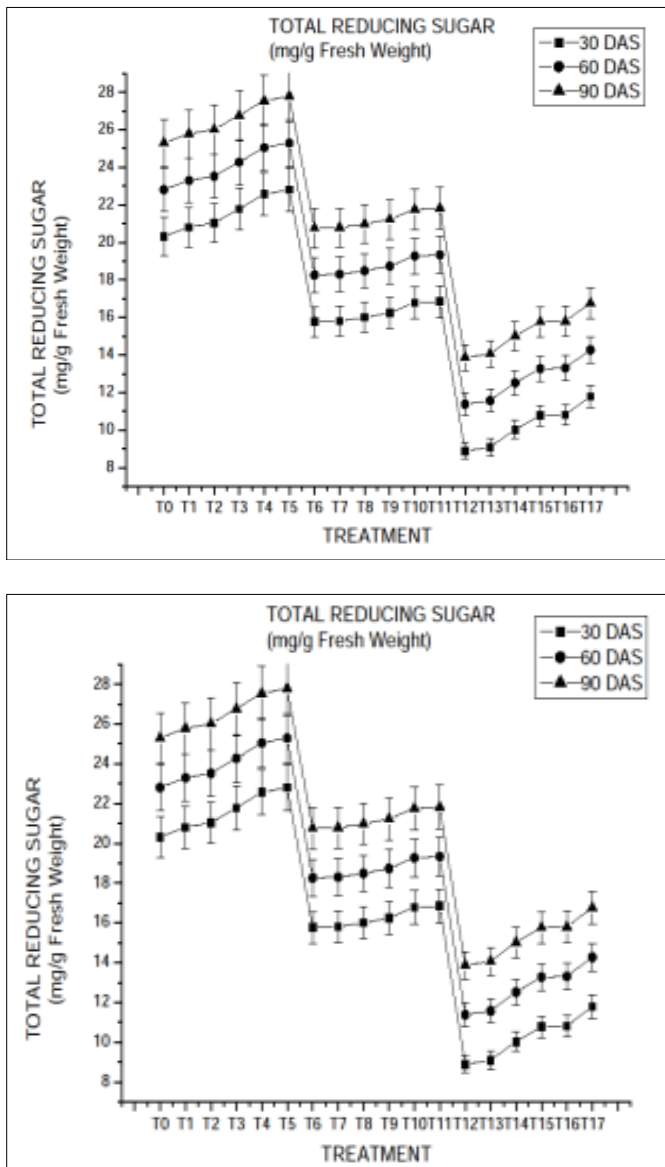
### Reagents

1. Alkaline copper tartrate
  - (a) Dissolved 2.5 g anhydrous sodium carbonate, 2g sodium bicarbonate, 2.5g potassium sodium tartrate and 20 g anhydrous sodium sulphate in 80 ml water and made up to 100 ml.
  - (b) Dissolved 15g copper sulphate in a small volume of distilled water. Added one drop of sulphuric acid and made up to 100 ml. Mixed 4ml of (b) and 96 ml of solution (a) before use.
2. Arsenomolybdate reagent: Dissolved 2.5 g ammonium molybdate in 45 ml water, and 2.5 ml sulphuric acid and mixed well. Then added 0.3 g disodium hydrogen arsenate. The mixture was mixed well and incubated at 37°C for 24 to 48 h.
3. Standard stock glucose solution: 100mg of glucose dissolved in 100ml distilled water. Working standard was prepared by dilution of 10 ml of standard stock to 100ml with distilled water (100µg/ml)

100 mg of plant sample was homogenized in 10 ml of 80 % ethanol. The supernatant was collected after centrifugation of homogenates at 5000 rpm for 20 min. The collected supernatant was evaporated on water bath. 10 ml of water was added. Then 0.1 ml of aliquot was pipetted out in separate test tubes. The final volume was made to 1 ml with the help of distilled water. The alkaline copper tartrate reagent (1 ml) was added in the test tube. The tubes were placed in a boiling water bath for 10 minutes. After cooling, 1 ml of arsenomolybdate reagent was added in the test tube. Finally the solution was diluted to 10 ml with double distilled water. The absorbance reading of blue colour was taken at 620 nm. For blank the distilled water was used along with the entire reagents except enzyme extract. The amount of total reducing sugar was calculated with the help of standard curve. 100 mg of the glucose was dissolved in 100 ml of distilled water or working standard was prepared by dilution of 10 ml of standard glucose stock with 100 ml of distilled water. From this stock solution different concentrations of the sugar solution prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in separate test tubes. The final volume of these test tubes was made by 1 ml by adding of distilled water. The standard curve was prepared by plotting the absorbance value at 620 nm on y-axis, against the concentration of glucose on x-axis.

### Results and Discussion

Effect of polyamine (putrescine), mycorrhiza and their combination on total reducing sugar (mg g<sup>-1</sup> Fresh Weight) was studied in sorghum variety CSV15 during the two subsequent years under the cadmium stress. Data were recorded at 30, 60 and 90 days after sowing (DAS) (Fig.1a & 1b). During the first year, it is evident that the average total reducing sugar content was significantly reduced by 22.39%, 19.94% and 17.98% when exposed to heavy metal stress (T6) as compared to control (T0) at 30, 60 and 90 DAS of interval. Similarly, when plants were exposed to higher dose of heavy metal (T12) then its total reducing sugar was significantly reduced by 56.25, 50.10% and 45.17% as compared to control (T0) on the dates of proposed interval. Exogenous application of endomycorrhiza in the soil (T7) showed the mitigation effect by increasing the total reducing sugar content by 0.196%, 0.175% and 0.158% as compared to T6 at 30, 60 and 90 DAS. When treatment, T13 was compared to T12, the total reducing sugar content increased significantly by 0.98%, 0.87% and 0.79% at proposed DAS. In comparison to T6, the exogenous application of putrescine (T8) showed mitigating effect by increasing total reducing sugar content by 1.13%, 1.00% and 0.90% on proposed DAS. The average total reducing sugar content was significantly enhanced as compared to T6 by 2.36%, 2.10% and 1.89% when treated with higher dose of putrescine (T9) with respect to T8. Similarly, when treatment, T14 was compared with T12, the total reducing sugar content increased significantly with 5.61%, 4.99% and 4.50% at proposed DAS. The average total reducing sugar was significantly enhanced as compared to T12 by 9.30%, 8.28% and 7.47% when treated with higher dose of putrescine (T15) with respect to T14. The combination of putrescine and mycorrhiza showed the best mitigation effect in treatment T10 by increasing total reducing sugar content by 4.97%, 4.42% and 3.99% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6 then significant total reducing sugar content was increased by 5.31%, 4.73% and 4.26%, respectively. Similar effect was seen in the treatment (T16) with respect to treatment T12. In this treatment (T16), the total reducing sugar content was found to increase significantly by 9.44%, 8.41% and 7.58%, respectively at proposed DAS. The treatment T17 was found to show better results; significant increase in total reducing sugar content by 14.22%, 12.66% and 11.42% with respect to T12 was observed. So, the combination of putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the total reducing sugar content. The similar trends were found during the study made in the second year of the experiment. Similar increase in reducing sugar and starch at higher levels of Zn treatment in *Hyptis suaveolens* was reported by Kumar *et al.*, 2011b, Siddique *et al.*, 2018, Pathak *et al.*, 2017, Kumar and Dwivedi, 2018d. These changes could be attributed to zinc induced disturbance of carbohydrate metabolism and interference with the export of photo-assimilate from source to the sink as reported by Kumar *et al.*, 2013, 2012, 2011a) in *Phaseolus vulgaris* exposed to excess metal ion. Accumulation of reducing sugars was reported in maize under Ni stress (Kumar *et al.*, 2016a, b) <sup>[11, 12]</sup>, in rice under Cd stress (Kumar, 2018, Kumar and Dwivedi, 2014, Kumar *et al.*, 2018d) <sup>[1-3]</sup>.



**Fig.1a & 1b:** Total Reducing Sugar ( $\text{mg g}^{-1}$  fresh weight) of sorghum during *Kharif* of two subsequent year of experiment [left to right]

Where, DAS=Days after sowing. Data are in the form of Mean  $\pm$  SEM. S=Significance at  $P \leq 0.05$  and  $P \leq 0.01$ , NS= Non Significant at  $P \leq 0.05$  and  $P \leq 0.01$  using Origin 6.1. T0= Control, T1=Control + Mycorrhiza, T2=Control + 2.5mM Putrescine, T3=Control + 5mM Putrescine, T4= Control + 2.5mM Putrescine + Mycorrhiza, T5=Control + 5mM Putrescine + Mycorrhiza, T6=0.07%  $\text{Cd}(\text{NO}_3)_2$ , T7=0.07%  $\text{Cd}(\text{NO}_3)_2$  + Mycorrhiza, T8=0.07%  $\text{Cd}(\text{NO}_3)_2$  + 2.5mM Putrescine, T9=0.07%  $\text{Cd}(\text{NO}_3)_2$  + 5mM Putrescine, T10=0.07%  $\text{Cd}(\text{NO}_3)_2$  + 2.5mM Putrescine + Mycorrhiza, T11=0.07%  $\text{Cd}(\text{NO}_3)_2$  + 5mM Putrescine + Mycorrhiza, T12=0.15%  $\text{Cd}(\text{NO}_3)_2$ , T13=0.15%  $\text{Cd}(\text{NO}_3)_2$  + Mycorrhiza, T14=0.15%  $\text{Cd}(\text{NO}_3)_2$  + 2.5mM Putrescine, T15=0.15%  $\text{Cd}(\text{NO}_3)_2$  + 5mM Putrescine, T16=0.15%  $\text{Cd}(\text{NO}_3)_2$  + 2.5mM Putrescine + Mycorrhiza, T17= 0.15%  $\text{Cd}(\text{NO}_3)_2$  + 5mM Putrescine + Mycorrhiza

### Conclusion

Polyamines like putrescine and mycorrhiza *Glomus* impart significant mitigation of cadmium induced toxicity in sorghum mediated through their defensive role in plants by increasing the total soluble sugar in the sorghum leaves.

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