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Effect of polyamines and endo-mycorrhiza on chlorophyll a, b ratio and total carotenoids in leaves of *Sorghum* grown under cadmium toxicity

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Abstract
The present study was carried out to evaluate the compatibility of polyamines and mycorrhiza in the mitigation of 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 chlorophyll a, b ratio and average of total carotenoids (mg g^{-1} fresh weight). The reverse responses were observed d by the external application of putrescine (2.5 and 5.0 mM) and mycorrhiza (Glomus; 150 inoculants per kg of soil).

Keywords: Agriculture, cadmium, density, economy, foliar, gap, higher

Introduction

Sorghum is one of the most important staple foods for the world's poorest and most food-unsecured people across the semi-arid tropics (Kumar *et al*., 2016a) [11]. Heavy metal contamination threats 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) [8, 16, 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 supermolecule and protecting alkaloids (Kumar and Dwivedi, 2018a, b, c, d, Kumar *et al*., 2018b) [4, 5, 3, 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) [4, 6, 15]. 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) [1, 9, 10, 17]. Metallic element (Cd) may be an extremely deadly element and has been hierarchal seventh among the highest twenty toxins (Kumar and Dwivedi, 2018a) [4]. Metallic element may be a doubtless deadly metal and so its transfer from plants to humans is of major concern.

Material 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 Origin6.1-advance scientific graphing and knowledge analysis [Origin Lab Corporation, One RoundHouse Plaza, Northhampton, MA 01060]. Multivariate analysis was performed for interaction between mycorrhiza and metallic element treatments. One way multivariate analysis was performed.

Chlorophyll content (mg g^{-1} fresh weight)

The chlorophyll content in the leaf of *Sorghum* was estimated by the method of Arnon DI. (1949). Chlorophyll is extracted in 80% acetone and the absorbance is measured at 645 nm and 663 nm. The amount of chlorophyll is calculated using the absorbance coefficient. Chlorophyll was extracted from 100 mg of the sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 10 min. The extraction was repeated until the residue became colourless. The volume in the flask was made up to 100 ml with 80% acetone. The absorbance of the extract was read using spectrophotometer (Spectramax M2) at 645 and 663nm against 80% acetone blank. The amount of the chlorophyll content was calculated by using the formula as given below.

Chlorophyll 'a' (mg/g Fresh Weight) = $12.7(A663)-2.69(A645) \times \frac{V}{1000 \times W}$

Chlorophyll 'b' (mg/g Fresh Weight) = $22.9(A645)-4.68(A663) \times \frac{V}{1000 \times W}$

Total chlorophyll (mg/g Fresh Weight) = $20.2(A645) + 8.02(A663) \times \frac{V}{1000 \times W}$

where,
V= final volume of the extract
W= fresh weight of the leaves
A= Absorbance at the specific wavelength
The value are expressed as the mg/g fresh weight

Total Carotenoids content (mg g^{-1} fresh weight)

The method described by Jensen A. (1978) for determination of total Carotenoids in the plant sample was followed. The total carotenoids was extracted and portioned in organic solvent (acetone or methanol) on the basis of their solubility. Carotenoids that are bound as esters are hydrolyzed using aqueous 60% KOH. The amount of the carotenoids present in sample was estimated calorimetrically at 450 nm using ß-carotene as a standard. Cut the fresh plant material and grind a known amount (2g) in a mortar with 20 ml of either distilled acetone or methanol. Filter on a Buchner funnel through Whatman No. 42 filter paper. Repeat the extraction until the tissue is free from pigments. Pool the filtrate thrice with equal volume of peroxide free ether using a separator funnel. Evaporate the combined ether layer (which contains the Carotenoids) under reduced pressure at 35ºC in a rotary evaporator or in a hot water bath. Dissolve the residue in minimum quantity of ethanol. Add 60% aqueous KOH at the rate of 1ml for every 10ml, of the ethanol extract to saponify. Keep the mixture in dark and leave it overnight at room temperature. Add equal volume of water and partition with ether. Evaporate the combined ether layer as before and dissolve the residue in minimum volume of ethanol. Measure the absorbance of this solution at 450nm and calculate the Carotenoids content in the sample using a calibration curve prepared against a high purity of ß-carotene. One gram of ß-carotene was dissolved in 100 ml of acetone or methanol solvent. From this stock solution different concentrations of ß-carotene solution were prepared by taking 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock 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 450 nm on y-axis, against the concentration of ß-carotene on x-axis.

Results and Discussion

Effect of polyamine (putrescine), mycorrhiza and their combination on chl a/b ratio (mg/g 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 & b). During first year, it is evident that the average chl a/b ratio was significantly reduced by 27.2%, 22.6% and 19.2% 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 chl a/b ratio was significantly reduced by 54%, 45.3% and 41.8% 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 chl a/b ratio by 2.86%, 2.38% and 2.02% as compared to T6 at 30, 60 and 90 DAS. When treatment, T13 was compared to T12, the chl a/b ratio increased significantly by 2.86%, 2.38% and 2.02% at proposed DAS. In comparison to T6, the exogenous application of putrescine (T8) showed mitigating effect by increasing chl a/b ratio by 8.59%, 7.15% and 6.07% on proposed DAS. The average chl a/b ratio was significantly enhanced as compared to T6 by 11.46%, 9.54% and 8.09% when treated with higher dose of putrescine (T9) with respect to T8. Similarly, when treatment, T14 was compared with T12, the chl a/b ratio increased significantly with 4.29%, 3.57% and 3.03% at proposed DAS. The average chl a/b ratio was significantly enhanced as compared to T12 by 7.16%, 5.96% and 5.06% 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 chl a/b ratio by 12.89%, 10.73% and 9.10% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6 then significant chl a/b ratio was increased by 414.32%, 11.93% and 10.12%, respectively. Similar effect was seen in the treatment (T16) with respect to treatment T12. In this treatment (T16), the chl a/b ratio was found to increase significantly by 10.02%, 8.35% and 7.08%, respectively at proposed DAS. The treatment T17 was found to show better results; significant increase in total carotenoids content by 11.46%, 9.54% and 8.09% 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 chl a/b ratio. The similar trends were found during the study made in the subsequent second year. Kumar *et al*., 2018a, b, c [4, 5, 3], reported that, Chlorophyll a+b content was increased by 100µM Cd but reduced by 200 µM Cd in mature maize leaves and reduced by both concentrations in aging leaves (Kumar, 2018; Kumar and Dwivedi, 2014, 2018a, b, c, d; Kumar *et al*., 2011a, b, 2012, 2013, 2016a, b, Pathak, 2017) [1, 4, 5, 3, 14, 8, 16, 15]. Low concentration of cadmium (0.05 and 0.1 µM) also has stimulating effect on chlorophyll synthesis and photosynthetic activity (Siddique *et al*., 2018, Kumar *et al*., 2018) [17, 1].

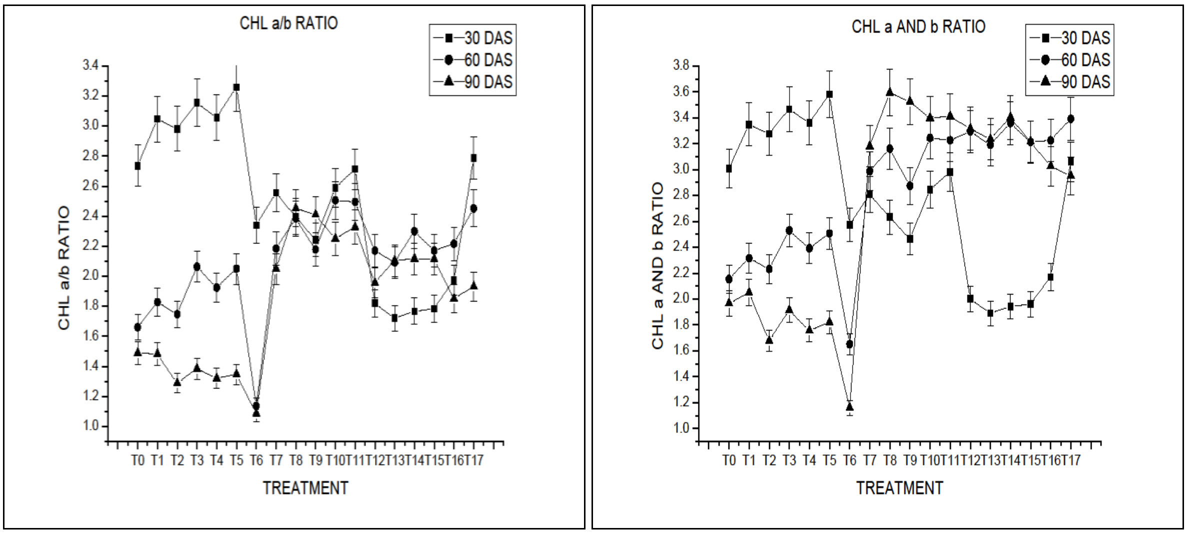

Fig 1a & b: Chlorophyll a/b ratio of *Sorghum* during *Kharif* season of subsequent two years [left to right]

where, DAS=Days after sowing. Data are in the form of Mean ± 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% $Cd(NO_3)_2$, T7=0.07% $Cd(NO_3)_2$ + Mycorrhiza, T8=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine, T9=0.07% $Cd(NO_3)_2$ + 5mM Putrescine, T10=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T11=0.07% $Cd(NO_3)_2$ + 5mM Putrescine + Mycorrhiza, T12=0.15% $Cd(NO_3)_2$, T13=0.15% $Cd(NO_3)_2$ + Mycorrhiza, T14=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine, T15=0.15% $Cd(NO_3)_2$ + 5mM Putrescine, T16=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T17= 0.15% $Cd(NO_3)_2$ + 5mM Putrescine + Mycorrhiza

Effect of polyamine (putrescine), mycorrhiza and their combination on total carotenoids content (mg/g Fresh Weight) was studied in *Sorghum* variety CSV15 during the first years under the cadmium stress. Data were recorded at 30, 60 and 90 days after sowing (DAS) (fig.2a & b). During first year, it is evident that the average total carotenoids content was significantly reduced by 27.2%, 22.6% and 19.2% 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 carotenoids content was significantly reduced by 54%, 45.3% and 41.8% 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 carotenoids content by 2.86%, 2.38% and 2.02% as compared to T6 at 30, 60 and 90 DAS. When treatment, T13 was compared to T12, the total carotenoids content increased significantly by 2.86%, 2.38% and 2.02% at proposed DAS. In comparison to T6, the exogenous application of putrescine (T8) showed mitigating effect by increasing total carotenoids content by 8.59%, 7.15% and 6.07% on proposed DAS. The average total carotenoids content was significantly enhanced as compared to T6 by 11.46%, 9.54% and 8.09% when treated with higher dose of putrescine (T9) with respect to T8. Similarly, when treatment, T14 was compared with T12, the total carotenoids content increased significantly with 4.29%, 3.57% and 3.03% at proposed DAS. The average total carotenoids content was significantly enhanced as compared to T12 by 7.16%, 5.96% and 5.06% 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 carotenoids content by 12.89%, 10.73% and 9.10% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6 then significant total carotenoids content was increased by 414.32%, 11.93% and 10.12%, respectively. Similar effect was seen in the treatment (T16) with respect to treatment T12. In this treatment (T16), the total carotenoids content was found to increase significantly by 10.02%, 8.35% and 7.08%, respectively at proposed DAS. The treatment T17 was found to show better results; significant increase in total carotenoids content by 11.46%, 9.54% and 8.09% 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 carotenoids content. The similar trends were found during the study made in the second year. Pathak *et al*., 2017 [15], reported that, carotenoids which protect the chlorophyll from photo-oxidative destruction and a reduction in carotenoids could have serious consequences on chlorophyll pigments in presence of Cd (Kumar and Dwivedi 2016a, b; Kumar, 2018, Kumar *et al*., 2012, 2013, 2018 a, b, c, d) [11, 12, 1, 9, 10, 4, 5, 3, 14]. In maize, Cd inhibits chlorophyll biosynthesis (Pathak *et al*., 2017) [15]. It reduces both the total chlorophyll and the carotenoids contents, through the latter decrease to a lower extent, thus leading to a drop in the chlorophyll: carotenoids ratio (Siddique *et al*., 2018) [17].

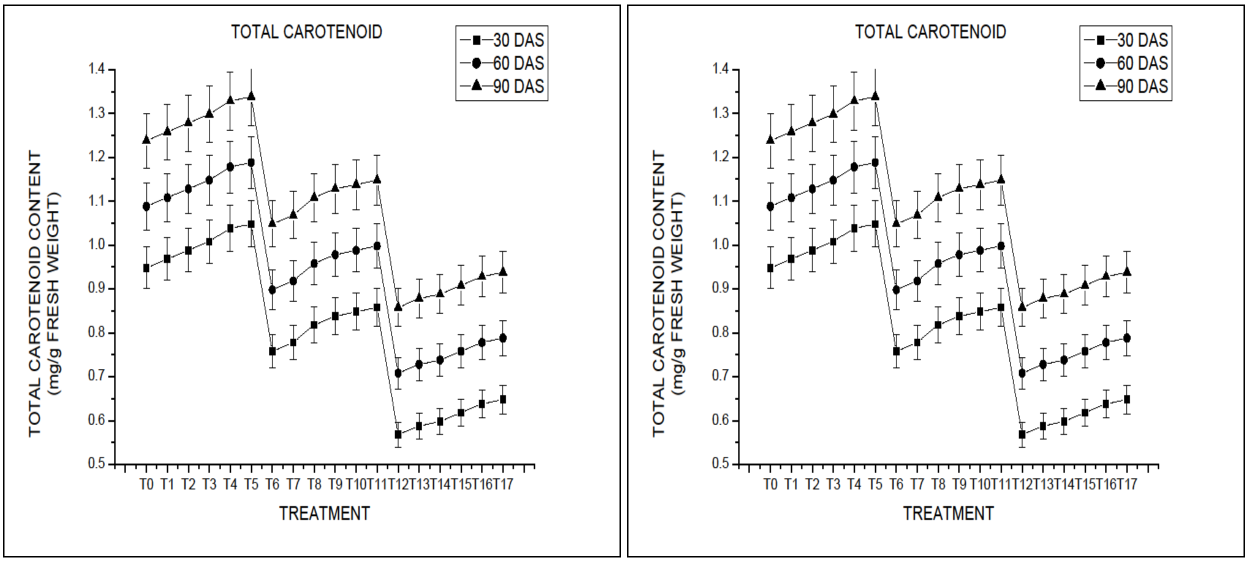

Fig 2a & b: Total carotenoids of *Sorghum* during *Kharif* season of subsequent two years [left to right]

where, DAS=Days after sowing. Data are in the form of Mean ± 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% $Cd(NO_3)_2$, T7=0.07% $Cd(NO_3)_2$ + Mycorrhiza, T8=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine, T9=0.07% $Cd(NO_3)_2$ + 5mM Putrescine, T10=0.07% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T11=0.07% $Cd(NO_3)_2$ + 5mM Putrescine + Mycorrhiza, T12=0.15% $Cd(NO_3)_2$, T13=0.15% $Cd(NO_3)_2$ + Mycorrhiza, T14=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine, T15=0.15% $Cd(NO_3)_2$ + 5mM Putrescine, T16=0.15% $Cd(NO_3)_2$ + 2.5mM Putrescine + Mycorrhiza, T17= 0.15% $Cd(NO_3)_2$ + 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 chlorophyll a & b ratio and total carotenoids content in the *Sorghum* leaves.

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