Title: The Cumulative Dose of AMF and GB is More Effective in The Amelioration of Cr(VI) Toxicity in Sorghum (Sorghum Bicolor L.) Than Individually

Praveen Kumar (praveenkhola@hau.ac.in)
Chaudhary Charan Singh Haryana Agricultural University

Research Article

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Abstract

Industrial and anthropogenic activities are the major source of heavy metal toxicants in agricultural soils. Among, heavy metal toxicants, hexavalent chromium is the most toxic toxicant that negatively affects plant's metabolic activities and yield. It reduces the plant growth and development by influencing the antioxidant defence system's activities. In the present experiment, two different soil applied dozes of GB *viz.* 50 and 100mM, and AMF, both individually and in combination were tested for their capability to ameliorate Cr toxicity in sorghum. The promotive behaviour of these treatments for antioxidant defence system was analysed at vegetative (35 DAS) and grain filling stage (95 DAS) in three varieties of sorghum *viz.* SSG 59-3, HJ 513 (multi-cut) and HJ 541 (single-cut) under 2 and 4 ppm Cr toxicity. At the same time resultant effects of this behaviour on Cr accumulation, grain yield and indices of oxidative stress was also studied. In this experiment antioxidant defence system includes enzymes *viz.* SOD, APX, CAT, GR, POD and metabolites *viz.* glutathione, ascorbate, proline, β-carotene and indices of oxidative stress includes parameters *viz.* PPO, H_2O_2 and MDA. The results delineated that Cr accumulation and indices of oxidative stress were increased with increasing concentration of Cr stress in all the varieties at both growth stages. Chromium stress at high concentration (4 ppm), decreased the grain yield (71.69 %) as compared with control. Due to 4 ppm Cr stress, PPO activity, MDA and H_2O_2 accumulation increased significantly (72.29 %, 73.15 %, 79 % respectively, at 35 DAS and 70.36 %, 74.78 %, 79.83 % respectively, at 95 DAS). GB and AMF individually increased antioxidant activity but in combination, further significantly increased antioxidant defence system's activity which in turn decreased indices of oxidative stress and reduced the Cr toxicity and increased grain yield of sorghum in all varieties at both the growth stages. However, treatment of 100mM GB with AMF was observed most significant in decreasing oxidative stress and improved antioxidant system's activities and grain yield as compared with all other treatments at both growth stages in all the varieties. SSG 59-3 cultivar showed lowest chromium content (1.60 and 8.61 ppm), indices of oxidative stress and highest antioxidant system's activity as compared to HJ 513 followed by HJ 541 variety, at 35 and 95 DAS respectively. Thus, among the varieties SSG 59-3 was found most tolerant as compared to HJ 513 followed by HJ 541 variety. These findings suggest that both GB and AMF, either individually or combined can play a positive role to reduce oxidative stress and increased yield attributes under Cr toxicity in sorghum.

Introduction

Sorghum crop [*Sorghum bicolor* (L.) Moench] a member of family Poaceae, is grown worldwide in over 41.14 million hectares of area and accounts for production of 58.72 million metric tons of grains with an average yield of 1.43 metric tons per hectare [1]. India ranks second in terms of area under sorghum cultivation. In India, Sorghum is cultivated in over 5.00 million hectares and accounts for production of 4.50 million metric tons of grains with an average yield of 0.90 metric tons per hectares [2]. Sorghum is one of the five top cereal crops and is consumed after rice, wheat, maize and barley [3]. It is an important kharif season crop which is directly or indirectly utilized for nourishment of humans, animals [4]. Sorghum is a C4 plant that is highly efficient in converting solar energy to chemical energy, and also in

water use efficiency [5]. The food quantity supplied from sorghum is 4480723.16 tonnes per year or 9.8g per capita per day, in India [6]. All these features make it a capable crop to meet the growing food demands, globally.

But now a day's increased chromium contamination of agricultural soils is showing damaging effects on crop growth [7]. Chromium toxicity has become a global concern. It exist in two stable forms *viz*. trivalent Cr (III) and the hexavalent Cr (VI). Both stable forms of Cr may cause toxicity to plants, but Cr (VI) is considered most toxic form of Cr due to its high solubility and more unstable nature [8]. Chromium is generally found linked with oxygen as oxyanions of chromates (CrO_4^{2-}) or dichromates ($Cr_2O_7^{2-}$) in organic matter in soil and aquatic environments [9]. The two main forms of Cr can interchange and coexist in a dynamic balance regulated by oxidation/reduction, precipitation/dissolution and adsorption/desorption. The redox reaction is particularly active in the rhizosphere, where different bacterial reductase enzymes are present, and/or released by plant roots [10]. The main sources of Cr (VI) in the biosphere are leather and paint industries. Annually, approx. 2000 to 32000 tons of Cr is released in the environment from Indian tanning industries only [11]. The allowable dose of Cr in water are set as 8 μg L^{-1} for Cr (III) and 1 μg L^{-1} for Cr (VI). Chromium toxicity value ranges from 21 to 47 ppm whereas the permissible value is 0.05 to 0.5 ppm both in water and soil in Haryana [12].Chromium (VI) acts as a strong oxidant possessing higher redox potential between 1.33 to 1.38 eV causing rapid ROS generation and resultant toxic effects in plants and animal [13]. Major Cr contaminated cities in India include Ranipet (Tamil Nadu), Kanpur (Uttar Pradesh), Vadodara (Gujarat), Talcher (Odisha), Sonepat, Dharuhera, Shahbad, Faridabad, Gurgaon, Yamunanagar, Karnal, Panchkula, and Panipat (Haryana) where poor plant growth of field crops has been observed [14]. Chromium concentration above 0.5 ppm in soil and water starts producing toxic effects in plants [12]. Therefore the present research work was planned on 2 and 4 ppm of chromium levels.

Chromium stress in plants is characterized by the decrease in photosynthesis, nutrient uptake, damaging of roots and finally plant death [15,16]. Some of well-established phytotoxic manifestations include generation of reactive oxygen species (ROS), replacement of enzyme cofactors and transcription factors, inhibition of antioxidative enzymes, cellular redox imbalance, ionic transport imbalance, DNA damage and protein oxidation [17,18]. These active molecules are involved in the free radical chain reaction of membrane lipids and proteins, thus causing their oxidative decomposition [19,20]. Plants possess several antioxidant defence systems to protect their cells against ROS and one such system is accumulation of a variety of small organic metabolites that are collectively referred to as compatible solutes [21]. Compatible solutes include sugars, polyols, glycine betaine (GB), amino acids (proline, histidine) and related compounds [22]. It has been reported that level of GB increased in plants subjected to abiotic stresses [23,24]. GB is a quaternary ammonium compound that is found in plants and mammals etc. [25]. GB functions as osmoprotectant which suppresses production of ROS. GB counteracts the oxidative stress in plants by elevating the level of proline and antioxidant enzymes like catalase (CAT), Peroxidase (POD) and Superoxide dismutase (SOD). It is a very effective osmoregulating substance [26]. Its level varies considerably among plant species. Many plant species, do not accumulate GB, either in normal or

under stressful conditions. In some plants, the natural accumulation of GB, is not enough to protect them from abiotic stresses. Under such conditions, exogenous application of GB may help to reduce the adverse effects of various environmental stresses [27]. GB is environmentally safe, nontoxic, and water-soluble [28]. There is strong evidence that GB plays an important role in plants against tolerance to abiotic stresses [29]. Previous studies on amelioration of heavy metal toxicity using GB in plants, suggested that 50 and 100 mM concentration was effective in the amelioration of heavy metal toxicity [30]. Furthermore, Arbuscular mycorrhizal fungi are recognized as biological agents that potentially increase the tolerance of plants to heavy metal toxicity [31]. The reduction of growth due to chromium interference with nutritional elements uptake can be improved through mycorrhizal inoculation. Karagiannidis and Hadjisavva-Zinoviadi, [32] showed that arbuscular mycorrhizal fungi (AMF) can enhance yield by simultaneously reducing the chromium content in crop plants. However, no one have reported about amelioration of hexavalent Cr toxicity by using combined dose of GB and AMF in sorghum. In this research, we tested the hypothesis that whether the combination of GB and AMF ameliorates Cr toxic effects and improves the yield in sorghum. Outcomes of our research would possibly depict a potential way to prevent Cr toxicity in sorghum.

Materials And Methods

Plant material selection

The present research was conducted in screen house of the department of biochemistry, college of basic sciences & humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana (India). Three varieties of sorghum (*Sorghum bicolor* L.) viz. HJ-541, HJ 513 and SSG 59-3 were procured from forage section of university. These varieties were selected because they are the only source of forage in dryland during the summer season and they are widely grown in Haryana region. Also, SSG 59-3 is sweeter than HJ 513 (multi-cut) variety and HJ 541 (single-cut) variety. Moreover, HJ 541 is suitable for both grain and fodder yield while HJ 513 is more suitable for grain yield. However, there are no reports about the sensitivity of these three cultivars for GB and AMF, under Cr (VI) toxicity. The toxic effects of hexavalent Cr, observed on sorghum plant growth along with possible reasons are depicted in Fig 1.

Experimental details and raising of the crop

Three varieties of sorghum at two growth stages viz. vegetative (35 DAS) and grain filling (95 DAS) stages were tested for amelioration of chromium toxicity (2 & 4 ppm) by exogenous application of GB (50 & 100 mM) and AMF in soil both individually and their combination, in completely randomized block design. The seeds of uniform size were selected and surface sterilized with 0.01 % mercuric chloride ($HgCl_2$) solution for 10 minutes, followed by 5 times washing with distilled water. The plants were raised in earthen pots lined with polyethylene bags filled with 5 kg sandy loam, acid (5 % HCL) washed soil. The sterilised seeds were sown at 2 cm depth in the pots. Two weeks old seedlings of same size were transferred to other pots containing 5 kg soil. Soil properties are mentioned in Table 1. Separate pots were kept for control plants. Three replications were maintained for each treatment and control. All pots were

irrigated with equal quantities of water and nutrient solution as per recommended package of practices (POP).

Table 1. Physicochemical properties of soil used during present experiment

Property	Value & unit	Evaluation
Texture	-	Sandy loam
Sand	71.70 %	-
Silt	18.96 %	-
Clay	9.34 %	-
pH	8.2	Basic
OC	0.32	Low
EC	0.17 DS $meter^{-1}$	Normal
Nitrogen (N)	3 mg kg^{-1} soil	Low
Phosphorus (P)	8 mg kg^{-1} soil	Low
Potassium (K)	84 mg kg^{-1} soil	Normal
Zink (Zn)	0.61 mg kg^{-1} soil	Normal
Iron (Fe)	0.7 mg kg^{-1} soil	Low
Copper (Cu)	0.18 mg kg^{-1} soil	Normal
Manganese (Mn)	2.73 mg kg^{-1} soil	Normal
Chromium (Cr)	0.016 mg kg^{-1} soil	Low

Chemicals and reagents

The chemicals and reagents used during this research work were of high analytical grade. All the chemicals were procured from Sigma Chemicals Co. USA, Sisco Research Laboratories (SRL), Hi-Media and E. Merck Ltd.

Treatments and growth conditions

During present research, the treatments were provided on the basis of procedures followed in previous experiments [15]. The detailed composition of treatments used in this experiment is given in Table 2.

Table 2. Treatments details of AMF and GB provided in soil prior to plantation.

Treatment Name	Treatment Composition	
C	Control	Control + AMF
T1	GB (50 mM)	GB (50 mM) + AMF
T2	GB (100 mM)	GB (100 mM) + AMF
T3	Cr (2 ppm)	Cr (2 ppm) + AMF
T4	Cr (2 ppm) + GB (50 mM)	Cr (2 ppm) + GB (50 mM) + AMF
T5	Cr (2 ppm) + GB (100 mM)	Cr (2 ppm) + GB (100 mM) + AMF
T6	Cr (4 ppm)	Cr (4 ppm) + AMF
T7	Cr (4 ppm) + GB (50 mM)	Cr (4 ppm) + GB (50 mM) + AMF
T8	Cr (4 ppm) + GB (100 mM)	Cr (4 ppm) + GB (100 mM) + AMF

Chromium stress treatments: Potassium dichromate salt ($K_2Cr_2O_7.7H_2O$) procured from Sigma Ltd. company, was used with distilled water to make two different levels of Cr stress solution (2 and 4 ppm). The soil in each pot was treated with 1 litre of respective, out of these two different levels of Cr stress solutions just after plantation of seedling. Level of respective stress was maintained by supplying respective Cr solution in the respective pots within the 7 days interval.

Glycine betaine treatments: Exogenously GB (50 and 100 mM) stalk solutions were prepared with distilled water and 1 litre of this from each was supplied in soil of respective pots just after plantation of seedling. The level of respective concentration of GB was maintained by supplying respective GB solution in the respective pots within a week interval.

Arbuscular mycorrhizal fungi (AMF) treatment: The AMF was supplied exogenously in soil before plantation of seedling. The treatment of AMF was provided by mixing 10 g of medium containing AMF in soil per pot. Generally, AMF can grow itself in the moist medium of soil and may increase their levels with time passes. So it was applied only once at the time of plantation of seedling in pots.

Plant sampling and analysis

The plant samples from control and each treatment, were collected at 35 and 95 DAS. A complete plant was collected in an ice cooled thermacol box. It was further divided in to leaf, shoot and root. Fresh leaves were used for the estimation of antioxidative enzymes, metabolites and indices of oxidative stress parameters. Shoot samples were hand homogenised and used immediately for the estimation of enzymes activity. Leaf, stem and root samples were dried in an oven for 72 h at 70 °C then Cr contents were estimated separately. The data was analysed by using a three-factorial, analysis of variance ANOVA, CRD design in SPSS software. Significant ($P \leq 0.05$) differences between treatments were determined using critical difference.

Determination of soil properties

The soil was analysed for texture, pH, electrical conductivity, organic carbon, N, P, K, Fe, Mn, Cu, Zn and Cr (Table 2). Texture was determined by International Pipette method [65]. The pH of the soils was measured with glass electrode using soil suspension of 1:2 (soil: water) and electrical conductivity in supernatant as given in [66]. Organic carbon was determined by wet-oxidation method of Walkely and Black, [67]. Available nitrogen (N) was determined by alkaline permanganate method [68], available P content was determined by extracting the soil samples using 0.5M $NaHCO_3$ and analysed by spectrophotometer [69] and available potassium was extracted by using neutral normal ammonium acetate and the content was determined by aspirating the extract into flame photometer. The available forms of Fe, Mn, Cu, Zn and Cr were extracted by DTPA at pH 7.3 and determined using atomic absorption spectrometer [70].

Determination of chromium contents

Chromium content was estimated in plant tissue (leaf, stem and roots) sample by using atomic absorption spectroscopy technique [70]. Five hundred mg tissue sample along with 20 ml digestion mixture (nitric acid and perchloric acid in 4:1 ratio, respectively) was digested overnight in a 100 ml conical flask at room temperature, followed by heating on an electric heater until a very small amount and colourless mixture (2-3 ml) was left in the flask. After cooling the total volume was made up to 25 ml with distilled water. The chromium content was determined in this digested mixture by calibration of standards of Cr (VI) in the form of potassium dichromate in the range 0 – 6 mg L^{-1} in water, and comparing with samples through atomic absorption spectroscopy (AAS). The results were expressed in ppm.

Determination of the enzymatic antioxidants

Following enzymatic antioxidants parameters were studied at vegetative and grain filling stage in sorghum plants.

Extract preparation for the estimation of enzymatic antioxidants: The complete extraction procedure was carried out below 4^0C. Two g of fresh and cleaned leaf tissue was homogenised in 10 ml of 0.1 M potassium phosphate buffer (pH-7.0) by using previously chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected as crude extract and stored in refrigerator for total soluble protein estimation. It was used for enzyme assay at same time.

Superoxide dismutase (SOD): The enzyme is a metalloprotein, which catalyses the dismutation of superoxide radical to H_2O_2 and molecular oxygen. It is a key antioxidant in aerobic cells and establishes the first line of defence against reactive oxygen species (ROS). Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) following the method of Beauchamp and Fridovich, [71]. The 3.0 ml reaction mixture contained 2.5 ml of 60 mM Tris-HCl (pH 7.8), 0.1 ml each of 420 mM L-methionine, 1.80 mM NBT, 90 µM riboflavin, 3.0 mM EDTA and enzyme extract. Riboflavin was added at the end. The tubes were shaked properly and placed 30 cm

below light source consisting of three 20 W-fluorescent lamps (Phillips, India). The reaction was started by switching-on the light and terminated after 40 min of incubation by switching-off the light. After terminating the reaction, the tubes were covered with black cloth to protect them from light. A non-irradiated reaction mixture was kept that did not develop any colour and served as control. A separate blank was prepared for each sample, simultaneously by taking boiled enzyme extract. The reaction mixture without enzyme extract had developed maximum colour and its absorbance was decreased with the addition of enzyme. The amount of inhibition was used to quantify the enzyme. The absorbance were record at 560 nm. The Log A560 were plotted as a function of volume of enzyme extract used for reaction mixture. The volume of enzyme extract used in 50% inhibition of the photo-chemical reaction was considered as one enzyme unit. One enzyme unit was defined as the amount of enzyme required to inhibit the photo-reduction of one µmole of NBT. The enzyme activity was expressed in terms of unit g^{-1} fresh weight and were converted to unit mg^{-1} protein by estimating the total soluble proteins in the sample. The percent inhibition was calculated by following formula of Asada *et al*. [72].

$$Percent\ inhibition = \frac{V - v}{v} \times 100$$

V = Rate of assay reaction in absence of SOD.

v = Rate of assay reaction in presence of SOD.

Ascorbate peroxidase activity (APX): Ascorbate peroxidase is most widely distributed antioxidant enzyme. It reduces hydrogen peroxide to water using reduced ascorbate as the electron donor. It plays an important role in scavenging ROS than other antioxidative enzymes since ascorbate, in addition to reacting with H_2O_2 may react with superoxide, singlet oxygen and hydroxyl radical. Ascorbate peroxidase was assayed by the method of Nakano and Asada, [73]. Three ml reaction mixture contained 2.7 ml of 100 mM potassium phosphate buffer (pH 7.0), 0.1 ml L-ascorbate and 0.15 ml H_2O_2. The reaction was initiated by adding 50 µl of enzyme extract. Decrease in absorbance were recorded at 290 nm spectrophotometrically for 2 min against a suitable blank. A separate blank was prepared for each sample, simultaneously by taking boiled enzyme extract. The enzyme activity was calculated, using the molar extinction coefficient (Absorbance of one molar solution) of 2.8 mM^{-1} cm^{-1} for ascorbate in the standard equation for absorbance. One enzyme unit corresponds to the amount of enzyme required to oxidize one nmol of ascorbic acid min^{-1}.

Standard equation for absorbance as $A = \varepsilon \times ⍁ \times c$

Where, A is the amount of light absorbed by the sample at a given wavelength, ε is the molar extinction coefficient, ⍁ is the distance that the light travels through the solution, and c is the concentration of the absorbing species.

Catalase activity (CAT): The enzyme catalase scavenges highly toxic hydrogen peroxide, produced in a number of reactions in the cell. Thus preventing metabolic machinery of the cell. It detoxifies hydrogen

peroxide without overwhelming cellular reducing equivalents and provides cell with energy efficient mechanism to remove hydrogen peroxide. It exists profusely in plant tissues and its activity is connected with peroxisomes where, it removes hydrogen peroxide produced during photorespiration. The activity of enzyme was measured by slightly modified method of Sinha, [74]. The reaction mixture contained 0.55 ml of 0.1 M potassium phosphate buffer (pH 7.0), 0.4 ml of 0.2 M hydrogen peroxide and 50 µl of enzyme extract. It was mixed thoroughly and incubated for one minute at room temperature followed by addition of 3.0 ml dichromate reagent to it. A separate reaction was run for control, comprising 0.6 ml potassium phosphate buffer and 0.4 ml hydrogen peroxide (0.2 M), without enzyme extract. The tubes were kept in boiling water bath for 10 min. After cooling, the absorbance were recorded at 570 nm using a suitable blank containing boiled enzyme extract. The absorbance of sample were subtracted from that of control and the amount of hydrogen peroxide was calculated from standard curve. One enzyme unit correspond to the amount of enzyme required to breakdown one µmole of hydrogen peroxide min^{-1} or mg^{-1} protein.

Glutathione reductase activity (GR): Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in a NADPH dependent reaction. Glutathione reductase was assayed using the procedure of Halliwell and Foyer, [75]. The assay mixture (3.0 ml) contained 2.5 ml of assay buffer buffer, 0.2 ml EDTA, 0.15 ml of 50 mM oxidized glutathione, 0.1 ml of 30 mM NADPH and 50 µl of enzyme extract. Assay reaction was initiated by adding NADPH at the end. Decrease in absorbance were recorded simultaneously, at 340 nm wavelength against a suitable blank containing boiled enzyme extract. Amount of NADPH oxidized were calculated by using an extinction coefficient (Absorbance of one molar solution) of 6.12 mM^{-1} cm^{-1} in the standard equation for absorbance. One unit activity of enzyme was correspond to the amount of enzyme required in the oxidation of one nmol of NADPH min^{-1}.

Standard equation for absorbance as $A = \varepsilon \times \ell \times c$

Where, A is the amount of light absorbed by the sample at a given wavelength, ε is the molar extinction coefficient, ℓ is the distance that the light travels through the solution, and c is the concentration of the absorbing species.

Peroxidase activity (POD): Peroxidase is non-specific in nature. It utilize different compounds as substrates to metabolize H_2O_2 preferably some phenolic compounds. During aging process, peroxidase catalyses cell wall softening reactions and plays an important role in response to environmental stresses. Peroxidase was assayed by the method of Shannon *et al*. [76]. Enzyme was assayed by putting 3.5 ml of assay buffer, 0.3 ml of o-dianisidine and 0.1 ml of diluted enzyme extract, in a cuvette of 5ml capacity. The solution was mixed well. The assay reaction was initiated by adding 0.1 ml of 0.2% hydrogen peroxide followed by recording the change in absorbance at 430 nm wavelength, simultaneously. A separate blank was prepared for each sample, simultaneously by taking boiled enzyme extract. The enzyme activity was expressed as change in 0.01 absorbance min^{-1} mg^{-1} protein.

Polyphenol oxidase (PPO): Polyphenol oxidase catalyses, o-hydroxylation of monophenols (phenol molecules with benzene ring containing, single hydroxyl substituent) to o-diphenols (phenols, with two hydroxyl substituents). They can further catalyse, the oxidation of o-diphenols to o-quinones. Polyphenol oxidase enzyme activity was assayed by the method of Taneja and Sachar, [77]. The assay mixture contained 1.8 ml of assay buffer, 2 ml catechol solution as substrate and 0.2 ml enzyme extract in glass test tubes. These test tubes were incubated at 37°C for 1 hour to take place the assay reaction followed by measuring absorbance at 430 nm on a UV-Vis spectrophotometer. A separate blank was prepared for each sample, simultaneously by taking boiled enzyme extract. The enzyme activity was expressed as change in 0.01 absorbance min^{-1} mg^{-1} protein.

Determination of the antioxidant metabolites

Following antioxidative metabolites were studied at vegetative and grain filling stage in sorghum plants under different treatments.

Glutathione: It is a low molecular weight thiol commonly found in both eukaryotic and prokaryotic cells. It is a most important water soluble antioxidant involved in preserving low redox potential and a highly reduced intracellular environment. It also take part in scavenging reactive oxygen species. The level of oxidized, reduced and total glutathione was estimated by the method of Smith, [78].

Extract preparation: One g of fresh leaf tissue was homogenised in 10 ml of 5% (w/v) sulphosalicylic acid using glass beads as abrasive, at 4ºC. Then, it was centrifuged at 30,000 x g for 20 min (4ºC) and the supernatant was collected for glutathione determination.

Assay: Total glutathione (GSH+GSSG), was determined by adding 0.1ml of 0.5 M potassium phosphate buffer (pH 7.5), 0.5 ml of 0.1 M sodium phosphate buffer (pH7.5) containing 5 mM EDTA, 0.1ml of 2mM NADPH, 0.1ml of glutathione reductase, 0.15 ml of 0.6 mM DTNB and 0.05 ml supernatant in a cuvette. The content was mixed thoroughly before the addition of supernatant, and the reaction was initiated by adding supernatant at the end of addition process. A separate blank tube was prepared by avoiding the addition of supernatant. The reduction rate of DTNB was monitored at 412 nm for 3 minutes. Total glutathione content was calculated from a standard curve of GSH (200-400 ng) plotted against the rate of increase of absorbance at 412 nm. Further, the oxidised glutathione (GSSG) content was determined by adding 1.5ml potassium phosphate buffer (0.5M, pH 7.5) and 0.2ml 4-vinylpyridine to 1ml supernatant in a test tube. The mixture was allowed to react for 1 hr to remove reduced glutathione (GSH). The GSSG content was measured using the same procedure as for total glutathione determination but with a GSSG standard curve (50-200 ng). Reduced glutathione (GSH) content was calculated by subtracting GSSG from the total glutathione content.

Proline: Proline is a basic amino acid found in high percentage in proteins. Free proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline, one of the hypotheses refers to breakdown of protein into amino acids and conversion to proline for storage. Many workers have reported a several-fold increase in the proline

content under physiological and pathological stress conditions. The proline content was estimated by the method of Bates *et al*. [79].

Extract preparation: One g of fresh leaves sample were homogenised in 10 ml of 3 % sulphosalicylic acid and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and used for proline estimation.

Assay: The extract was filtered through Whatman No. 2 filter paper. Two mL of filtrate along with 2mL of glacial acetic acid and 2mL acid ninhydrin were transferred in a test tube followed by heating in the boiling water bath for 1hr. The reaction was terminated by placing the tube in ice bath. Four mL toluene was added to the reaction mixture and stirred well for 20-30 sec. Toluene layer was separated and cooled to room temperature. The red coloured intensity of toluene was measure at 520 nm. Amount of proline present in the samples were determined from the standard curve (0.04 – 0.2 µg ml^{-1}) of proline.

$$Proline\ content\ (\mu moles\ per\ g\ tissue) = \frac{\mu g\ proline\ per\ ml \times ml\ toluene \times 5}{115.5 \times g\ sample}$$

Where 115.5 is the molecular weight of proline.

Ascorbic acid: Ascorbic acid is an important antioxidant, when present in reduced form. It is widely distributed in fresh fruits like guava, mango, ber, papaya and leafy vegetables such as cabbage and spinach. Ascorbic acid was determined by the slightly modified procedure of Oser, [80].

Extract preparation: One g of the plant tissue was homogenised in 6 ml of ice-cold 0.8 N $HClO_4$ and centrifuged at 4^0C, 10000 rpm for 30 minutes. The supernatant was collected and neutralized with 5M K_2CO_3. It was centrifuged again at same conditions (4^0C temperature, 10000 rpm for 30 minutes). Thus a clear supernatant was obtained, which were used for estimation of ascorbic acid content.

Assay: For estimation of total ascorbate, 1 ml extract was treated with equal volume (i.e. 1 ml) of 10% TCA. It was incubated in ice for 5 minutes. It was further mixed with 1 ml each of 5 M NaOH, 10 mM dithiothreitol (DTT) and 0.5% (w/v) N-ethyl maleimide (NEM) and 2 ml sodium phosphate buffer (pH7.4) in a final volume of 7 ml followed by 1 ml of 2% dinitrophenyl hydrazine and a drop of 10% thiourea, addition. Then the tubes were shaken vigorously and kept in boiling water bath for 15 minutes and cooled. After cooling 80% H_2SO_4 was added to the tubes at 4^oC and vortexed. Then the absorbance were recorded at 530 nm against a suitable blank without the sample extract. The amount of ascorbate was determined by using a reference curve (0-100 nmoles) of ascorbate and expressed as µmoles g^{-1} fresh weight.

β-Carotene: It is a red-orange coloured pigment, found plentiful in cereals, vegetables, and fruits. β-carotene is a precursor of retinol (vitamin A). The absorption of β-carotene increases, if it is eaten with fats. The amount of β-carotene was determined by the method of AOAC, [81].

Assay: A homogeneous suspension was made by dispersing 10g of shoot sample in 50 ml of water-saturated n-butanol (The n-butanol and water were mixed in the ratio of 6:2 (v/v) and shacked vigorously. Then it was allowed to stand, till it separates into two phases. The upper clear layer was water saturated n-butanol). After vigorous shaking, it was allowed to stand overnight (16 hrs) at room temperature in dark. It was shacked again followed by filtration through Whatman filter paper No. 1. The total volume of filtrate was made up to 100 ml. The absorbance (A) of the clear filtrate was measured at 440 nm in Spectronic-20/spectrophotometer against a blank of saturated n-butanol. The amount of β-carotene were calculated from the following equation:

$$\beta - carotene\ content\ (ppm) = 0.0105 + 23.5366 \times A$$

Detection of indices of oxidative stress

Following metabolites were studied as indices of oxidative stress at vegetative and grain filling stage in different treatments during the experimental analysis.

Hydrogen peroxide (H_2O_2): It is an important oxidant that initiates localized oxidative destruction which leads to disturbance of metabolic functions and damages of cellular integrity at site where it gathers. Hydrogen peroxide acts as a second messenger. It can diffuse across the cells and tissues; causes induction of proteins and genes, involved in stress defence system like CAT, APX. It is relatively stable.

Extraction: Two g tissue was macerated in 5ml of ice cold 0.01 M phosphate buffer (pH 7.0) and centrifuged at 8000 x g for 10 minutes. Supernatant was collected and used for the estimation of H_2O_2 content [75].

Assay: Fifty µl of extract were added to 1.95 ml of 0.01M potassium phosphate buffer (pH 7.0) and 2 ml of dichromate reagent to the mixture. It was kept in boiling water bath for 10 min and then cooled. After cooling, the absorbance were taken at 570 nm wavelength against a reagent blank without sample extract and the quantity of H_2O_2 were calculated from the standard calibration curve (10 to 160 µmole of H_2O_2).

Malondialdehyde (MDA): MDA content in plants reflects the extent of oxidative damage and hence membrane deterioration. It is produced during lipoxygenase (LOX) reaction.

Extraction: One g tissue were homogenized in 5 ml of TCA (0.1 % trichloroacetic acid; w/v) and centrifuged at 8000 x g for 15 min. The supernatant was used for MDA estimation by the method of Heath and Packer, [82].

Assay: The MDA estimation reaction was started by putting 1 ml of the supernatant, 4 ml of 20% TCA containing 0.5% 2-thiobarbituric acid (TBA). The content was heated in a boiling water bath at 95ºC for 30 minutes with constant stirring. Then it was cooled quickly in ice bath followed by centrifugation at 8000 x g for 10 min. The supernatant was decanted and the absorbance were recorded at 532 nm

against distilled water as blank. The values for non-specific absorption at 600 nm were subtracted from it and the concentration of MDA was calculated by using the molar extinction coefficient at 155 mM^{-1} cm^{-1}.

Grain yield determination

The grain yield was determined on 100 grains weight basis. One hundred grains from each replication were selected randomly and weighed, separately for each treatment, by using laboratory weighing balance. The average value of all replications was calculated and expressed as the yield in grams per 100 grains weight basis.

Statistical analysis

The present study was carried out in a completely randomized design (CRD) with three replications per treatment. All the results were analysed by using IBM SPSS Statistics 23 software for windows [83]. Comparison between different treatments was evaluated with a post hoc test followed by Tukey test. In the present study, the value for P was ascertained significant at ≤ 0.05.

Results

The present investigation was carried out on three varieties of sorghum viz. HJ 541 (single-cut), HJ 513 and SSG 59-3 (multi-cut) to check out the effects of GB (50 & 100 mM), individually and in combination with AMF (10 g) on Cr (VI) toxicity (2 & 4 ppm) given through soil spiking at the time of sowing. The data were collected at 35 and 95 DAS, except for grains weight which were analysed at maturity. The observations were recorded for chromium accumulation; antioxidant defence system enzymes viz. SOD, APX, CAT, GR, POD and metabolites viz. glutathione, proline, ascorbate, β-carotene; indices of oxidative stress parameters viz. H_2O_2, MDA, PPO; and grain yield. Plant growth was greatly reduced under Cr stress as compared to control plants (Fig 1).

Changes in Cr content of different parts of the sorghum plants

Chromium content of roots, stem and leaves were determined at two different growth stages (35 to 95 DAS) in three varieties (HJ541, HJ 513 & SSG 59-3) of sorghum (Fig 2). Chromium content of all these parts increased with increasing Cr stress, in all the varieties at both growth stages. Chromium content in these parts increased significantly with plant age (35 to 95 DAS) at both levels (2 & 4 ppm) of Cr (VI) in all the varieties. Maximum increase of Cr content was observed at 4 ppm Cr stress in all parts at both growth stages in all varieties. The highest Cr content was observed in roots at 4 ppm Cr stress, during 95 DAS stage (37.54 ppm), followed by stem (18.30 ppm) and leaves (13.67 ppm). However, the exogenous application of GB and AMF, either individually or in combination, reduced Cr content in all plant parts, in all the varieties at both growth stages. Maximum decrease in Cr content of roots, stems and leaves was observed in plants provided with the combination of 100 mM GB and AMF at both the growth stages in all the varieties under both Cr stresses (2 & 4 ppm). At 4 ppm Cr stress, the Cr content of roots, stem and leaves was reduced up to 29.48, 14.39 and 10.09 ppm with 100 mM GB and AMF combined application

at 95 DAS growth stage. Among the varieties, HJ 541 variety showed highest Cr content (42.88, 20.20, 14.59 ppm in roots stem & leaves, respectively) followed by HJ 513 (37.56, 18.29, 14.32 ppm in roots stem & leaves, respectively) and lowest in SSG 59-3 variety (32.18, 16.41, 12.11 ppm in roots stem & leaves, respectively).

Effect of GB and AMF treatments on anti-oxidative system in sorghum under chromium toxicity

The oxidative stress was measured in terms of polyphenol oxidase (PPO) activity, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents and antioxidant enzymes and metabolites. PPO causes oxidation of phenolic compounds and increased the oxidative stress. Indices of oxidative stress increased with plant age (35 DAS to 95 DAS) at both levels (2 & 4 ppm) of Cr stress in all the varieties. To observe effects of GB and AMF on the biochemical qualities of the membrane and oxidative stress in response to Cr stress, PPO activity and contents of H_2O_2 and MDA were examined (Fig 3). An increase in PPO activity, H_2O_2 and MDA content were observed under Cr stress as compared with controls at both the growth stages, in all the varieties. However, GB and AMF, individually and their combined application declined PPO activity, H_2O_2 and MDA content as compared with Cr alone treatment at both the growth stages in all the varieties. Maximum enhancements in these traits were found when Cr was applied at 4 ppm. At this level PPO activity, H_2O_2 and MDA contents were increased by 36.18 %, 38.12 % and 38.92 % respectively, without GB and AMF as compared with plants supplied with 100 mM GB combined with AMF at 35 DAS growth stage. Similar trends were observed from the analysis of plants at 95 DAS growth stage in all the varieties (Fig 3). Among the varieties, HJ 541 variety showed highest level of these indices of oxidative stress (29.27 units of PPO, 68.80 and 2.80 µmol of H_2O_2 and MDA respectively), followed by HJ 513 (19.73 units of PPO, 47.89 and 2.25 µmol of H_2O_2 and MDA respectively) and lowest in SSG 59-3 variety (13.15 units of PPO, 37.16 and 2.08 µmol of H_2O_2 and MDA respectively) at 95 DAS. Treatments of GB and AMF decreased indices of oxidative stress, significantly in all the varieties at both growth stages. However, treatments of GB and AMF combined showed lowest values of these parameters as compared to all other treatments, at both the stages in all varieties. Among all the treatments, 100 mM GB with AMF was observed most effective in lowering down the indices of oxidative stress.

Furthermore, Cr stress increased antioxidant enzymes SOD, APX, CAT, GR and POD activities in all the varieties at both the stages of growth (Fig 4). Antioxidative enzyme activities increased as the level of Cr stress increased; it was maximum at 4 ppm of Cr stress and tended to increase afterwards because this increase was not enough to protect the plants from oxidative damage caused by toxicity of hexavalent Cr. The GB and AMF either alone or their combined application further augmented the activities of these enzymes in control as well as in Cr stressed plants in all the varieties at both growth stages. Maximum increase in the activity of these antioxidative enzymes was observed in plants provided with 100 mM GB and AMF in combination, at both the growth stages in all the varieties. However, activities of these enzymes decreased with plant age (35 DAS to 95 DAS) at both levels (2 & 4 ppm) of Cr stress, in all the varieties. At 4 ppm of Cr stress SOD, APX, CAT, GR and POD activities increased up to 87.19 %, 56.28 %, 75.38 %, 85.35 % and 85.50 % respectively, without GB and AMF, whereas with combination of 100 mM

GB and AMF, these increased up to 92.92 %, 68.99 %, 83.66 %, 90.80 % and 90.63 % respectively, as compared with controls during 35 DAS stage. Similar results were obtained during 95 DAS stage in all the varieties for enzymatic activities. Among the varieties, SSG 59-3 variety showed highest activity of these antioxidative enzymes, followed by HJ 513 and lowest in HJ 541 variety (Fig 4).

The antioxidative defence systems include both enzymatic and non-enzymatic antioxidant components. Apart from enzymatic, non-enzymatic antioxidants such as Glutathione (GSH and GSSG), Ascorbate (AsA), Proline and β-carotene, are crucial for plant defence against oxidative stress. They play a key role as antioxidant buffers. Glutathione reductase is responsible for maintaining the supply of reduced glutathione. It is one of the most abundant reducing thiols in majority of cells. GSH plays a key role in the cellular control of ROS. The major role of APX is detoxifying hydrogen peroxide in plant cells via, ascorbate-glutathione cycle, in which, ascorbate acts as a specific electron donor for APX enzymes in catalyzing the conversion of H_2O_2 into H_2O.

To determine the ameliorative effect of GB and AMF against hexavalent Cr in sorghum, non-enzymatic antioxidant components were also analysed. Non-enzymatic antioxidant components, namely total glutathione, reduced glutathione (GSH), oxidized glutathione (GSSG), ascorbate, proline and β-carotene were studied. Among them, except β-carotene all other metabolites, increased significantly with increasing concentration of Cr stress at both the growth stages, in all the varieties (Figs 5 and 6). The β-carotene content decreased significantly with increasing concentration of Cr (VI), at both the growth stages in all the varieties (Fig 6). All other properties observed were similar to other antioxidative metabolites contents. Along with β-carotene and except GSSG, further increase in the content of these metabolites was observed on exogenous application of GB and AMF, either individually or in combination, at both the growth stages in all the varieties. In contrast, GSSG content decreased on GB and AMF application, either individually or in combination, at both the growth stages in all the varieties (Fig 5).

Maximum increase in GSH, AsA, proline and β-carotene contents (88.93 %, 87.50 %, 64.04 %, 40 % at 35 DAS and 90.99 %, 90.48 %, 62.68 %, 42.85 % at 95 DAS respectively), observed in plants provided with the combination of 100 mM GB and AMF, while the content of GSSG decreased maximally (65.76 % and 47.64 % at 35 and 95 DAS respectively) at same treatment. The level of all these metabolites decreased with plant age at both levels of Cr (VI), but GSSG was increased in all the varieties (Fig 5). Among varieties, SSG 59-3 variety showed highest level of GSH, AsA and β-carotene, followed by HJ 513 and lowest in HJ 541 variety while reverse order was observed for GSSG. These findings exemplify the role of GB and AMF for regulating the membrane stability and generation of ROS in cells under the conditions of Cr stress.

Effect of GB and AMF treatments on grain yield (100 grains weight) in sorghum under chromium toxicity

There was a progressive decrease in grain yield with increasing concentration of Cr (VI), at both the growth stages, in all the varieties (Fig 7). Increase in grain yield was observed on exogenous application of GB and AMF, either individually or in combination, at both the growth stages in all the varieties.

Maximum increase was observed in plants provided with the combination of 100 mM GB and AMF, at both the growth stages in all the varieties.

The results demonstrate that weight of hundred grains decreased significantly with increasing concentrations of Cr (VI) in all the varieties. However, application of GB with and without AMF resulted in significant increase in weight of hundred grains, in all the varieties. Maximum increase was observed with treatment of 100 mM GB with AMF (2.83, 2.33 & 2.03 g in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (2.55, 2.19 & 1.84 g in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with AMF (1.76, 1.29 & 1.18 g in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (1.47, 1.27 & 1.11 g in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF only (1.06, 0.86 & 0.61 g in control, 2 ppm Cr & 4 ppm Cr, respectively). Highest grain yield was observed in SSG 59-3 (1.83 g) followed with HJ 513 and HJ 541. The lowest grain yield was observed in HJ 541 (1.19 g).

Discussion

Chromium toxicity in cultivable lands has become a serious problem all over the world [33]. It reduces growth and yield of the sorghum crop [34]. There are many reports on Cr (VI) toxicity causing hazardous effects in plants. However, reports on amelioration of chromium toxicity by using GB and AMF together are scanty in literature. In this study, the ameliorative effect of exogenously applied GB and AMF (individually and in combination) against Cr (VI) toxicity was investigated on antioxidative defence system in sorghum. During present research, increasing levels of Cr treatments was resulted in increased Cr content in sorghum. It seems that after application of only 2 and 4 ppm of Cr, the Cr content in roots, stem and leaves increased many folds i.e. more than the highest treatment of 4 ppm. The reason behind this might be the lower weight of dried sorghum plant as compared to weight of soil (5 kg pot^{-1}) because the concentration of matter changes with respect to weight of medium, when it is expressed in terms of weight. It increases as weight of medium decreases. Similar reports have been reported earlier also [35,36]. The Cr content was higher in roots followed by stem and leaves indicated that sorghum plants might have abundant resistance against Cr stress as reported by other researcher in chickpea [37]. Reduction in Cr content of plant samples might be due to GB and AMF, either individually or in combination maintains cell membranes integrity and protects cells from damages which in turn limits the entry of Cr in to the cell. The reduction in Cr absorption by plants on GB application might also be due to shielding nature of GB towards cell membranes that reduces chromium movement to cells [38,39]. Similar results have been reported for Pb and Cd contents in mung bean [40], rice [41] and wheat [42].

Karagiannidis and Hadjisavva, [32] reported that AMF inoculation increased nutrient uptake and supresses Cr, Mn, Fe, Co, Ni, and Pb absorption in duram wheat. It suggested, other possibility in reduction of Cr absorption with AMF and GB application might be the competition between nutrients and Cr for entry in to the cells. Many reports on heavy metal resistant microorganisms have indicated exceptional ability of AMF to promote the growth of host plant under stressful conditions [31,43]. Moreover, AMF also has been recognized as a potential biological agent that increases the tolerance capacity of host plant under heavy metal stress.

It was noticed that Cr enhanced ROS generation such as H_2O_2 and hydroxyl compounds which in turn increases MDA level and PPO activity. It was reported earlier that Cr is non-essential for plants and generates toxic stress by causing reduction of molecular oxygen and producing intermediate products called ROS such as superoxide radicals, hydroxyl radicals and H_2O_2. Interestingly, generation of ROS is the first line of defence reaction exhibited by any plant cell in response to stress. They further induce the synthesis of other biomolecules (metabolites) and activation of enzymes of various pathways as a defence mechanism. The level of these compounds signifies the extent of stress and are known as indices of oxidative stress. Membrane lipids and proteins are more liable to be attacked by ROS making them reliable indicators of oxidative stress in plants.

In present study activities of antioxidant enzymes and metabolites were increased with increasing levels of Cr treatments (Figs 4-6). But this increase was not sufficient in scavenging the ROS generated under Cr stress as was evident from increased H_2O_2, MDA and PPO activities at same treatments of Cr. Further, exogenous application of AMF and GB both individually or in combination enhanced antioxidant enzymes and metabolites activities at same Cr treatments in Sorghum and alleviates chromium induced toxicity as was evident from reduced H_2O_2, MDA and PPO activities on GB and AMF application (Fig 3). The reason behind promotive role of GB and AMF towards antioxidants activities might be the inhibition of Cr absorption and increased nutrient absorption as studied by Jabeen *et al*. [44] in mung bean under Cr toxicity. Moreover, GB itself acts as compatible solutes and AMF helps in accumulation of them that functions as osmoprotectants and counteracts the oxidative stress by elevating the levels of antioxidant enzymes and metabolites [45]. Hisyam *et al*. [46] have also reported increased antioxidant system activities on exogenous GB application to counteract the stress caused by water defciency in rice plants. Wang *et al*. [47] were also of similar view that GB acts as osmoprotectant, which in turn protects the plant cells from osmotic stresses and resulted in decreased PPO activity, while working on GB accumulation in wheat. Raza *et al*. [48] and Gill *et al*. [15] also got similar reports on exogenous GB application in wheat and brassica under Cr toxicity. These reports are supportive of the findings of present investigation.

In the present experiment loss of grain yield on Cr application was noticed that might be due to excessive production of ROS which is toxic to plants and cause oxidative damage to cellular constituents that resulted in loss of growth and yield as reported by Khaliq *et al*. [49], who studied the effect of Cd toxicity in duram wheat. The other reason might be increased PPO activity which causes oxidation of polyphenols that reduces the chances of plants growth and reduces the yield under stressful conditions [50,51]. Apart from that H_2O_2 is also very toxic compound and a higher content of it, produces injuries through lipid peroxidation in plant cells which in turn increases MDA content in plants that might also be the cause for reduced yield during stressed conditions [52,53]. The decrease in grain yield under Cr toxicity may also be due to increased Cr absorption with increasing Cr stress in plant that caused damaging of roots, chlorosis, necrosis, loss of mineral nutrition, and loss of water balance, ultimately resulted in to reduced yield of plants as also suggested by Ali *et al*. [54] in barley, Gill *et al*. [55] in oilseed rape cultivars under Cr toxicity and Kanwal *et al*. [56] in wheat under lead toxicity. The reduction in yield under Cr (VI) toxicity has also been reported widely in literature [30,57,58,59].

The results of present investigation revealed an increased yield on GB and AMF application both individually or in combination (Fig 7). This increase in grain yield might be resulted due to reduction in Cr uptake on GB and AMF application which in turn decreased stress level by maintaining proper stomatal conductance, chloroplast ultrastructure, RuBisCo activity, photosynthetic capacity and proper nutrient uptake [60]. Glycine betaine increased the antioxidant systems activity which in turn prevents plants from oxidative damages caused by ROS generated due to stressed conditions that might resulted in enhanced grain yield [61-63] demonstrated similar effects on AMF inoculation in sunflower under Cd toxicity, as were observed during present investigation. Similarly, GB application increased the growth and yield in rice plants under Cd toxicity [41]. Bharwana *et al*. [64] also obtained similar results that foliar application of GB increased the yield of cotton plants grown under lead (Pb) toxicity. However, the mechanism(s) involved in enhancement of growth and yield of the plant by GB and AMF application are still not clear. In present experiment, variety SSG 59-3 showed highest grain yield as compared to HJ 513 and HJ 541 (Fig 7). This might be ascribed to highest level of antioxidant enzymes and metabolites activities (Figs 4-6), and lowest level of Cr accumulation and indices of oxidative stress parameters (Figs 2 and 3) in SSG 59-3 variety followed by HJ 513 and lowest in HJ 541.

To sum up, our findings revealed that Cr stresses significantly reduced the grain yield, antioxidant enzymes and metabolites activities. Indices of oxidative stress parameters were dominant due to Cr toxicity. However, the exogenous application of GB and AMF both individually and in combination significantly enhanced the grain yield and reduced the indices of oxidative stress parameters by improving antioxidant enzymes and metabolites activities under Cr stresses. The GB and AMF application also reduced the Cr accumulation and transport. No reports are available about the mechanism of GB and AMF combination in sorghum under Cr stress. Hence, further studies are needed at field level in order to see the role of GB and AMF combinations and its mechanism towards various plant species under heavy metal stresses.

Conclusions

To conclude, Cr (VI) toxicity (2 & 4 ppm) produced biochemical changes in sorghum (*Sorghum bicolor* L.) plants resulted in increased ROS levels in all the varieties at both vegetative and grain filling stage. The deleterious effects increased with the increasing concentration of Cr. This may be due to increased Cr uptake which resulted in increased indices of oxidative stress. Through, the components of the antioxidant defence system increased under Cr toxicity. However it seems that it was not sufficient to combat the toxicity stress. As revealed by high level of indices of oxidative stress parameters of the plant. Exogenous application of GB and AMF, however improved the stress tolerance due to further increase in enzymes and metabolites of antioxidant defence system and reduction in indices of oxidative stress. The treatment of GB at both 50 and 100 mM level, applied in soil, significantly ameliorated Cr toxicity. However, AMF (10 g) concomitantly with GB, at both 50 & 100 mM level, further ameliorated the effects of Cr toxicity in sorghum plants at both growth stages (35 & 95 DAS). But the AMF application with GB at 100 mM level was found more beneficial at both growth stages. The combination of GB (100 mM) alongwith AMF (10 g) was observed most effective and best concentration among all the treatments, for

the amelioration of Cr toxicity in sorghum plants at both growth stages. However, the effects were found more prominent at 35 DAS than 95 DAS. Based on results obtained in present investigation, the variety SSG 59-3 was observed to be more tolerant to Cr toxicity followed by HJ 513 and HJ 541. Further studies in field conditions are necessary to confirm the mechanisms and findings of this experiment.

Declarations

Data availability

All data generated or analysed during this study are included in this article file.

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Author contributions

P.K. performed the experiments, analysed the data, drafted the manuscript and prepared all the Figures 1–7 and Tables 1-2. Thereafter, reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to P.K.

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Figures

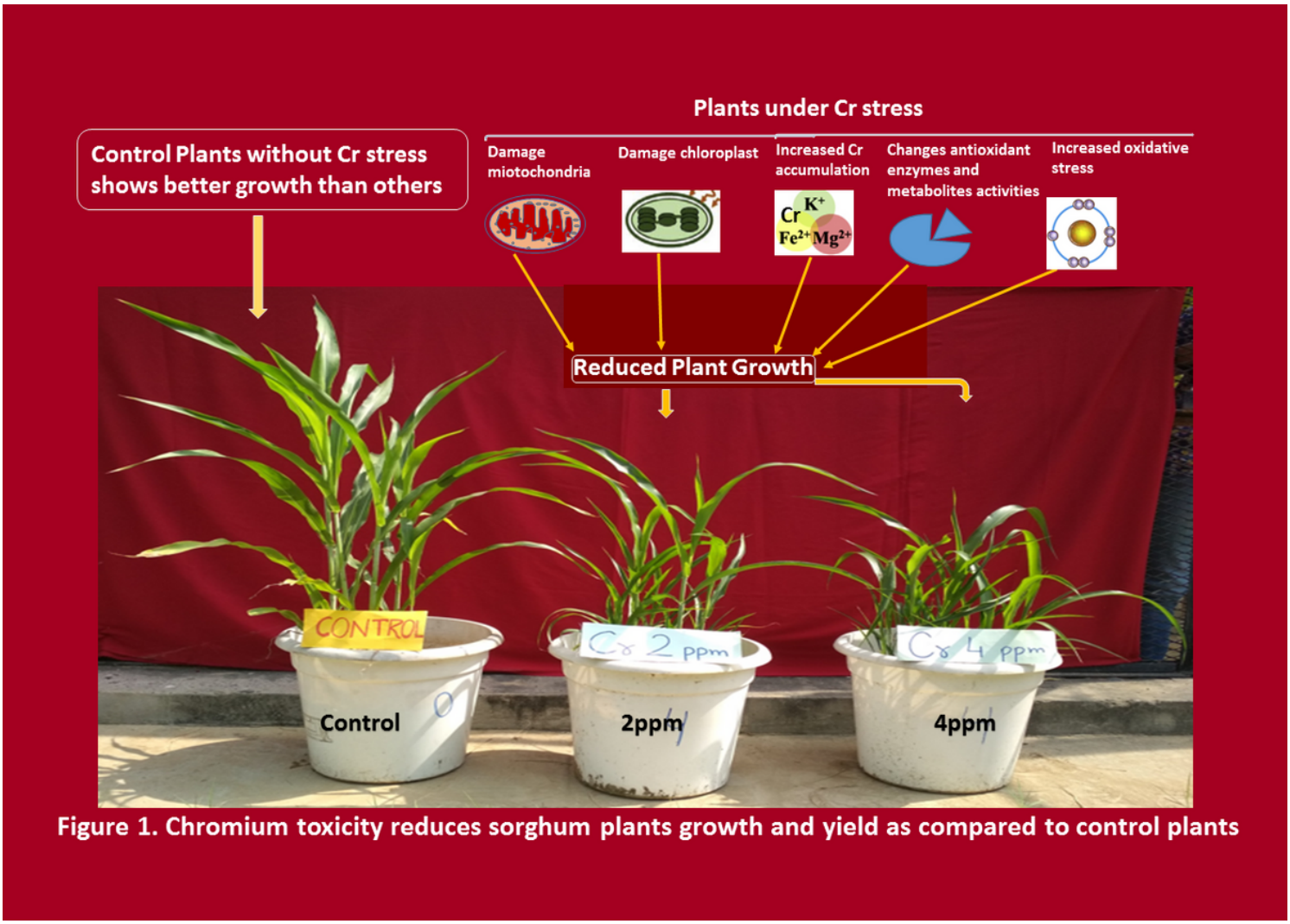

Figure 1. Chromium toxicity reduces sorghum plants growth and yield as compared to control plants

Figure 1

Chromium toxicity reduces Sorghum plants growth and yield as compared to control plants.

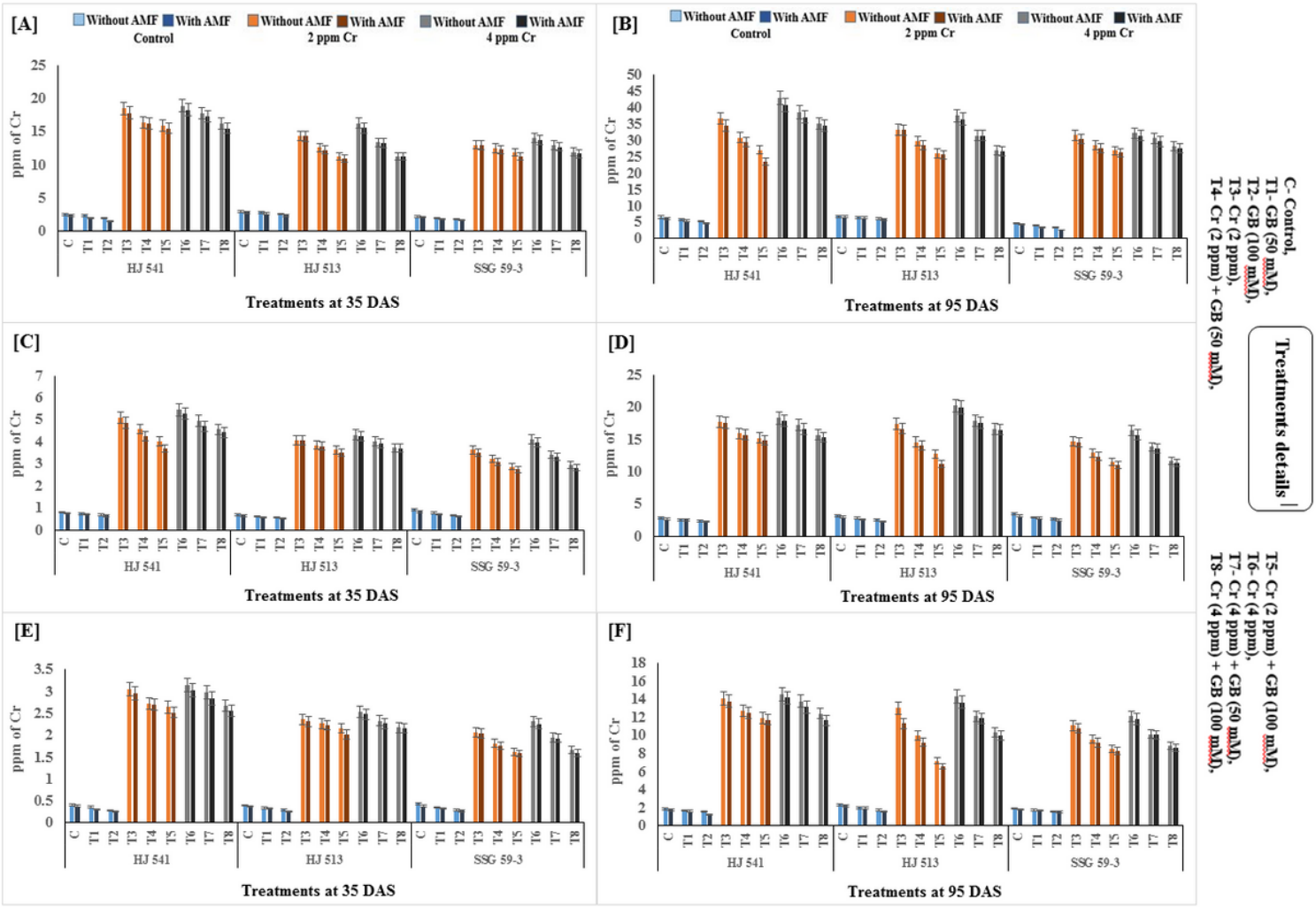

Figure 2

Effects of GB and AMF treatments on Cr accumulation in roots (A and B), stems (C and D) and leaves (E and F) of different varieties of sorghum under Cr (VI) toxicity at 35 & 95 DAS, respectively. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).

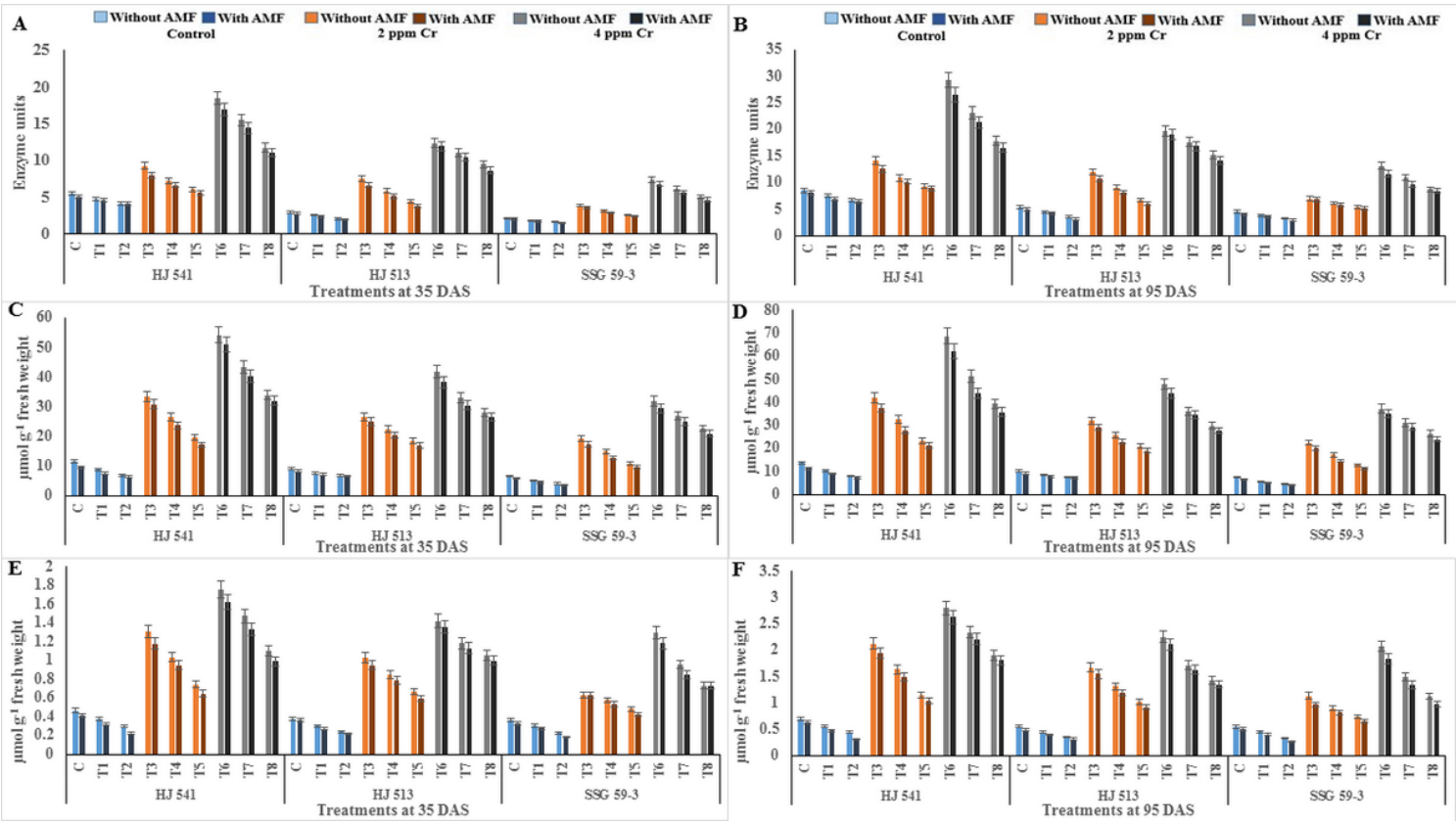

Figure 3

Effects of GB and AMF treatments on indices of oxidative stress viz. PPO activity (A and B), H2O2 content (C and D) and MDA level (E and F) in different varieties of sorghum under Cr (VI) toxicity at 35 & 95 DAS, respectively. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).

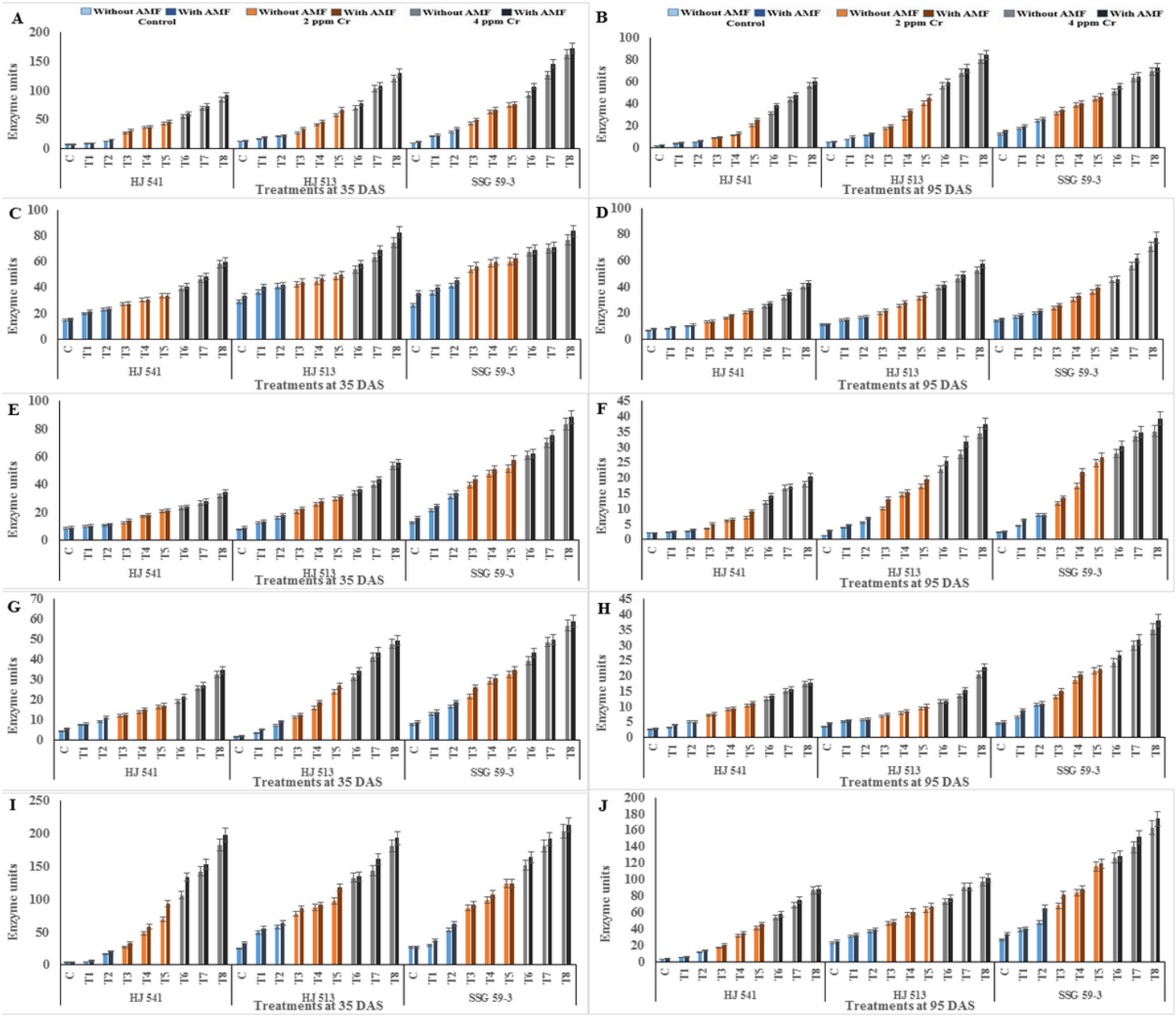

Figure 4

Effects of GB and AMF treatments on activities of antioxidant enzymes viz. SOD (A and B), APX (C and D), CAT (E and F), GR (G and H) and POD (I and J) in different varieties of sorghum under Cr (VI) toxicity at 35 & 95 DAS, respectively. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).

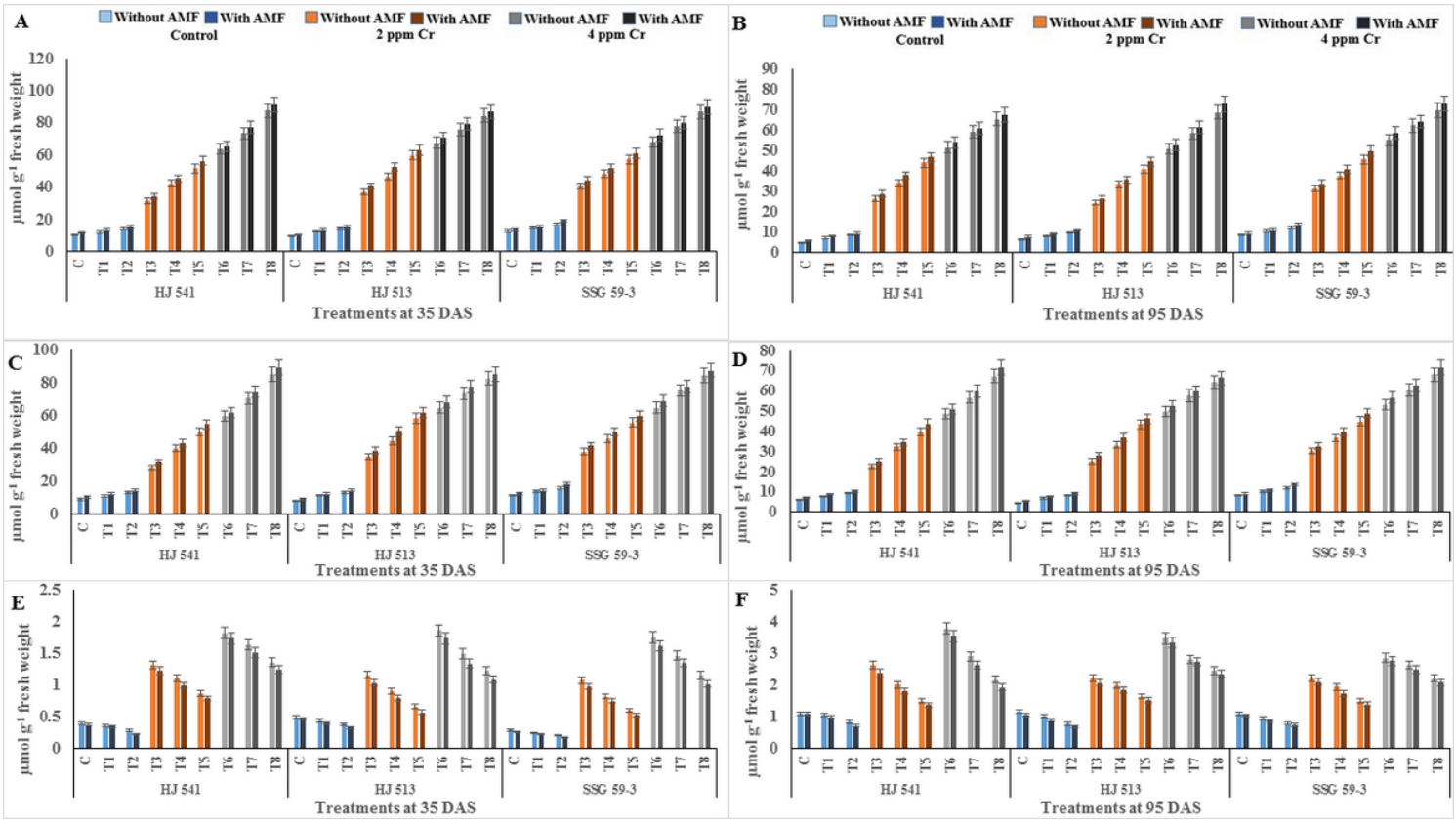

Figure 5

Effects of GB and AMF treatments on antioxidant metabolites viz. total glutathione (A and B), GSH (C and D) and GSSG (E and F) in different varieties of sorghum under Cr (VI) toxicity at 35 & 95 DAS, respectively. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).

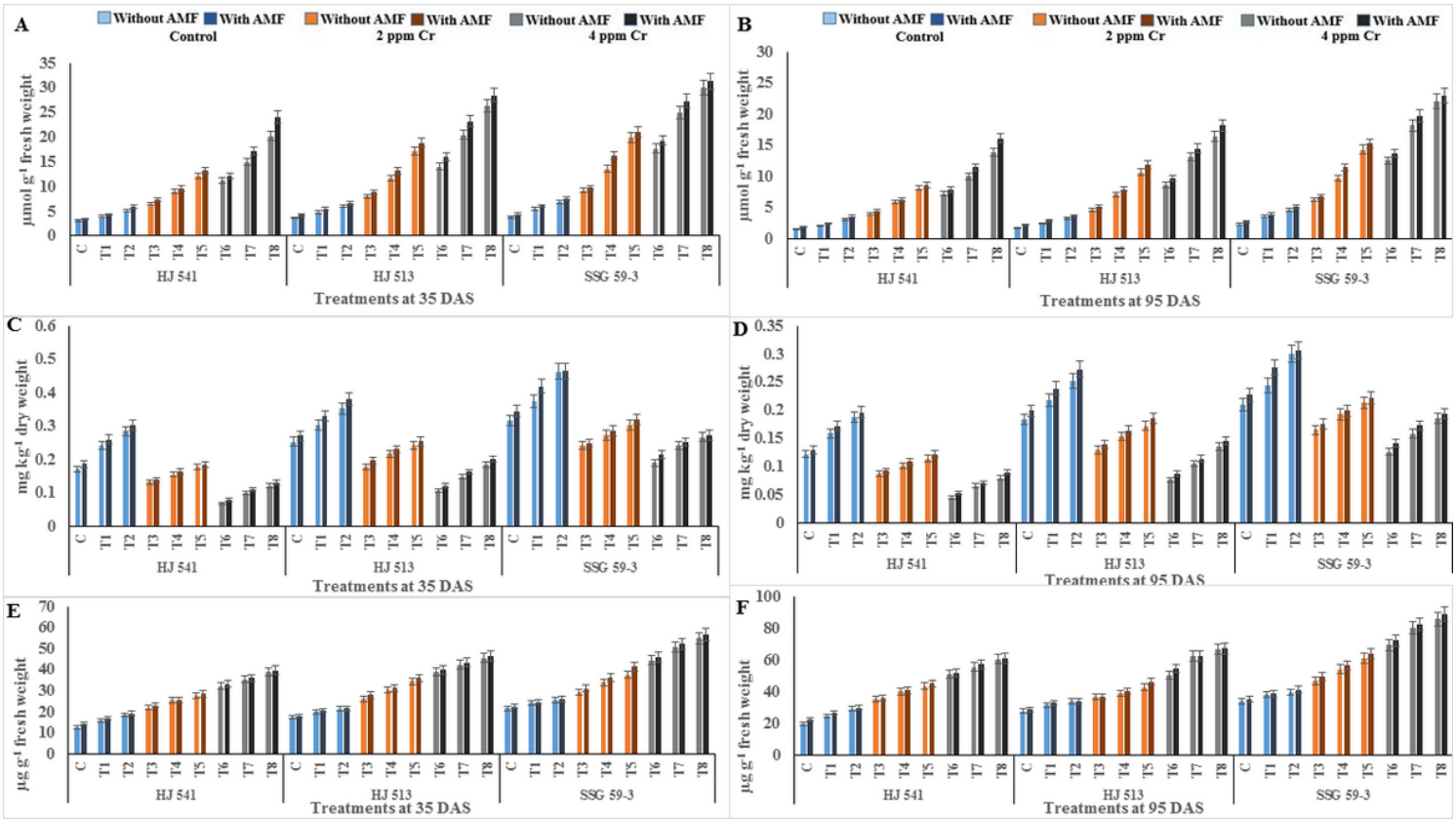

Figure 6

Effects of GB and AMF treatments on antioxidant metabolites viz. ascorbate (A and B), β-carotene (C and D) and proline (E and F) in different varieties of sorghum under Cr (VI) toxicity at 35 & 95 DAS, respectively. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).

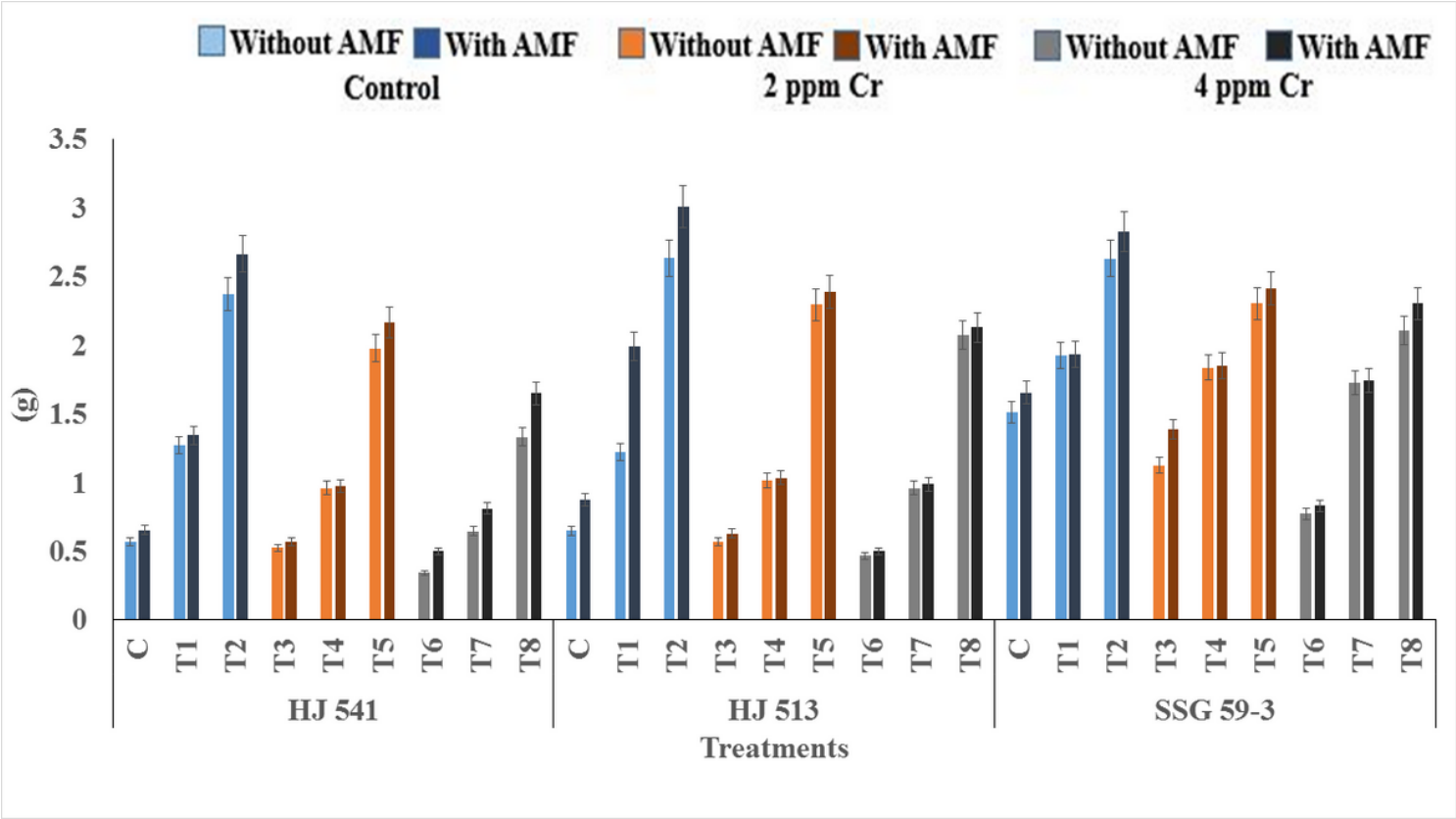

Figure 7

Effects of GB and AMF treatments on grain yield (hundred grains weight in g) in different varieties of sorghum under Cr (VI) toxicity. Values represent the mean ± S.E., N = 3, from three independent experiments. Significance difference was at $P \leq 0.05$ (ANOVA).