Exogenous AMF with Glycine Betaine Reduces Cr (VI) Uptake and Alleviates Chromium Toxicity by Supressing the Oxidative Stress in Three Genetically Different Sorghum (Sorghum bicolor L.) Cultivars

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Research article

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Abstract

Background:

Chromium is most toxic pollutant that negatively affects plant's metabolic activities and yield. It reduces plant growth by influencing the antioxidant defense system's activities. The aim of the present research was to examine the ameliorative capability of exogenous GB and AMF spiked in soil, either individually or in combination against Cr toxicity. The ameliorative effects were studied in terms of Cr uptake, grain yield, antioxidative defense system parameters (viz. enzymes – SOD, APX, CAT, GR, POX and metabolites – proline, glutathione, ascorbate, β-carotene) and indices of oxidative stress parameters (viz. PPO, $H_2O_2$, and MDA).

Results:

The results delineated that Cr uptake and indices of oxidative stress were increased with increasing concentration of Cr stress in all the varieties (HJ 541, HJ513 & SSG 59-3) at both the growth stages (35 & 95 DAS). At higher concentration (4 ppm), Cr stress decreased the grain yield (45-50%) as compared with controls. PPO activity, MDA and $H_2O_2$ content increased at both growth stages in all the varieties. However, antioxidative enzymes and metabolite activities increased due to Cr stress but this increase was not sufficient to counteract with ROS generated under Cr stress which was enhanced on the application of AMF and GB either individually or in combination (spiked in soil). It decreased the indices of oxidative stress and ameliorated the Cr toxicity and increased grain yield (65-70%) in all the varieties.

Conclusions:

Both GB and AMF, improved the plant growth and stress tolerance capacity of the plant. GB at both 50 and 100 mM level, significantly ameliorated Cr (VI) toxicity. However, AMF concomitantly with GB further boost up the amelioration behavior of the plant against Cr toxicity, at both growth stages in all the varieties. The combination of 100 mM GB with 10 g AMF was observed most effective among all the treatments. Among the varieties SSG 59-3 had the lowest chromium uptake, indices of oxidative stress, and highest antioxidative system's activity as compared to HJ 513 followed by HJ 541 variety. Thus AMF and GB either individually or in combination may be used to maintain plant yield attributes under Cr toxicity.

1. Background

Sorghum crop [Sorghum bicolor(L.) Moench] a member of the family Poaceae, is grown worldwide and India ranks second in terms of area under sorghum cultivation with an area over 5.00 million hectares which accounts for an average yield of 0.90 metric tons per hectares (USDA, 2019). Sorghum is a native of Africa (Elangovan et al. 2009). It is one of the top cereal crops (rice, wheat, maize, and barley) in the world (Rao et al. 2010). It is an important Kharif season crop that is consumed in India for the nourishment of humans as well as for animals directly or indirectly (Singh et al. 2010). As it is a C4 plant
that makes it highly efficient in converting solar energy to chemical energy even in water scared conditions, usually (O et al. 2012). It supplied 9.8 g per capita per day of food, in India (FAOSTAT, 2019). These characteristics make sorghum a capable crop to meet the growing food demands, globally (Kleih et al. 2000).

But currently, the increased contamination of agricultural soils with chromium has reduced the crop growth and yield and it has become a global concern (Adrees et al. 2015). The main sources of Cr (VI) toxicity in the biosphere are leather and paint industries which are responsible for 40% of the total Cr influx in the environment (Barnhart, 1997). Annually, approx. 2000 to 32000 tons of Cr is released in the environment from Indian tanning industries only (Nigam et al. 2015). Chromium exists in two stable forms viz. trivalent Cr (III) and the hexavalent Cr (VI). Both forms may cause toxicity to plants, but Cr (VI) is considered the most toxic form of Cr due to its high solubility and more unstable nature (Zhang et al. 2019). The trivalent form is less toxic due to its easy bioavailability as it is mostly stable, less mobile and it readily forms insoluble hydroxide/oxides above pH ~ 5.5 (He et al. 2009). It is mainly found bound to the organic matter in soil and aquatic environments (Ertani et al. 2017). Chromium (VI) is generally found linked with oxygen as oxyanions of chromates (CrO$_4^{2-}$) or dichromates (Cr$_2$O$_7^{2-}$). 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 (Kumar et al. 2018).

Chromium concentration in agricultural soils varies up to values as high as 350 mg kg$^{-1}$ in the world and 21 to 47 ppm in Haryana, whereas the permissible value is 0.05 to 0.5 ppm both in water and soil (Singh et al. 2016; Zaidi and Pal, 2017). Its concentration above 0.5 ppm in soil and water starts producing toxic effects in plants (Singh et al. 2016). Therefore the present research work was planned on 2 and 4 ppm of chromium levels. It 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 animals. It is listed among the top 129 environmental pollutants and, in the hexavalent form, it is considered as one of the 14 most harmful substances for the health of living organisms (USEPA, 2000).

Chromium stress in plants is characterized by the decrease in photosynthesis, nutrient uptake, damaging of roots, and finally, plant death (Gill et al. 2015; Hussain et al. 2019). Some of the well-established phytotoxic manifestations include the 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 (Kumar et al. 2010; Cuypers et al. 2011; Gangwar et al. 2011; Huang et al. 2012). These active molecules (ROS) are involved in the free radical chain reaction of membrane lipids and proteins, thus causing their oxidative decomposition (Kanoun-Boulé et al. 2009; Cuypers et al. 2011). Plants possess several antioxidant defense systems to protect their cells against ROS and one such system is the accumulation of a variety of small organic metabolites that are collectively referred to as compatible solutes (Ashraf and Foolad, 2007). Compatible solutes include sugars, polyols, glycine betaine (GB), amino acids (proline, histidine), and related
compounds (Hanson, 1992). It has been reported that the level of GB increased in plants subjected to abiotic stresses (Dhir et al. 2012). GB is a quaternary ammonium compound that is found in plants and mammals etc. (Chen and Murata, 2011). GB functions as an osmoprotectant that suppresses production of ROS. It counteracts the oxidative stress in plants by elevating the level of proline and antioxidant enzymes like catalase (CAT), Peroxidase (POX), and Superoxide dismutase (SOD). It is a very effective osmoregulating substance (Kumar et al. 2019). 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. Under such conditions, the exogenous application of GB may help to reduce the adverse effects (Islam et al. 2009; Hossain et al. 2010). GB is environmentally safe, non-toxic, and water-soluble (Makela et al. 1998). There is strong evidence that GB plays an important role in plants against tolerance to abiotic stresses (Giri, 2011). 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 (Ali et al. 2015). Furthermore, Arbuscular mycorrhizal fungi are recognized as biological agents that potentially increase the tolerance of plants to heavy metal toxicity (Vivas et al. 2003a; Vivas et al. 2003b; Vivas et al. 2003c; Vivas et al. 2005; Vivas et al. 2006). The reduction of growth due to chromium interference with nutritional elements uptake can be improved through mycorrhizal inoculation. Karagiannidis and Hadjisavva-Zinoviadi, (1998) showed that arbuscular mycorrhizal fungi (AMF) can enhance yield by simultaneously reducing the chromium content in crop plants.

2. 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 AMF (10 g) individually and in combination with GB (50 & 100 mM), on Cr (VI) toxicity (2 & 4 ppm) given through soil spiking at the time of sowing. The data were collected except for grains weight which were analysed at maturity. The observations were recorded at 35 and 95 DAS for (i) chromium content; (ii) antioxidative defence system enzymes viz. superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, peroxidase and metabolites viz. glutathione, proline, ascorbate, β-carotene; (ii) indices of oxidative stress parameters viz. hydrogen peroxide, MDA, polyphenol oxidase and (iii) grain yield. The results obtained during present investigation are presented below under following headings.

2.1 Effect of GB and AMF treatments on Cr content in sorghum under chromium toxicity.

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

2.2.1 Effect of GB and AMF treatments on anti-oxidative enzymes.

2.2.2 Effect of GB and AMF treatments on anti-oxidative metabolites.

2.3 Effect of GB and AMF treatments on indices of oxidative stress in sorghum under chromium toxicity.

2.4 Effect of GB and AMF treatments on grain yield in sorghum under chromium toxicity.
2.1 Effect of GB and AMF treatments on Cr content in sorghum under chromium toxicity:

Chromium content of roots, stems and leaves increased with increasing concentration of Cr (VI), in all the varieties at both the growth stages (Figs. 1–6). Exogenous application of AMF and GB, either individually or in combination, decreased Cr content in roots, stems and leaves, in all the varieties at both the growth stages. Chromium content in roots, stems and leaves increased significantly with plant age (35 to 95 DAS) at both levels (2 & 4 ppm) of Cr (VI) in all the varieties. Maximum decrease in Cr content of roots, stems and leaves observed in plants provided with the combination of AMF and GB at both the growth stages in all the varieties. Among the varieties HJ 541 variety showed highest Cr content in their roots, shoots and leaves, followed by HJ 513 and lowest in SSG 59 – 3 variety (Figs. 1–6).

2.1.1. Effects on chromium content in roots:
The results presented in Fig. 1 demonstrate that Cr content increased (from 2.59 ppm in control to 15.31 & 16.38 ppm at 2 ppm Cr & 4 ppm Cr, respectively) significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS. However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 35 days after sowing (DAS). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (1.87, 12.57 & 12.79 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (2.14, 13.02 & 13.13 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (2.19, 13.59 & 14.44 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (2.41, 13.83 & 14.69 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (2.46, 15.01 & 15.84 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (12.07 ppm) followed by HJ 513 (9.61 ppm) and SSG 59 – 3 (9.03 ppm) for Cr content at 35 DAS in plants provided with GB spiked in soil. All the varieties showed significant (CD \((0.05) = 0.074\)) differences in Cr content at 35 DAS under all the treatments (Fig. 1). The interaction between variety – treatment and treatment – fungi were observed significant (CD \((0.05) = 0.221\) & CD \((0.05) = 0.181\), respectively). But the other interaction such as variety – fungi and overall interaction (V × T × F) were found non-significant.

Similarly, Cr content increased significantly (Fig. 2) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 6.04 ppm in control to 33.76 & 37.54 ppm at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 95 DAS. Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (4.39, 25.13 & 29.48 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (4.94, 26.59 & 30.00 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (5.12, 28.48 & 32.67 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (5.49, 29.64 & 33.45 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (5.75, 32.63 & 36.21 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean Cr content values of all treatments (combined) for each variety, decreased significantly in HJ 541, HJ 513 and SSG 59 – 3 (24.70, 22.46 & 20.73 ppm, respectively). All the varieties, treatments and fungi showed significant (CD \((0.05) = 0.189\), CD \((0.05) = 0.328\) & CD \((0.05) = 0.155\), respectively) differences in
Cr content at 95 DAS. The interaction between variety & treatments, variety & fungi, treatment & fungi and overall interaction (V × T × F) were significant (CD (0.05) = 0.568, CD (0.05) = 0.268, CD (0.05) = 0.464 & CD (0.05) = 0.803) at 95 DAS (Fig. 2).

To sum up, the Cr content was found lower in SSG 59 – 3 (9.03, 20.73 ppm at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The highest Cr content was observed in HJ 541 (12.07, 24.70 ppm at 35 & 95 DAS, respectively). It was observed from the results that the Cr content was increased (10.24 to 22.63 ppm) with increase in growth stages (35 to 95 DAS, respectively) of plants. The GB treatments decreased Cr content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB combined with AMF spiked in soil showed lower Cr content as compared to all other treatments, at both the stages in all varieties (Fig. 1, 2).

2.1.2. Effects on chromium content in stem:
The Cr content of the stem increased (from 0.81 ppm in control to 4.26 & 4.62 ppm at 2 ppm Cr & 4 ppm Cr, respectively) significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS. However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 35 DAS (Fig. 3). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.59, 3.32 & 3.64 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (0.64, 3.51 & 3.75 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (0.67, 3.71 & 3.99 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.70, 3.88 & 4.12 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.74, 4.14 & 4.50 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (3.34 ppm) followed by HJ 513 (2.80 ppm) and SSG 59 – 3 (2.45 ppm) for Cr content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD (0.05) = 0.026, CD (0.05) = 0.045 & CD (0.05) = 0.021) differences in Cr content at 35 DAS (Fig. 3). The interaction between variety - treatment was found significant (CD (0.05) = 0.078). But the other interactions such as variety – fungi, treatment – fungi and overall interaction (V × T × F) were found non-significant.

Similarly, Cr content increased significantly (Fig. 4) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 3.15 ppm in control to 16.61 & 18.30 ppm at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 95 DAS. Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (2.30, 12.36 & 14.39 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (2.50, 13.12 & 14.67 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (2.60, 14.03 & 15.91 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (2.72, 14.45 & 16.31 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (2.86, 16.21 & 17.84 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean Cr content of all treatments (combined) for each variety, decreased significantly in HJ 541, HJ 513 and SSG 59 – 3 (11.82, 11.75 & 9.81 ppm, respectively). All the varieties, treatments and fungi showed significant (CD (0.05) = 0.087, CD (0.05) = 0.150 & CD (0.05) = 0.071, respectively) differences in Cr
content at 95 DAS. The interaction between variety & treatments, variety & fungi, treatment & fungi and overall interaction (V × T × F) were significant (CD (0.05) = 0.260, CD (0.05) = 0.123, CD (0.05) = 0.212 & CD (0.05) = 0.368) at 95 DAS (Fig. 4).

To sum up, the Cr content was found lower in SSG 59 – 3 (2.45, 9.81 ppm at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The highest Cr content was observed in HJ 541 (3.34, 11.82 ppm at 35 & 95 DAS, respectively). It was observed from the results that the Cr content was increased (2.87 to 11.13 ppm) with increase in growth stages (35 to 95 DAS, respectively) of plants. The GB treatments decreased Cr content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB combined with AMF spiked in soil showed lower Cr content as compared to all other treatments, at both the stages in all varieties (Fig. 3, 4).

2.1.3. Effects on chromium content in leaves:
The results presented in Fig. 5 reveal that Cr content in the third leaf from the top of the plant increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 0.41 ppm in control to 2.49 & 2.67 ppm at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 35 days after sowing (DAS). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.26, 2.04 & 2.10 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (0.29, 2.14 & 2.17 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (0.32, 2.23 & 2.34 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.35, 2.27 & 2.42 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.38, 2.44 & 2.59 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (1.99 ppm) followed by HJ 513 (1.63 ppm) and SSG 59 – 3 (1.37 ppm) for Cr content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD (0.05) = 0.013, CD (0.05) = 0.023 & CD (0.05) = 0.011) differences in Cr content at 35 DAS (Fig. 5). The interactions between variety – treatment, variety – fungi and treatment - fungi were found significant (CD (0.05) = 0.040, CD (0.05) = 0.019 & CD (0.05) = 0.033). But the overall interaction (V × T × F) was found non-significant.

Similarly, Cr content increased significantly (Fig. 6) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 2.02 ppm in control to 12.76 & 13.67 ppm at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in Cr content, in all the varieties at 95 DAS. Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (1.42, 8.86 & 10.09 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (1.60, 9.19 & 10.55 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (1.74, 10.31 & 11.70 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (1.78, 9.19 & 12.02 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (1.90, 11.99 & 13.23 ppm in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean Cr content of all treatments (combined) for each variety, decreased significantly in HJ 541, HJ 513 and SSG 59 – 3 (9.24, 7.85 & 7.18 ppm, respectively). All the varieties, treatments and fungi showed
significant \( \text{CD}_{(0.05)} = 0.059, \text{CD}_{(0.05)} = 0.102 \) & \( \text{CD}_{(0.05)} = 0.048 \), respectively) differences in Cr content at 95 DAS. The interactions between variety & treatments, variety & fungi, treatment & fungi and overall interaction \((V \times T \times F)\) were found significant \( \text{CD}_{(0.05)} = 0.176, \text{CD}_{(0.05)} = 0.083, \text{CD}_{(0.05)} = 0.144 \) & \( \text{CD}_{(0.05)} = 0.249 \) at 95 DAS (Fig. 6).

To sum up, the Cr content was found lower in SSG 59 – 3 (1.37, 7.18 ppm at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The highest Cr content was observed in HJ 541 (1.99, 9.24 ppm at 35 & 95 DAS, respectively). It was observed from the results that the Cr content was increased (1.66 & 8.09 ppm) with increase in growth stages (35 to 95 DAS, respectively) of plants. The GB treatments decreased Cr content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB combined with AMF spiked in soil showed lower Cr content as compared to all other treatments, at both the stages in all varieties (Fig. 5, 6).

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

2.2.1 Effect of GB and AMF treatments on anti-oxidative enzymes:

Antioxidative enzymes namely superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) activity increased with increasing concentration of Cr (VI), at both the growth stages, in all the varieties (Figs. 7–16). However, this increase in antioxidative enzymes activity was not enough to protect the plants from increasing toxicity of Cr (VI). Further increase in the activity of these antioxidative enzymes was observed on exogenous application of GB (soil spiked & foliar spray) and AMF, either individually or in combination, at both the growth stages in all the varieties. The activity of these enzymes decreased with plant age (35 DAS to 95 DAS) at both levels (2 & 4 ppm) of Cr (VI), in all the varieties. Maximum increase in the activity of these antioxidative enzymes observed in plants provided with AMF and GB in combination, at both the growth stages in all the varieties. Among the varieties, SSG 59 – 3 variety showed highest activity of these antioxidative enzymes, followed by HJ 513 and lowest in HJ 541 variety (Figs. 7–16).

2.2.1.1. Effects on Superoxide dismutase activity:

The activity of enzyme superoxide dismutase increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 9.3 units mg\(^{-1}\) protein in control to 31.9 & 72.6 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in superoxide dismutase activity, in all the varieties at 35 DAS (Fig. 7). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (23.7, 63.1 & 131.4 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (20.2, 58.5 & 122.2 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (17.1, 50.4 & 109.3 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr,
respectively), 50 mM GB only (15.2, 46.7 & 99.6 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (10.4, 38.1 & 81.7 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 - 3 (72.6 units mg⁻¹ protein) followed by HJ 513 (54.8 units mg⁻¹ protein) and HJ 541 (39.5 units mg⁻¹ protein) for superoxide dismutase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD (0.05) = 0.49, CD (0.05) = 0.85 & CD (0.05) = 0.40, respectively) differences in superoxide dismutase activity at 35 DAS (Fig. 7). The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) were observed significant (CD (0.05) = 1.48, CD (0.05) = 0.70, CD (0.05) = 1.21 & CD (0.05) = 2.09, respectively).

Similarly, superoxide dismutase activity increased significantly (Fig. 8) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 6.10 units mg⁻¹ protein in control to 18.82 & 45.82 units mg⁻¹ protein at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in superoxide dismutase activity, in all the varieties at 95 DAS. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (14.83, 39.01 & 72.33 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (13.20, 35.03 & 68.54 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (11.13, 28.91 & 61.20 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (9.28, 25.23 & 58.14 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (7.44, 21.13 & 50.94 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean superoxide dismutase activity values of all treatments (combined) for each variety, decreased significantly in SSG 59 - 3, HJ 513 and HJ 541 (40.23, 36.20 & 21.41 units mg⁻¹ protein, respectively). All the varieties, treatments and fungi showed significant (CD (0.05) = 0.531, CD (0.05) = 0.919 & CD (0.05) = 0.433, respectively) differences in superoxide dismutase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD (0.05) = 1.592, CD (0.05) = 0.751, CD (0.05) = 1.300 & CD (0.05) = 2.252, respectively) effects at 95 DAS (Fig. 8).

To sum up, the superoxide dismutase activity was found higher in SSG 59 - 3 (72.6, 40.23 units mg⁻¹ protein at 35 & 95 DAS, respectively; when compared with HJ 513 and HJ 541. The lowest superoxide dismutase activity was observed in HJ 541 (39.5, 21.41 units mg⁻¹ protein at 35 & 95 DAS, respectively). It was observed from the results that the superoxide dismutase activity was decreased (55.6 to 32.62 units mg⁻¹ protein) with increase in growth stages (35 to 95 DAS) of plant. The GB treatments increased superoxide dismutase activity significantly in all the varieties (HJ 541, HJ 513 & SSG 59 - 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest superoxide dismutase activity as compared to all other treatments, at both the stages in all varieties (Fig. 7, 8).

### 2.2.1.2. Effects on Ascorbate peroxidase activity:
The ascorbate peroxidase activity increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 23.36 units mg\(^{-1}\) protein in control to 41.31 & 53.44 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in ascorbate peroxidase activity, in all the varieties at 35 DAS (Fig. 9). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (36.90, 48.67 & 75.34 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (34.94, 47.27 & 69.94 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (33.72, 45.93 & 62.84 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (30.56, 44.54 & 59.93 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (28.21, 42.74 & 56.04 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 – 3 (56.38 units mg\(^{-1}\) protein) for ascorbate peroxidase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.355, CD\((0.05) = 0.615 & CD\((0.05) = 0.290, respectively) differences in ascorbate peroxidase activity at 35 DAS (Fig. 9). The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) were observed significant (CD\((0.05) = 1.065, CD\((0.05) = 0.502, CD\((0.05) = 0.869 & CD\((0.05) = 1.506, respectively) at 35 DAS.

Similarly, ascorbate peroxidase activity increased significantly (Fig. 10) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 10.79 units mg\(^{-1}\) protein in control to 19.06 & 36.72 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in ascorbate peroxidase activity, in all the varieties at 95 DAS. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (16.75, 31.77 & 59.27 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (15.58, 29.50 & 54.64 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (14.42, 26.48 & 49.07 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (13.39, 24.02 & 44.74 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (11.69, 20.71 & 38.34 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean ascorbate peroxidase activity values of all treatments (combined) for each variety, decreased significantly in SSG 59 – 3, HJ 513 and HJ 541 (36.32, 29.71 & 20.12 units mg\(^{-1}\) protein, respectively). All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.292, CD\((0.05) = 0.506 & CD\((0.05) = 0.238, respectively) differences in ascorbate peroxidase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD\((0.05) = 0.876, CD\((0.05) = 0.413, CD\((0.05) = 0.715 & CD\((0.05) = 1.239, respectively) effects at 95 DAS (Fig. 10).

To sum up, the ascorbate peroxidase activity was found higher in SSG 59 – 3 (56.38, 36.32 units mg\(^{-1}\) protein at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest ascorbate peroxidase activity was observed in HJ 541 (32.88, 20.12 units mg\(^{-1}\) protein at 35 & 95 DAS,
respectively). It was observed from the results that the ascorbate peroxidase activity was decreased (46.43 to 28.72 units mg\(^{-1}\) protein) with increase in growth stages (35 to 95 DAS) of plant. The GB treatments increased ascorbate peroxidase activity significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined, showed highest ascorbate peroxidase activity as compared to all other treatments, at both the stages in all varieties (Fig. 9, 10).

2.2.1.3. Effects on Catalase activity:

The catalase enzyme activity increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 9.72 units mg\(^{-1}\) protein in control to 24.32 & 39.49 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in catalase activity, in all the varieties at 35 DAS (Fig. 11). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (21.33, 36.70 & 59.51 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) followed by 100 mM GB only (19.56, 34.21 & 56.22 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (16.39, 32.47 & 49.09 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (14.68, 30.29 & 45.67 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (11.68, 27.14 & 41.01 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 – 3 (48.52 units mg\(^{-1}\) protein) followed by HJ 513 (27.79 units mg\(^{-1}\) protein) and HJ 541 (18.59 units mg\(^{-1}\) protein) for catalase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.293, CD\((0.05) = 0.507 & CD\((0.05) = 0.239, respectively) differences in catalase activity at 35 DAS (Fig. 11). The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) were observed significant (CD\((0.05) = 0.878, CD\((0.05) = 0.414, CD\((0.05) = 0.716 & CD\((0.05) = 1.241, respectively at 35 DAS.

Similarly, catalase enzyme activity increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 1.90 units mg\(^{-1}\) protein in control to 8.49 & 20.95 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in catalase activity, in all the varieties at 95 DAS (Fig. 12). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (6.14, 18.55 & 32.51 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (5.37, 16.39 & 29.25 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (4.59, 14.69 & 27.98 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (3.51, 12.62 & 25.97 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (2.54, 10.61 & 23.43 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean catalase activity of all treatments (combined) for each variety, decreased significantly in SSG 59 – 3, HJ 513 and HJ 541 (19.41, 16.41 & 8.43 units mg\(^{-1}\) protein, respectively). All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.090, CD\((0.05) = 0.157 & CD\((0.05) =
0.074, respectively) differences in catalase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD \((0.05) = 0.271, CD \((0.05) = 0.128, CD \((0.05) = 0.222 & CD \((0.05) = 0.384, respectively) effects at 95 DAS (Fig. 12).

To sum up, the catalase activity was found higher in SSG 59 - 3 (48.52, 19.41 units mg\(^{-1}\) protein at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest catalase activity was observed in HJ 541 (18.59, 8.43 units mg\(^{-1}\) protein at 35 & 95 DAS, respectively). It was observed from the results that the catalase activity was decreased (31.63 to 14.75 units mg\(^{-1}\) protein) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased catalase activity significantly in all the varieties (HJ 541, HJ 513 & SSG 59 - 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest catalase activity as compared to all other treatments, at both the stages in all varieties (Fig. 11, 12).

### 2.2.1.4. Effects on Glutathione reductase activity:

The activity of glutathione reductase increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 4.38 units mg\(^{-1}\) protein in control to 14.86 & 29.91 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in glutathione reductase activity, in all the varieties at 35 DAS (Fig. 13). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (12.90, 26.28 & 47.63 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (10.88, 24.20 & 45.77 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (9.06, 21.45 & 40.19 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (7.83, 19.71 & 38.47 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (5.49, 16.92 & 33.05 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 - 3 (30.59 units mg\(^{-1}\) protein) followed by HJ 513 (21.32 units mg\(^{-1}\) protein) and HJ 541 (16.26 units mg\(^{-1}\) protein) for glutathione reductase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD \((0.05) = 0.175, CD \((0.05) = 0.302 & CD \((0.05) = 0.143, respectively) differences in glutathione reductase activity at 35 DAS (Fig. 13). The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) were observed significant (CD \((0.05) = 0.524, CD \((0.05) = 0.247, CD \((0.05) = 0.428 & CD \((0.05) = 0.741, respectively) at 35 DAS.

Similarly, glutathione reductase enzyme activity increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 3.58 units mg\(^{-1}\) protein in control to 9.10 & 16.19 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in glutathione reductase activity, in all the varieties at 95 DAS (Fig. 14). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (7.38, 14.52 & 26.30 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by
100 mM GB treatment only (7.12, 13.81 & 24.41 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (6.20, 12.77 & 20.92 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (4.98, 11.94 & 19.57 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (4.18, 10.08 & 17.35 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean glutathione reductase activity of all treatments (combined) for each variety, decreased significantly from SSG 59 − 3 to HJ 513 and HJ 541 (19.14, 9.81 & 9.44 units mg⁻¹ protein, respectively). All the varieties, treatments and fungi showed significant (CD (0.05) = 0.123, CD (0.05) = 0.212 & CD (0.05) = 0.100, respectively) differences in glutathione reductase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD (0.05) = 0.368, CD (0.05) = 0.173, CD (0.05) = 0.300 & CD (0.05) = 0.520, respectively) effects at 95 DAS (Fig. 14).

To sum up, the glutathione reductase activity was found higher in SSG 59 − 3 (30.59, 19.14 units mg⁻¹ protein at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest glutathione reductase activity was observed in HJ 541 (16.26, 9.44 units mg⁻¹ protein at 35 & 95 DAS, respectively). It was observed from the results that the glutathione reductase activity was decreased (22.72 to 12.80 units mg⁻¹ protein) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased glutathione reductase activity significantly in all the varieties (HJ 541, HJ 513 & SSG 59 − 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest glutathione reductase activity as compared to all other treatments, at both the stages in all varieties (Fig. 13, 14).

### 2.2.1.5. Effects on Peroxidase activity:

The results presented in Fig. 15 conclude that peroxidase enzyme activity increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 18.9 units mg⁻¹ protein in control to 64.2 & 130.4 units mg⁻¹ protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in peroxidase activity, in all the varieties at 35 DAS. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (49.1, 111.9 & 201.9 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (42.9, 97.5 & 189.5 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (33.9, 85.9 & 169.1 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (28.2, 78.6 & 156.0 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (22.1, 70.6 & 144.4 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 − 3 (110.1 units mg⁻¹ protein) followed by HJ 513 (99.8 units mg⁻¹ protein) and HJ 541 (72.6 units mg⁻¹ protein) for peroxidase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD (0.05) = 0.96, CD (0.05) = 1.66 & CD (0.05) = 0.78, respectively) differences in peroxidase activity at 35 DAS (Fig. 15). The interactions between variety – treatments,
variety – fungi, treatments - fungi and overall interaction (V × T × F) were observed significant (CD (0.05) = 2.88, CD (0.05) = 1.36, CD (0.05) = 2.35 & CD (0.05) = 4.07, respectively) at 35 DAS.

Similarly, peroxidase enzyme activity increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 17.6 units mg⁻¹ protein in control to 43.9 & 84.3 units mg⁻¹ protein at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in peroxidase activity, in all the varieties at 95 DAS (Fig. 16). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (39.5, 77.5 & 121.4 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (32.1, 73.7 & 115.9 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (26.4, 61.4 & 106.0 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (25.0, 57.7 & 99.7 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (21.2, 50.4 & 87.7 units mg⁻¹ protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean peroxidase activity of all treatments (combined) for each variety, decreased significantly from SSG 59 − 3 to HJ 513 and HJ 541 (94.1, 59.1 & 37.0 units mg⁻¹ protein, respectively).

All the varieties, treatments and fungi showed significant (CD (0.05) = 0.42, CD (0.05) = 0.73 & CD (0.05) = 0.34, respectively) differences in peroxidase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD (0.05) = 1.26, CD (0.05) = 0.59, CD (0.05) = 1.03 & CD (0.05) = 1.78, respectively) effects at 95 DAS (Fig. 16).

To sum up, the peroxidase activity was found higher in SSG 59 − 3 (110.1, 94.1 units mg⁻¹ protein at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest peroxidase activity was observed in HJ 541 (72.6, 37.0 units mg⁻¹ protein at 35 & 95 DAS, respectively). It was observed from the results that the peroxidase activity was decreased (94.2 to 63.4 units mg⁻¹ protein) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased peroxidase activity significantly in all the varieties (HJ 541, HJ 513 & SSG 59 − 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest peroxidase activity as compared to all other treatments, at both the stages in all varieties (Fig. 15, 16).

**2.2.2 Effect of GB and AMF treatments on anti-oxidative metabolites:**

Among the antioxidative metabolites namely total glutathione, reduced glutathione, oxidized glutathione, ascorbate and β-carotene, except β-carotene all other antioxidative metabolites increased with increasing concentration of Cr (VI), at both the growth stages (35 DAS & 95 DAS), in all the varieties (Figs. 17–26). Except oxidized glutathione, further increase in the content of these antioxidative metabolites was observed on exogenous application of GB and AMF, either individually or in combination, at both the growth stages in all the varieties. Except oxidized glutathione, the maximum increase in contents of all other antioxidative metabolites observed in plants provided with the combination of AMF and GB, at both the growth stages in all the varieties. Except the content of oxidized glutathione all other antioxidative
metabolites decreased with plant age (35 DAS to 95 DAS) at both levels (2 & 4 ppm) of Cr (VI), in all the varieties. Except oxidized glutathione, among all the varieties, SSG 59 – 3 variety showed highest level of these antioxidative metabolites, followed by HJ 513 and lowest in HJ 541 variety (Figs. 17–26).

Oxidized glutathione content decreased on GB and AMF application, either individually or in combination, at both the growth stages in all the varieties. Oxidised glutathione increased with plant age in all the varieties (Figs. 21–22). Among the varieties, HJ 541 variety showed highest content of oxidized glutathione, followed by HJ 513 and lowest in SSG 59 – 3 variety (Figs. 21–22).

The β-carotene content decreased significantly with increasing concentration of Cr (VI), at both the growth stages in all the varieties (Figs. 25–26). Increase in β-carotene content observed on exogenous application of GB and AMF, either individually or in combination, at both the growth stages in all the varieties. All other properties observed were similar to other antioxidative metabolites contents (Figs. 17–24).

### 2.2.2.1. Effects on Glutathione content:

The glutathione content increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 10.75 µmol g\(^{-1}\) fresh weight in control to 36.37 & 66.41 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in glutathione content, in all the varieties at 35 DAS (Fig. 17). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (16.53, 60.07 & 89.24 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (15.10, 56.19 & 86.30 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (14.12, 49.77 & 78.94 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (13.12, 45.64 & 75.69 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (11.86, 39.73 & 69.33 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 – 3 (48.37 µmol g\(^{-1}\) fresh weight) followed by HJ 513 (46.62 µmol g\(^{-1}\) fresh weight) and HJ 541 (44.20 µmol g\(^{-1}\) fresh weight) for glutathione content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD \((0.05) = 0.376, CD \((0.05) = 0.652 & CD \((0.05) = 0.307\) differences in glutathione content at 35 DAS (Fig. 17). The interactions between variety – treatments and treatments - fungi were observed significant (CD \((0.05) = 1.129 & CD \((0.05) = 0.922, respectively) at 35 DAS. However, the interaction between variety – fungi and overall interaction (V × T × F) were found non-significant.

Similarly, glutathione content increased significantly (Fig. 18) with increasing concentrations of Cr in all the varieties at 95 DAS also (from 6.73 µmol g\(^{-1}\) fresh weight in control to 27.38 & 52.37 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in glutathione content, in all the varieties at 95
Das. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (11.41, 47.01 & 71.07 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (10.23, 43.54 & 67.94 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (9.48, 38.07 & 62.20 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (8.66, 35.10 & 59.92 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (7.67, 29.84 & 55.13 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean glutathione content of all treatments (combined) for each variety, decreased significantly from SSG 59 - 3 to HJ 513 and HJ 541 (38.21, 34.61 & 34.49 µmol g\(^{-1}\) fresh weight, respectively). All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.300, CD\((0.05) = 0.520 & CD\((0.05) = 0.245, respectively) differences in glutathione content at 95 DAS. The interactions between variety – treatments and treatments - fungi showed significant (CD\((0.05) = 0.900 & CD\((0.05) = 0.735, respectively) effects at 95 DAS (Fig. 18). However, the interactions between variety – fungi and overall interaction (V × T × F) were found non-significant.

To sum up, the glutathione content was found higher in SSG 59 - 3 (48.37, 38.21 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest glutathione content was observed in HJ 541 (44.20, 34.49 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively). It was observed from the results that the glutathione content was decreased (46.40 to 35.77 µmol g\(^{-1}\) fresh weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased glutathione content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 - 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest glutathione content as compared to all other treatments, at both the stages in all varieties (Fig. 17, 18).

2.2.2.2. Effects on Reduced glutathione content:

The results (Fig. 19) demonstrate that reduced glutathione content increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 9.63 µmol g\(^{-1}\) fresh weight in control to 33.80 & 62.96 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in reduced glutathione content, in all the varieties at 35 DAS. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (15.77, 58.53 & 87.06 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (14.26, 54.49 & 83.97 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (13.19, 47.83 & 76.26 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (12.12, 43.54 & 72.81 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (10.81, 37.35 & 66.09 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59 - 3 (46.39 µmol g\(^{-1}\) fresh weight) followed by HJ 513 (44.89 µmol g\(^{-1}\) fresh weight) and HJ 541 (42.14 µmol g\(^{-1}\) fresh weight) for reduced glutathione content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\((0.05)
Similarly, reduced glutathione content increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 6.29 µmol g\(^{-1}\) fresh weight in control to 26.01 & 50.58 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in reduced glutathione content, in all the varieties at 95 DAS (Fig. 20). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (11.12, 46.26 & 69.88 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (9.92, 42.69 & 66.64 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean reduced glutathione content of all treatments (combined) for each variety, decreased significantly from SSG 59 – 3 to HJ 513 and HJ 541 (37.24, 33.64 & 33.49 µmol g\(^{-1}\) fresh weight, respectively). All the varieties, treatments and fungi showed significant differences in reduced glutathione content at 95 DAS. The interactions between variety – treatments and treatments - fungi showed significant effects at 95 DAS (Fig. 20). However, the interactions between variety – fungi and overall interaction (V x T x F) were found non-significant.

To sum up, the reduced glutathione content was found higher in SSG 59 – 3 (46.39, 37.24 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest reduced glutathione content was observed in HJ 541 (42.14, 33.49 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively). It was observed from the results that the reduced glutathione content was decreased (44.47 to 34.79 µmol g\(^{-1}\) fresh weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased reduced glutathione content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed highest reduced glutathione content as compared to all other treatments, at both the stages in all varieties (Fig. 19, 20).

### 2.2.2.3. Effects on Oxidized glutathione content:

Oxidized glutathione content increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 0.38 µmol g\(^{-1}\) fresh weight in control to 1.18 & 1.81 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in oxidized glutathione content, in all the varieties at 35 DAS (Fig. 21). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.23,
0.62 & 1.11 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (0.28, 0.70 & 1.24 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (0.32, 0.84 & 1.40 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.34, 0.94 & 1.52 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.36, 1.07 & 1.69 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The lowest mean value of all the treatments (combined) was observed in SSG 59−3 (0.80 µmol g$^{-1}$ fresh weight) followed by HJ 513 (0.91 µmol g$^{-1}$ fresh weight) and HJ 541 (0.97 µmol g$^{-1}$ fresh weight) for oxidized glutathione content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD$^{(0.05)}$ = 0.009, CD$^{(0.05)}$ = 0.015 & CD$^{(0.05)}$ = 0.007, respectively) differences in oxidized glutathione content at 35 DAS (Fig. 21). The interactions between variety – treatments and treatments - fungi were observed significant (CD$^{(0.05)}$ = 0.027 & CD$^{(0.05)}$ = 0.022, respectively) at 35 DAS. However, the interaction between variety – fungi and overall interaction (V × T × F) were found non-significant.

Likewise, oxidized glutathione content (Fig. 22) increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 1.11 µmol g$^{-1}$ fresh weight in control to 2.35 & 3.36 µmol g$^{-1}$ fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in oxidized glutathione content, in all the varieties at 95 DAS. Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.71, 1.42 & 2.12 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (0.79, 1.54 & 2.28 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (0.91, 1.78 & 2.61 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (1.01, 1.97 & 2.77 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (1.06, 2.17 & 3.21 µmol g$^{-1}$ fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean oxidized glutathione content of all treatments (combined) for each variety, decreased significantly from HJ 541 to HJ 513 and SSG 59−3 (1.91, 1.89 & 1.74 µmol g$^{-1}$ fresh weight, respectively). All the varieties, treatments and fungi showed significant (CD$^{(0.05)}$ = 0.017, CD$^{(0.05)}$ = 0.029 & CD$^{(0.05)}$ = 0.014, respectively) differences in oxidized glutathione content at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) showed significant (CD$^{(0.05)}$ = 0.051, CD$^{(0.05)}$ = 0.024, CD$^{(0.05)}$ = 0.041 & CD$^{(0.05)}$ = 0.072, respectively) effects at 95 DAS (Fig. 22).

To sum up, the oxidized glutathione content was found lowest in SSG 59−3 (0.80, 1.74 µmol g$^{-1}$ fresh weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. Maximum oxidized glutathione content was observed in HJ 541 (0.97, 1.91 µmol g$^{-1}$ fresh weight at 35 & 95 DAS, respectively). It was observed from the results that oxidized glutathione content was increased (0.89 to 1.84 µmol g$^{-1}$ fresh weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments decreased oxidized glutathione content significantly in all the varieties (HJ 541, HJ 513 & SSG 59−3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed more...
decrease in oxidized glutathione content as compared to all other treatments, at both the stages in all varieties (Fig. 21, 22).

2.2.2.4. Effects on Ascorbate content:

Ascorbate content increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 3.49 µmol g\(^{-1}\) fresh weight in control to 7.89 & 14.31 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in further significant increase in ascorbate content, in all the varieties at 35 DAS (Fig. 23). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (6.72, 17.71 & 27.93 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (6.00, 16.36 & 25.54 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (5.29, 13.00 & 22.52 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (4.75, 11.39 & 20.15 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (4.04, 8.60 & 15.80 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59-3 (15.24 µmol g\(^{-1}\) fresh weight) followed by HJ 513 (13.16 µmol g\(^{-1}\) fresh weight) and HJ 541 (10.18 µmol g\(^{-1}\) fresh weight) for ascorbate content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\(_{(0.05)}\) = 0.136, CD\(_{(0.05)}\) = 0.183 & CD\(_{(0.05)}\) = 0.086, respectively) differences in ascorbate content at 35 DAS (Fig. 23). The interactions between variety – treatments, treatments – fungi and overall interaction (V × T × F) were observed significant (CD\(_{(0.05)}\) = 0.318, CD\(_{(0.05)}\) = 0.259 & CD\(_{(0.05)}\) = 0.449, respectively). The interaction between variety & fungi was found non-significant.

In the same way, ascorbate content (Fig. 24) increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 1.82 µmol g\(^{-1}\) fresh weight in control to 4.88 & 9.42 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in further significant increase in ascorbate content, in all the varieties at 95 DAS. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (4.08, 11.92 & 19.13 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (3.62, 11.01 & 17.46 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (3.06, 8.50 & 15.21 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (2.66, 7.47 & 13.73 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (2.20, 5.42 & 10.42 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean ascorbate content of all treatments (combined) for each variety, decreased significantly from SSG 59-3 to HJ 513 and HJ 541 (10.82, 7.97 & 6.54 µmol g\(^{-1}\) fresh weight, respectively). All the varieties, treatments and fungi showed significant (CD\(_{(0.05)}\) = 0.058, CD\(_{(0.05)}\) = 0.100 & CD\(_{(0.05)}\) = 0.047, respectively) differences in ascorbate content at 95 DAS (Fig. 24). The interactions between variety – treatments, variety – fungi, treatments - fungi and overall interaction (V × T × F) were also found significant (CD\(_{(0.05)}\) = 0.173, CD\(_{(0.05)}\) = 0.082, CD\(_{(0.05)}\) = 0.141 & CD\(_{(0.05)}\) = 0.245, respectively).
To sum up, the ascorbate content was found higher in SSG 59–3 (15.24, 10.82 µmol g$^{-1}$ fresh weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest ascorbate content was observed in HJ 541 (10.18, 6.54 µmol g$^{-1}$ fresh weight at 35 & 95 DAS, respectively). It was observed from the results that the ascorbate content was decreased (12.86 to 8.45 µmol g$^{-1}$ fresh weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased ascorbate content significantly in all the varieties (HJ 541, HJ 513 & SSG 59–3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed more increase in ascorbate content as compared to all other treatments, at both the stages in all varieties (Fig. 23, 24).

2.2.2.5. Effects on β-carotene content:

The results presented in Fig. 25 demonstrate that β-carotene content decreased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 0.25 mg kg$^{-1}$ dry weight in control to 0.18 & 0.12 mg kg$^{-1}$ dry weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant increase in β-carotene content, in all the varieties at 35 days after sowing (DAS). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (0.38, 0.25 & 0.20 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (0.37, 0.24 & 0.19 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (0.34, 0.23 & 0.17 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.31, 0.21 & 0.16 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.27, 0.19 & 0.14 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in SSG 59–3 (0.30 mg kg$^{-1}$ dry weight) followed by HJ 513 (0.23 mg kg$^{-1}$ dry weight) and HJ 541 (0.17 mg kg$^{-1}$ dry weight) for β-carotene content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD (0.05) = 0.001, CD (0.05) = 0.002 & CD (0.05) = 0.001, respectively) differences in β-carotene content at 35 DAS (Fig. 25). The interactions between variety – treatments, variety - fungi, treatments – fungi and overall interaction (V × T × F) were observed significant (CD (0.05) = 0.003, CD (0.05) = 0.002, CD (0.05) = 0.003 & CD (0.05) = 0.005, respectively).

Similarly, β-carotene content decreased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 0.17 mg kg$^{-1}$ dry weight in control to 0.13 & 0.08 mg kg$^{-1}$ dry weight at 2 ppm Cr & 4 ppm Cr, respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant increase in β-carotene content, in all the varieties at 95 DAS (Fig. 26). Maximum increase was observed with treatment of 100 mM GB with 10 g AMF (0.26, 0.18 & 0.14 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (0.25, 0.17 & 0.19 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (0.23, 0.16 & 0.12 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.21, 0.15 & 0.11 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.19, 0.14 & 0.09 mg kg$^{-1}$ dry weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean β-carotene content of all treatments (combined), decreased significantly from SSG 59–3 to HJ 513 and HJ
541 (0.21, 0.17 & 0.11 mg kg$^{-1}$ dry weight, respectively). All the varieties, treatments and fungi showed significant (CD$_{(0.05)}$ = 0.001, CD$_{(0.05)}$ = 0.002 & CD$_{(0.05)}$ = 0.001, respectively) differences in β-carotene content at 95 DAS (Fig. 26). The interactions between variety – treatments, variety – fungi and treatments - fungi were observed significant (CD$_{(0.05)}$ = 0.003, CD$_{(0.05)}$ = 0.001 & CD$_{(0.05)}$ = 0.002, respectively). However, the overall interaction (V × T × F) was found non-significant.

To sum up, the β-carotene content was found higher in SSG 59 – 3 (0.30, 0.21 mg kg$^{-1}$ dry weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The lowest β-carotene content was observed in HJ 541 (0.17, 0.11 mg kg$^{-1}$ dry weight at 35 & 95 DAS, respectively). It was observed from the results that the β-carotene content was decreased (0.23 to 0.16 mg kg$^{-1}$ dry weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments increased β-carotene content significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed more increase in β-carotene content as compared to all other treatments, at both the stages in all varieties (Fig. 25, 26).

### 2.3 Effect of GB and AMF treatments on indices of oxidative stress in sorghum under chromium toxicity:

The oxidative stress was measured in terms of polyphenol oxidase (PPO) activity, hydrogen peroxide (H$_2$O$_2$) and MDA contents under Cr (VI) toxicity as compared with control in sorghum.

PPO causes oxidation of phenolic compounds and increased the oxidative stress in sorghum plants. Indices of oxidative stress compounds namely PPO, H$_2$O$_2$ and MDA contents increased with increasing concentration of Cr (VI), at both the growth stages (35 DAS & 95 DAS), in all the varieties (Figs. 27–32). Decrease in these indices of oxidative stress was observed on exogenous application of GB and AMF, either individually or in combination, at both the growth stages in all the varieties. All these indices of oxidative stress increased with plant age (35 DAS to 95 DAS) at both levels (2 & 4 ppm) of Cr (VI) in all the varieties. Maximum increase in all these indices of oxidative stress observed in plants under Cr (VI) toxicity only, at both the growth stages in all the varieties. Among the varieties, HJ 541 variety showed highest level of these indices of oxidative stress, followed by HJ 513 and lowest in SSG 59 – 3 variety (Figs. 27–32).

#### 2.3.1. Effects on Poly-phenol oxidase activity:

The results demonstrate that polyphenol oxidase activity increased significantly (Fig. 27) with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 3.53 units mg$^{-1}$ protein in control to 6.86 & 12.74 units mg$^{-1}$ protein at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in polyphenol oxidase activity, in all the varieties at 35 days after sowing (DAS). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (2.52, 3.94 & 8.13 units mg$^{-1}$ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (2.61, 4.36 & 8.75 units mg$^{-1}$ protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (2.87, 4.87 & 10.16 units mg$^{-1}$ protein in control,
2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (3.04, 5.40 & 10.91 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (3.28, 6.07 & 11.91 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (8.84 units mg\(^{-1}\) protein) followed by HJ 513 (6.22 units mg\(^{-1}\) protein) and SSG 59 – 3 (3.60 units mg\(^{-1}\) protein) for polyphenol oxidase activity at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.050\), CD\((0.05) = 0.087\) & CD\((0.05) = 0.041\)) differences in polyphenol oxidase activity at 35 DAS (Fig. 27). The interactions between variety – treatment, variety – fungi, treatment – fungi and overall interaction (V × T × F) showed significant (CD\((0.05) = 0.150\), CD\((0.05) = 0.071\), CD\((0.05) = 0.123\) & CD\((0.05) = 0.213\), respectively) effects in polyphenol oxidase activity at 35 DAS.

Correspondingly, polyphenol oxidase activity also increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS (from 6.14 units mg\(^{-1}\) protein in control to 11.05 & 20.72 units mg\(^{-1}\) protein at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in polyphenol oxidase activity, in all the varieties at 95 DAS (Fig. 28). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (4.14, 6.73 & 13.08 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (4.47, 7.13 & 13.93 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (4.92, 8.09 & 15.97 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (5.27, 8.69 & 17.23 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (5.70, 10.02 & 19.10 units mg\(^{-1}\) protein in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean polyphenol oxidase activity of all treatments (combined), decreased significantly from HJ 541 to HJ 513 and SSG 59 – 3 (13.59, 10.07 & 6.73 units mg\(^{-1}\) protein, respectively). All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.057\), CD\((0.05) = 0.098\) & CD\((0.05) = 0.046\), respectively) differences in polyphenol oxidase activity at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments – fungi and their overall interaction (V × T × F) were found significant (CD\((0.05) = 0.170\), CD\((0.05) = 0.080\), CD\((0.05) = 0.139\) & CD\((0.05) = 0.241\), respectively) at 95 DAS (Fig. 28).

The activity of enzyme poly-phenol oxidase was found lowest in SSG 59 – 3 (6.22, 10.13 units at 35 & 95 DAS, respectively) as compared with HJ 513 and HJ 541. The activity of enzyme poly-phenol oxidase was observed highest in HJ 541 (8.84, 13.59 units at 35 & 95 DAS, respectively). It was observed from the results that the activity of enzyme poly-phenol oxidase was increased (6.22 to 10.13 units) with increase in growth stages (35 to 95 DAS, respectively) of plants. The GB treatments significantly reduced the activity of enzyme poly-phenol oxidase, in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at all the growth stages (35 & 95 DAS). Among all the treatments the activity of enzyme poly-phenol oxidase was lower in plants provided with GB and AMF combined at 35 and 95 DAS, respectively (Fig. 27, 28).

### 2.3.2. Effects on Hydrogen peroxide (H\(_2\)O\(_2\)) content:
The hydrogen peroxide increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 8.96 µmol g\(^{-1}\) fresh weight in control to 26.36 & 42.68 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in hydrogen peroxide, in all the varieties at 35 DAS (Fig. 29). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (5.45, 14.54 & 26.41 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (5.81, 16.19 & 28.18 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (6.28, 18.90 & 31.89 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (7.01, 21.27 & 34.38 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (7.75, 24.33 & 39.62 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (25.31 µmol g\(^{-1}\) fresh weight) followed by HJ 513 (20.69 µmol g\(^{-1}\) fresh weight) and SSG 59 – 3 (15.00 µmol g\(^{-1}\) fresh weight) for hydrogen peroxide at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\(_{0.05}\) = 0.184, CD\(_{0.05}\) = 0.319 & CD\(_{0.05}\) = 0.150, respectively) differences in hydrogen peroxide at 35 DAS (Fig. 29). The interactions between variety – treatment, variety – fungi and treatment – fungi showed significant (CD\(_{0.05}\) = 0.552, CD\(_{0.05}\) = 0.260 & CD\(_{0.05}\) = 0.451, respectively) effects in hydrogen peroxide at 35 DAS. However, the overall interaction (V × T × F) was found non-significant.

In the same way, hydrogen peroxide content increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 10.34 µmol g\(^{-1}\) fresh weight in control to 32.13 & 51.28 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in hydrogen peroxide, in all the varieties at 95 DAS (Fig. 30). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (6.14, 17.12 & 29.12 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (6.53, 19.02 & 31.86 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (7.18, 21.79 & 36.02 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (8.02, 25.20 & 39.56 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (8.90, 28.93 & 47.11 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean hydrogen peroxide of all treatments (combined), decreased significantly from HJ 541 to HJ 513 and SSG 59 – 3 (30.30, 23.33 & 17.40 µmol g\(^{-1}\) fresh weight, respectively). All the varieties, treatments and fungi showed significant (CD\(_{0.05}\) = 0.187, CD\(_{0.05}\) = 0.323 & CD\(_{0.05}\) = 0.152, respectively) differences in hydrogen peroxide at 95 DAS. The interactions between variety – treatments, variety – fungi, treatments – fungi and their overall interaction (V × T × F) were found significant (CD\(_{0.05}\) = 0.560, CD\(_{0.05}\) = 0.264, CD\(_{0.05}\) = 0.457 & CD\(_{0.05}\) = 0.792, respectively) at 95 DAS (Fig. 30).

To sum up, the hydrogen peroxide content was found lower in SSG 59 – 3 (15.00, 17.40 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively) when compared with HJ 513 and HJ 541. The highest hydrogen
peroxide content was observed in HJ 541 (25.31, 30.30 µmol g\(^{-1}\) fresh weight at 35 & 95 DAS, respectively). It was observed that hydrogen peroxide was increased (20.33 to 23.68 µmol g\(^{-1}\) fresh weight) with increase in growth stages (35 to 95 DAS) of plants. The GB treatments decreased hydrogen peroxide significantly in all the varieties (HJ 541, HJ 513 & SSG 59 – 3) at both the growth stages (35 & 95 DAS). However, treatments of GB and AMF combined showed lower hydrogen peroxide as compared to all other treatments, at both the stages in all varieties (Fig. 29, 30).

### 2.3.3. Effects on Malondialdehyde (MDA) content:

The results (Fig. 31) demonstrate that MDA content increased significantly with increasing concentrations of Cr (VI) in all the varieties at 35 DAS (from 0.40 µmol g\(^{-1}\) fresh weight in control to 0.99 & 1.49 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr, respectively). However, application of GB in soil (50 mM & 100 mM) with and without AMF (10 g) resulted in significant decrease in MDA, in all the varieties at 35 DAS. Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.21, 0.56 & 0.91 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB only (0.26, 0.63 & 0.96 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB along with 10 g AMF (0.29, 0.76 & 1.11 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.33, 0.82 & 1.21 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.36, 0.92 & 1.39 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The highest mean value of all the treatments (combined) was observed in HJ 541 (0.90 µmol g\(^{-1}\) fresh weight) followed by HJ 513 (0.77 µmol g\(^{-1}\) fresh weight) and SSG 59 – 3 (0.60 µmol g\(^{-1}\) fresh weight) for MDA content at 35 DAS in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant (CD\((0.05) = 0.006\), CD\((0.05) = 0.011\) & CD\((0.05) = 0.005\), respectively) differences in MDA at 35 DAS (Fig. 31). The interactions between variety – treatment, variety – fungi, treatment – fungi and overall interaction (V × T × F) showed significant (CD\((0.05) = 0.019\), CD\((0.05) = 0.009\), CD\((0.05) = 0.016\) & CD\((0.05) = 0.027\), respectively) effects in MDA at 35 DAS.

Similarly, MDA content increased significantly with increasing concentrations of Cr in all the varieties at 95 DAS also (from 0.60 µmol g\(^{-1}\) fresh weight in control to 1.64 & 2.38 µmol g\(^{-1}\) fresh weight at 2 ppm Cr & 4 ppm Cr; respectively). However, GB treatment (50 mM & 100 mM) in soil with and without AMF (10 g) resulted in significant decrease in MDA, in all the varieties at 95 DAS (Fig. 32). Maximum decrease was observed with treatment of 100 mM GB with 10 g AMF (0.29, 0.87 & 1.38 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), followed by 100 mM GB treatment only (0.37, 0.97 & 1.49 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB with AMF (10 g) treatment (0.42, 1.16 & 1.73 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively), 50 mM GB only (0.48, 1.28 & 1.85 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively) and AMF (10 g) only (0.54, 1.49 & 2.19 µmol g\(^{-1}\) fresh weight in control, 2 ppm Cr & 4 ppm Cr, respectively). The mean MDA of all treatments (combined), decreased significantly from HJ 541 to HJ 513 and SSG 59 – 3 (1.45, 1.15 & 0.92 µmol g\(^{-1}\) fresh weight, respectively). All the varieties, treatments and fungi showed
significant \((CD_{(0.05)} = 0.011, CD_{(0.05)} = 0.019 \& CD_{(0.05)} = 0.009, \text{respectively})\) differences in MDA at 95 DAS. The interactions between variety – treatments, variety – fungi and treatments – fungi were found significant \((CD_{(0.05)} = 0.033, CD_{(0.05)} = 0.015 \& CD_{(0.05)} = 0.027, \text{respectively})\) at 95 DAS (Fig. 32). However, the overall interaction \((V \times T \times F)\) was found non-significant.

To sum up, the MDA content was found lower in SSG 59 – 3 \((0.60, 0.92 \mu\text{mol g}^{-1} \text{fresh weight at 35 & 95 DAS, respectively})\) when compared with HJ 513 and HJ 541. The highest MDA content was observed in HJ 541 \((0.90, 1.45 \mu\text{mol g}^{-1} \text{fresh weight at 35 & 95 DAS, respectively})\). It was observed that MDA was increased \((0.75 \text{ to } 1.17 \mu\text{mol g}^{-1} \text{ fresh weight})\) with increase in growth stages \((35 \text{ to } 95 \text{ DAS})\) of plants. The GB treatments decreased MDA content significantly in all the varieties \((HJ 541, HJ 513 \& SSG 59 – 3)\) at both the growth stages \((35 \& 95 \text{ DAS})\). However, treatments of GB and AMF combined showed lower MDA as compared to all other treatments, at both the stages in all varieties (Fig. 31, 32).

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

There was a progressive decrease in grain weight with increasing concentration of Cr (VI), at both the growth stages \((35 \& 95 \text{ DAS})\), in all the varieties (Figs. 33). Increase 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 GB and AMF, at both the growth stages in all the varieties. Among the varieties, SSG 59 – 3 variety showed highest grain yield, followed by HJ 513 and lowest in HJ 541 variety (Figs. 33).

The results presented in Fig. 33 reveal that weight of hundred grains decreased significantly with increasing concentrations of Cr (VI) in all the varieties \((\text{from } 0.91 \text{ g in control to } 0.74 \& 0.53 \text{ g at } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\). However, application of GB in soil \((50 \text{ mM} \& 100 \text{ mM})\) with and without AMF \((10 \text{ g})\) resulted in significant increase in weight of hundred grains, in all the varieties. Maximum increase was observed with treatment of 100 mM GB with 10 g AMF \((2.83, 2.33 \& 2.03 \text{ g in control, } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\), followed by 100 mM GB only \((2.55, 2.19 \& 1.84 \text{ g in control, } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\), 50 mM GB along with 10 g AMF \((1.76, 1.29 \& 1.18 \text{ g in control, } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\), 50 mM GB only \((1.47, 1.27 \& 1.11 \text{ g in control, } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\) and AMF \((10 \text{ g})\) only \((1.06, 0.86 \& 0.61 \text{ g in control, } 2 \text{ ppm Cr} \& 4 \text{ ppm Cr, respectively})\). The highest mean value of all the treatments (combined) was observed in SSG 59 – 3 \((1.83 \text{ g})\) followed by HJ 513 \((1.41 \text{ g})\) and HJ 541 \((1.19 \text{ g})\) for weight of hundred grains, in plants provided with GB spiked in soil. All the varieties, treatments and fungi showed significant \((CD_{(0.05)} = 0.012, CD_{(0.05)} = 0.020 \& CD_{(0.05)} = 0.009)\) differences in weight of hundred grains (Fig. 33). The interactions between variety – treatment, variety – fungi, treatment – fungi and overall interaction \((V \times T \times F)\) were also observed significant \((CD_{(0.05)} = 0.035, CD_{(0.05)} = 0.016, CD_{(0.05)} = 0.028 \& CD_{(0.05)} = 0.049, \text{respectively})\).

To sum up, the weight of hundred grains was found higher in SSG 59 – 3 \((1.83 \text{ g})\) when compared with HJ 513 and HJ 541. The lowest weight of hundred grains was observed in HJ 541 \((1.19 \text{ g})\). It was
observed from the results that the weight of hundred grains was increased on AMF and GB application. However, treatments of GB combined with AMF spiked in soil showed higher weight of hundred grains as compared to all other treatments in all varieties (Fig. 33).

3. Discussion

Sorghum (*Sorghum bicolor* L.) is an important forage crop for the livestock sector. After wheat and rice, it is a major cereal crop consumed in India, particularly in dry season when other feed resources are in short supply. It is highly efficient in the utilization of solar energy and water use efficiency. The growth and yield of the crop is affected by biotic and abiotic stresses including Cr toxicity. Chromium toxicity in agriculture has become a serious problem in developing countries (Ali *et al.* 2011). Chromium is non-essential for plants and generates toxic stress by causing reduction of molecular oxygen and producing intermediate products called reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (H$_2$O$_2$). Excessive production of ROS is toxic to plants and cause oxidative damage to cellular constituents (Banu *et al.* 2009). Plants possess several antioxidant defence systems to protect their cells against ROS such as accumulation of a variety of small organic metabolites, collectively referred to as compatible solutes (Ashraf and Foolad, 2007). Compatible solutes include sugars, polyols, glycinebetaine, amino acids (proline, histidine) and related compounds (Rhodes and Hanson, 1993). One of the most studied compatible solute is glycine betaine (GB), N, N, N-trimethyl glycine, which is synthesized at elevated rates in response to abiotic stresses (Allard *et al.* 1998). It functions as osmoprotectant which suppresses production of free radicals and ROS. It counteracts the oxidative stress in plants by elevating the level of antioxidant enzymes and metabolites. There are many reports on Cr (VI) toxicity causing hazardous effects in plants. However, reports on amelioration of chromium toxicity by exogenous application of glycine betaine (GB) and Arbuscular mycorrhizal fungi (AMF) in sorghum are scanty in literature. Therefore, the aim of present investigation was to assess the ameliorative effect of exogenously applied GB and AMF on antioxidative defence system in sorghum grown under Cr (VI) toxicity. The results obtained during present studies are discussed here under the following heads.

3.1 Exogenous application of AMF and GB both individually or in combination reduces Cr (VI) content in Sorghum and alleviates chromium induced toxicity:

The results of present investigation indicated that the Cr content increased significantly with increasing concentration of Cr (VI) as compared to control, in roots, stem and leaves at both the stages in all the varieties (Figs. 1–6). The Cr content was highest in roots followed by stem and leaves, at both the stages in all the varieties. It seems that the Cr content in roots, stem and leaves increased many folds after soil application of only 2 and 4 ppm of Cr (VI). The reason for this increase might be the lower weight of dried sorghum plant as compared to weight of soil (5 kg pot$^{-1}$). The concentration of Cr (VI) changes with respect to weight of medium (soil to plant). It increases as weight of medium decreases from soil to dried plant. Similar reports have been reported earlier, that Cr content increased in plant tissue with increasing concentration of Cr (VI), under chromium toxicity in sorghum (Kumar *et al.* 2010; Bacaha *et al.* 2015; Kasmiyati *et al.* 2016). The increase in Cr content has also been reported in moong bean grown under
tannery effluents (Bera and Bokaria, 1999) and exogenous chromium treatments (Samantary, 2002; Banerjee et al. 2008).

Exogenous application of GB and AMF, decreased Cr content as compared to control, at both 2 & 4 ppm of Cr (VI) at both the stages in all the varieties (Figs. 1–6). Among all varieties the SSG 59 – 3 variety showed least Cr content in roots, stem and leaves as compared to HJ 513 and HJ 541 (Figs. 1–6). Glycine betaine mediated decrease in Pb and Cd content have been reported in mung bean (Hossain et al. 2010), rice (Cao et al. 2013) and cotton (Bharwana et al. 2014). The reduction in Cr absorption by plants on GB application might be due to the shielding nature of GB towards cell membranes which in turn reduces chromium movement and absorption in the cell (Giri, 2011). The other possibility in reduction of Cr absorption with GB application might be the competition between nutrients and Cr (VI) absorption by plant, as reported by Shahbaz and Zia, (2011), who have studied the effect of exogenous GB application on nutrients mineral status in rice under saline conditions.

Reports on heavy metal resistant microorganisms indicate exceptional ability to promote the growth of host plant under stressful conditions (Grichko and Glick, 2001). Among them AMF has been recognized as a potential biological agent that increases the tolerance capacity of host plant under heavy metal stress (Vivas et al. 2006). In the present investigation it has been observed that GB and AMF inoculation increased the yield by reducing chromium content (Figs. 33). Karagiannidis and Hadjisavva, (1998) reported that AMF inoculation increased nutrient uptake and supresses Cr, Mn, Fe, Co, Ni, and Pb absorption in duram wheat grown under different environmental conditions.

3.2 Exogenous application of GB and AMF both individually or in combination counteracts Cr (VI) induced alterations in the ROS by Antioxidant defence system of Sorghum:

The plants grown under Cr (VI) toxicity have shown increased generation of ROS, which reduces the growth of plants by causing oxidation of macromolecules like lipids, nucleic acids and proteins (Singh et al. 2013; Das et al. 2014). Highly reactive oxygen species are generated during various abiotic stresses and if not quenched or scavenged properly, they can cause oxidative harm to plant biomolecules (Apel and Hirt, 2004). Generation of ROS under Cr (VI) toxicity in plants, makes it phytotoxic in nature. It causes harmful oxidative injuries in plants (Rastgoo and Alemzadeh, 2011). To get rid of the harmful effects of reactive oxygen species (ROS), plants have developed a well organised and effective ROS quenching system composed of antioxidative enzymes (SOD, APX, CAT, GR, POD etc.) and antioxidative metabolites such as glutathione, ascorbate, β-carotene etc. (Mittler, 2002; Apel and Hirt, 2004). Studies have been conducted on effect of individual application of GB and AMF (separately) in inducing the tolerance capacity against abiotic stresses in plants (Sakamoto and Murata, 2000; Shirasawa et al. 2006; Chen and Murata, 2008). But there is no such report on the effects of GB and AMF combined application in inducing the tolerance capacity of plant against abiotic stresses or ameliorating the Cr (VI) toxic effects in plants especially sorghum.

3.2.1 Antioxidative enzymes:
In the present investigation, it was observed that the activity of antioxidative enzymes viz. SOD, APX, CAT, GR and POD increased significantly with increasing concentration of Cr (VI) as compared to control at both the stages (35 & 95 DAS) in all the varieties (Figs. 7–16). Similarly, many scientists have reported decreased activity of antioxidative enzymes under heavy metal toxicity (Ali et al. 2011; Farooq et al. 2013; Adrees et al. 2015; Gill et al. 2015). Mittler, (2002) has also reported development of an antioxidative defense system to quench ROS by augmenting the activities of antioxidative enzymes (SOD, APX, CAT, GR & POD). Emamverdian et al. (2015), Solanki and Dhankhar, (2011) and Sharma et al. (2012), have reported that the increased activity of antioxidative enzymes in plants under heavy metal stress depends on plant species and the level of their metal tolerance. However, Gill et al. (2015) observed that APX activity decreased while CAT activity increased in B. napus grown under Cr (VI) toxicity. Mobin and Khan, (2007) in Indian mustard and Khan et al. (2008) in wheat have demonstrated an increased APX activity, under Cd stress which supports the findings of present investigation. Ehasan et al. (2014) have also obtained similar results in B. napus under Cd toxicity.

Diwan et al. (2012); Suthar et al. (2014) and Habiba et al. (2015) have also reported increase in activity of antioxidative enzymes in wheat and soyabean, mung bean and B. napus, respectively, under heavy metal Cu and Cr (VI) grown under hydroponic system. Similarly, Gill et al. (2015) have reported increased activity of CAT enzyme under Cr (VI) toxicity in B. napus. The increase in activities of antioxidative enzymes in plants under Cr (VI) toxicity suggested increased efficiency of antioxidative defense system to quench ROS which in turn increased the probabilities of ROS decline (Michalak, 2006). The results of the present investigation revealed that the soil application of GB and AMF (individually and combined) further increased the activity of SOD, APX, CAT, GR and POD enzymes at both 2 & 4 ppm of Cr (VI) at both the stages in all the varieties as compared to control (Figs. 7–16). The activity of antioxidative enzymes was observed higher at 35 DAS as compared to 95 DAS.

Also the activity of antioxidative enzymes increased with increasing concentration of GB (50 to 100 mM) at both growth stages in all the varieties (Figs. 7–16). Similar results were reported during Cd stress in rice and mung bean plants by Hossain et al. (2010) and Cao et al. (2013), respectively. Bharwana et al. (2014) have also obtained similar results on exogenous GB application under Pb stress in cotton. Xin et al. (2011) have reported that exogenous GB application increased the activities of CAT, SOD and POD enzymes in maize during drought stress. Increase in CAT activity during chilling stress in tomato plants on exogenous GB application have also been reported by Park et al. (2004). Similar results have been reported by Hasanuzzamam et al. (2014) in rice seedling on exogenous GB application. The increase in antioxidative enzymes activity has also been reported in rice, maize and mung bean plants, under salt stressed conditions by exogenous GB application (Hossain and Fujita, 2010; Nawaz and Ashraf, 2010). Karagiannidis and Hadjisavva, (1998) observed that the AMF inoculated wheat plants showed decreased heavy metals (Mn, Fe, Co, Cr, Ni, Pb) uptake by plants, which in turn reduces the oxidative stress in plants and improved the plant growth in stressful conditions. Similarly, Hassan et al. (2013) observed reduced metal uptake in sunflower on AMF inoculation. These reports support the results obtained in present investigation.
The increase in antioxidative enzymes activity on GB and AMF (individually and combined) application may be due to reduction in Cr uptake, electrolyte leakage, oxidative stress and improvement in the absorption of essential nutrients by the plants as suggested by Giri, (2011) and Bharwana et al. (2014) while studying the effect of exogenous GB application in cotton under lead toxicity. Thus, AMF played important role in the alleviation of trace metal toxicity in host plants. The results of present investigation indicate that the soil treatments of GB along with AMF significantly increased the activity of antioxidative enzymes. Among all varieties the SSG 59 - 3 variety showed highest activity of antioxidative enzymes as compared to HJ 513 and HJ 541 (Figs. 7–16).

3.2.2 Antioxidative metabolites:

The stress tolerance mechanism in plants involves the coordination of multifaceted biochemical and physiological processes covering the variations in global gene expression (Dalcorso et al. 2010). The stressful conditions leads to dehydration of plant cells which in turn causes disturbance in the creation and abolition of ROS vis-à-vis alteration in the redox state of cell (Noctor et al. 2014). Plants evolve several strategies to manage normal level of ROS and redox state in the cell. One of such strategies is to maintain cellular redox homeostasis during unfavourable conditions by antioxidative metabolites such as ascorbates, glutathiones, tocopherols, carotenoids, flavonoids and ROS quenching enzymes like SOD, APX, CAT, GR, POD etc. (Yadav et al. 2005; Anwar et al. 2014; Hossain et al. 2011). Ascorbate and glutathione acts as cofactors of many important enzymes such as APX and glyoxylate cycle enzymes. Both can quench ROS directly or indirectly and also involved in gene expression regulation linked with either biotic or abiotic stress reactions to improve defence and survival (Hossain et al. 2012). Glutathione generally involves three possible ways in protecting plant cells from any stress such as metal or metalloid stress: Firstly- direct quenching of ROS, secondly- conjugating the toxic metals or metalloids or other xenobiotics to glutathione-s-transferase (GST) and thirdly- by acting as a precursor for the creation of phytochelatins (Hasanuzzaman et al. 2017).

In the present investigation, the glutathione content (reduced, oxidised & total) increased with increasing levels of Cr (VI) toxicity as compared to control at both the stages in all the varieties (Figs. 17–22). There are many reports on protective roles of glutathione against various toxic stresses of metal and metalloids in plants and a few has reported the increase in glutathione content against a particular metal stress in plants by enhancing the functioning of proteins involved in glutathione biosynthesis (Xiang and Oliver, 1998). Ruegsegger et al. (1990) reported 3 to 4 times increase in glutathione synthase activity during Cd toxicity in P. sativum. Time dependent increase in glutathione synthase functioning were also reported during Cd toxicity in P. sativum plants (Bergmann and Rennenberg, 1993).

Application of exogenous GB and AMF (individually and combined) further increased reduced glutathione (GSH) and total glutathione content while oxidised glutathione (GSSG) decreased, significantly as compared to control at both the stages in all the varieties (Figs. 17–22). The reduction in GSSG content may be due to increased GSH content on GB and AMF application in sorghum plants as suggested by Gietler et al. (2016), who studied that the quenching of ROS by GSH. This involved the generation of GSSG subsequent reduction to GSH by enzyme glutathione reductase, which in turn decreased the stress caused by Cr (VI) toxicity. Similarly, Wang et al. (2011) observed significant alleviation Cd toxicity stress
by the application of GSH in barley. Hisyam et al. (2017) have also reported increased antioxidant system activity to counteract the stress caused by water deficiency on exogenous GB application. It has been proposed that glutathione (GSH) prevents proteins from denaturation instigated by oxidation of thiols groups in proteins under stress conditions (Hasanuzzaman et al. 2014). However the mechanism involved in the defence reaction is still unclear.

Ascorbate quenches ROS by acting as an electron donor, during antioxidative reactions. One such reaction is catalysed by ascorbate peroxidase which oxidizes ascorbate to dehydrascorbate which in turn scavenges ROS during ascorbate-glutathione cycle. Along with glutathione and ascorbate, β-carotene also acts as an antioxidative compound and played important role in increasing stress tolerance (Abugri et al. 2013). During present investigation, the ascorbate content increased significantly with increasing concentration of Cr (VI) and further increase was observed on exogenous GB and AMF (individually and combined) application as compared to control at both the stages in all the varieties (Figs. 23–24). However, β-carotene decreased significantly with increasing concentration of Cr (VI) and increased on exogenous application of GB and AMF as compared to control at both 2 & 4 ppm of Cr (VI) in all the varieties at both the stages (Figs. 25–26). Yadav and Singh, (2013) also observed similar results in wheat under benzoic acid and Cd toxicity. Similar observations were also made in beans, mangroves and sal plants under Pb, Cd, Cu, Hg and As toxicity by Zengin and Munzuroglu, (2005); Huang et al. (2010) and Pant et al. (2011), respectively.

Strzalka et al. (2003) reported that carotenoids are the main pigments in plants that acts as an antioxidants in guarding plants from various stresses. They favour plant survival under stressful conditions by scavenging ROS and protecting the photosynthetic machinery of the plant (Havaux, 2014). The increase in glutathione, ascorbate and β-carotene content on exogenous GB and AMF (individually and combined) application may be due to involvement of GB in inducing the activity of antioxidative system by altering cellular signalling which in turn increased the synthesis of antioxidative enzymes and metabolites as was reported by Vasquez et al. (2006) in mango fruits, Nhung et al. (2010) in gac fruits and Su et al. (2015) in tomato fruits.

Toth et al. (2013) reported that ascorbic acid acts as a multifunctional biochemical that is involved in cell-wall synthesis, redox signalling and cell division. Ascorbic acid can directly quench ROS and also indirectly by ascorbate peroxidase mediated quenching of \( \text{H}_2\text{O}_2 \) (Sairam et al. 2005). Ascorbate peroxidase enzyme is the main component of ascorbate-glutathione pathway which plays very crucial role in the regulation of ROS level in the cell (Sharma et al. 2012). Thus exogenous application of GB and AMF (individually and combined) seems to improve the plant growth and yield by increasing the content of antioxidative metabolites and antioxidative enzymes which in turn reduced the ROS content and ameliorated the toxic effects of Cr (VI) in sorghum. Among all the varieties SSG 59–3 variety showed highest tolerance capacity against Cr (VI) toxicity, by expressing highest content of antioxidative metabolites as compared to HJ 513 and HJ 541 varieties (Figs. 17–26).
3.3 Exogenous GB and AMF both individually or in combination counteracts Cr (VI) induced indices of oxidative stress and mitigated the toxicity of chromium in Sorghum:

Generation of reactive oxygen species (ROS) is the first line of defense reaction exhibited by any plant cell in response to environment. 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.

Enzyme PPO causes oxidation of polyphenols, thus reducing the chances of plants survival under stressful conditions and signals as measure of stress (Rodríguez et al. 2015; Kulbat, 2016). Polyphenols have played crucial roles in plants growth and development by strengthening their defence mechanisms, particularly in fighting against stressful situations (Chalker et al. 2018). Gill and Tuteja, (2010) reported powerful antioxidant property of polyphenols which counteract the oxidative stress caused by unfavourable situations. Posmyk et al. (2009) demonstrated the heavy metal chelating ability of polyphenols while working on phenolic compounds content in red cabbage seedlings under copper stress. They protects plants from microbial, fungal and herbivores attack by acting as antibiotic, antifungal and herbivores repellent (Kulbat, 2016).

In the present investigation, polyphenol oxidase (PPO) activity, hydrogen peroxide ($H_2O_2$) and MDA content were analysed as indices of oxidative stress under Cr (VI) toxicity (2 ppm and 4 ppm) in sorghum at two growth stages viz. vegetative (35 DAS) and grain filling (95 DAS). It was observed that the PPO activity increased with increasing concentration of Cr (VI) as compared to control, at both the stages in all the varieties (Figs. 27–28). It might be due to increased Cr (VI) uptake and decreased nutrients uptake under Cr (VI) toxicity as reported by He et al. (2019), while studying the effect of Cd in tobacco plants. Exogenous application of GB and AMF (individually and combined) decreased the PPO activity as compared to control, at both 2 & 4 ppm of Cr (VI) at both the stages in all the varieties in sorghum (Figs. 27–28).

Wang et al. (2014) 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. Ashraf and Foolad, (2007) demonstrated that GB increased the components of antioxidative defense system and protects photosynthetic apparatus of the plant by acting as an osmoprotectant under stressful conditions. Rodriguez et al. (2015) observed similar results in banana fruits under chilling stress during postharvest stage. Similar results were also reported by Luo et al. (2011) in plum fruits by exogenous salicylic acid application. Similarly, Kumar et al. (2013) observed that 0.5% salicylic acid treatments successfully reduced the activity of PPO in litchi fruits during storage. These reports are supportive of the findings of present investigation. The decrease in activity of PPO on GB and AMF application might be due to Cr (VI) uptake and increase in essential nutrients uptake in sorghum as suggested by Raza et al. (2014) and Gill et al. (2015), who have studied the effect of
exogenous GB application in wheat and brassica under Cr (VI) toxicity. Among all the varieties SSG 59 – 3 variety showed lowest PPO activity as compared to HJ 513 and HJ 541 (Figs. 27–28).

The H$_2$O$_2$ is a very toxic compound and a higher level of it, produces injuries in plant cells by lipid peroxidation which in turn increases MDA content in plant samples during stressed conditions (Gratão et al. 2008; Upadhyay and Panda, 2010). The results of present investigation revealed that the H$_2$O$_2$ and MDA content increased significantly with increasing concentration of Cr (VI), as compared to control at both the stages in all the varieties (Figs. 29–32). Similar results in H$_2$O$_2$ and MDA content were observed by Bharwana et al. (2014) while working on Pb toxicity in cotton plants. The increase in MDA and H$_2$O$_2$ content was also reported by Kamran et al. (2015) in barely genotypes during chilling stress.

The increase in MDA and H$_2$O$_2$ content was also observed by Vitória et al. (2001) and Wahid et al. (2008), while studying on Cd toxicity in radish and mung beans, respectively. Similar observations (increase in MDA content) were reported by Radic et al. (2009) and Szollosi et al. (2009), in duckweed under Zn stress and in B. juncea under Cd stress, respectively. These reports are supportive of the findings of present investigation.

Exogenous application of GB and AMF (individually and combined) decreased the MDA and H$_2$O$_2$ content as compared to control at both 2 & 4 ppm of Cr (VI) at both the stages in all the varieties (Figs. 29–32). The decrease in H$_2$O$_2$ and MDA content might be due to reduction in Cr (VI) accumulation in roots, shoots and leaves, on GB and AMF application (Figs. 1–6) which in turn reduces the stressed conditions in sorghum. similar view have been put forward by Islam et al. (2009); Hossain et al. (2010) and Cao et al. (2013), who have studied the effect of exogenous GB application in tobacco, mung bean and rice under Cd toxicity, respectively. Similar results were reported by Allakhverdiev et al. (2007); Yang et al. (2007) and Islam et al. (2009) during heat stress, salt stress and Cd toxicity in tobacco plants, respectively. Nomura et al. (1998) observed that glycine betaine reduced the stress in plants by maintaining stomatal conductance, chloroplast ultrastructure, RuBisCo activity, photosynthetic capacity and proper nutrient uptake. Glycine betaine increased the antioxidative systems activity which in turn prevents plants from oxidative damages caused by ROS generated due to stressed conditions (Meloni et al. 2003; Park et al. 2004; Islam et al. 2009; Farooq et al. 2010).

In the present investigation, decreased H$_2$O$_2$, MDA and PPO activity upon GB and AMF application indicated the ameliorative effect under Cr (VI) toxicity in sorghum. Similar observations have been made by Molla et al. (2014) in lentil under Cd stress; Jabeen et al. (2016) in mung bean under Cr toxicity; Bharwana et al. (2014) in cotton under Pb stress and Ali et al. (2015) in wheat under Cr toxicity. Kotb and Elhamahmy, (2014) also reported similar results in wheat plants under salinity stress. Ragab et al. (2015) observed that foliar application of GB alleviated water stress in tomato plants grown in sandy soil by using similar mechanisms. Similar effects of GB were observed by Yildirim et al. (2015) in lettuce during salt stress. Hassan et al. (2013) demonstrated similar effects of AMF inoculation in sunflower under Cd toxicity, as were observed during present investigation.
The increase in H$_2$O$_2$ content observed in the present investigation might be due to increase in activities of superoxide dismutase enzyme (Figs. 7–8) as a result of tolerance mechanism and GB and AMF application. Shirasawa et al. (2006) have reported that superoxide dismutase (SOD) acts as main scavenger of ROS such as superoxides radicals, and convert them to H$_2$O$_2$ and O$_2$ molecules. This conversion of superoxides to hydrogen peroxides and oxygen molecules by superoxide dismutase enzyme, increases the H$_2$O$_2$ content which was also reported by Scandalios, (1993), while studying on oxygen stress and superoxide dismutases. Thus, the plant cells are protected from damages by decreasing the level of MDA and H$_2$O$_2$ resulting into improved plant growth. Among all the varieties SSG 59 – 3 variety showed lowest H$_2$O$_2$ and MDA content as compared to HJ 513 and HJ 541 (Figs. 29–32).

3.4 Exogenous GB and AMF both individually and in combination offsets Cr (VI) induced inhibition in yield and its attributes:

In the present investigation, grain yield (100 grains weight) decreased with increasing levels of Cr (VI) as compared to control at both the stages in all the varieties (Figs. 33). The reduction in yield under Cr (VI) toxicity has also been reported widely in literature by Dey et al. (2009); Diwan et al. (2012); Ali et al. (2013c); Gill et al. (2014) and Ali et al. (2015). Similar observations on reduction in yield, growth, biomass and chlorophyll content have also been reported in mung bean under Cr (VI) toxicity by Bera and Bokaria, (1999); Samantaray, (2002); Banerjee et al. (2008) and Suthar et al. (2014). Chromium (VI) toxicity has been reported to cause great losses in growth and yield of wheat and oat by Oliveira, (2012) and Farid et al. (2013a). Chromium toxicity induces oxidative stress in plants by increasing the accumulation of ROS which in turn causes the oxidation of macromolecules such as proteins, lipids and nucleic acids resulted in to decreased photosynthetic pigments and yield in plants as reported by Shanker et al. (2005); Dey et al. (2009); Rodriguez et al. (2012); Singh et al. (2013); Das et al. (2014); Ehsan et al. (2014) and Habiba et al. (2015) in pea leaves under Cr (VI) toxicity, in wheat under Cr (VI) toxicity and in Brassica napus L. under Cu toxicity. The decrease in grain yield may be due to increased Cr content in plant under Cr (VI) toxicity which in turn decreased plant growth by damaging of roots, chlorosis, necrosis, mineral nutrition, water balance and antioxidative enzyme activity ultimately resulted in to reduced yield of the plants as also suggested by Serraj and Sinclair, (2002), Sanchez-Rodriguez et al. (2012) in tomato under water stress, Ali et al. (2013c) in barley under Cr toxicity and Gill et al. (2014) in oilseed rape cultivars under Cr toxicity. The decrease in yield under Cr (VI) toxicity may be due to increased Cr uptake as compared to nutrient uptake by plants. Rizwan et al. (2012), Pradas-del-Real et al. (2013), Keller et al. (2015) and Khaliq et al. (2016) who studied the effect of Cd toxicity in duram wheat, Cr (III) & (VI) toxicity in Silene vulgaris (Moench), Cu toxicity in wheat and nickle toxicity in cotton, respectively, have also suggested that the decrease in yield under Cr (VI) toxicity.

Exogenous application of GB and AMF increased grain yield as compared to control at both 2 & 4 ppm of Cr (VI) at both the stages in all the varieties (Figs. 33). The increase in grain yield on GB and AMF application may be due to reduction in Cr uptake which results in to decreased stressed. Raza et al. (2014) have suggested increase in yield on exogenous GB application in wheat under drought stress may
be due to increase in the rate of photosynthesis, transpiration rate and nutrient uptake. Cao et al. (2013) have also reported that GB application increased the growth and yield in rice plants under Cd toxicity. Farid et al. (2013b) and Bharwana et al. (2014) also obtained similar results that the 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 application are still not clear. Among all varieties the SSG 59 – 3 variety showed highest grain yield as compared to HJ 513 and HJ 541 (Figs. 33).

4. 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 effect increased with the increasing concentration of Cr (VI). This may be due to increased Cr uptake which resulted in increased indices of oxidative stress. Through the components of the antioxidative defence system increased under Cr toxicity. However it seems that it was not sufficient to combat the toxicity stress. As revealed by increased ROS of the plant. Exogenous application of GB and AMF, however improved the plant growth and stress tolerance due to further increase in enzymes and metabolites of antioxidative defence system and reduction in indices of oxidative stress. The treatment of GB at both 50 and 100 mM level, application in soil, significantly ameliorated Cr (VI) toxicity. However, AMF (10 g) concomitantly with GB, at both 50 & 100 mM level, further ameliorated the effects of Cr (VI) 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) along with AMF (10 g) was observed most effective and best concentration among all the treatments, for the amelioration of Cr (VI) 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.

5. Materials And Methods

The present investigation was conducted in a screen house in the department of Biochemistry, College of Basic Sciences & Humanities (COBS&H), Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, Haryana (India).

5.1 Materials

5.1.1 Plant Material: Three varieties of sorghum (Sorghum bicolor L.) viz. HJ-541, HJ 513 and SSG 59 – 3 were procured from Forage Section, Department of Genetics and Plant Breeding, CCSHAU -Hisar, Haryana (India). 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 under Cr (VI) toxicity.
5.1.2 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.

5.1.3 Experimental details: Three varieties of sorghum (HJ 541, HJ 513 & SSG 59-3) 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 50 & 100 mM glycine betaine (in soil) treatments separately, with and without Arbuscular mycorrhizal fungi, in completely randomized block design. Three replications were maintained for each treatment and control. The data was analyzed by using a three-factorial, analysis of variance ANOVA, CRD design in SPSS software. Significant (P ≤ 0.05) differences between treatments were determined using critical difference.

5.1.4 Raising of the Crop: The seeds of uniform size were selected and surface sterilized with 0.01% mercuric chloride (HgCl₂) solution, followed by sowing. The plants were raised in earthen pots lined with polyethylene bags filled with 5 kg sandy loam soil in screen house. Separate pots were kept for control plants. All pots were irrigated with equal quantities of water and nutrient solution as per recommended package of practices (POP).

5.1.5 Treatments: The detailed composition of treatments used in this experiment is given in Table 1.
Table 1
Treatments details of AMF and GB provided prior to sowing in soil.

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

5.1.5.1 Chromium treatment: The soil in each pot was treated with different levels of Cr (VI) viz. 2 and 4 ppm, before sowing in the form of K$_2$Cr$_2$O$_7$.7H$_2$O salt (Sigma Ltd.).

5.1.5.2 Glycine betaine treatment: GB (50 and 100 mM) were supplied exogenously in soil prior to sowing.

5.1.5.3 Arbuscular mycorrhizal fungi (AMF) treatment: The AMF was supplied in the soil exogenously before sowing. The treatment of AMF was provided as 10 g of medium containing AMF per pot.

5.1.6 Sampling: The plant samples (leaf, shoot and root) 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 antioxidative system's parameters, indices of oxidative stress parameters and cellwall digesting enzymes, study. 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 the estimation of Cr content separately.

5.2 Methods

5.2.1 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 (Piper, 1950). 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 (Jackson, 2005). Organic carbon was determined by wet-oxidation method of Walkely and Black, (1934). Available nitrogen (N) was determined by alkaline permanganate method (Subbaiah and Asija, 1956), available P content was determined by extracting the soil samples using 0.5M NaHCO$_3$ and analysed by spectrophotometer (Olsen et al. 1954) and available potassium was extracted by using neutral normal ammonium acetate and the content was determined by aspirating the extract into flame photometer (Jackson, 2005). The available forms of Fe, Mn, Cu, Zn and Cr were extracted by DTPA at pH 7.3 and determined using atomic absorption spectrometer (Lindsay and Norvell, 1978).

### Table 2

| 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       |

**5.2.2 Chromium content:** Chromium content was estimated in plant tissue (leaf, stem and roots) sample by using atomic absorption spectroscopy technique (Lindsay and Norvell, 1978). 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.

**5.2.3 Assays of the Antioxidative defense system of Sorghum:**

Following antioxidative system parameters were studied at vegetative and grain filling stage in sorghum plants.

**5.2.3.1 Antioxidative enzymes:**

**Extraction**

The complete extraction procedure was carried out below 4\(^{0}\)C. 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.

**5.2.3.1.1 Superoxide dismutase (SOD) (EC 1.15.1.1):**

The enzyme is a metalloprotein, which catalyses the dismutation of superoxide radical to H\(_2\)O\(_2\) 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, (1971). 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 A\(_{560}\) 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. (1974).

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

Where

\( V = \) Rate of assay reaction in absence of SOD.

\( v = \) Rate of assay reaction in presence of SOD.

### 5.2.3.1.2 Ascorbate peroxidase activity (APX) (EC 1.11.1.11):

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 \( \text{H}_2\text{O}_2 \) may react with superoxide, singlet oxygen and hydroxyl radical. Ascorbate peroxidase was assayed by the method of Nakano and Asada, (1981). 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 \( \text{H}_2\text{O}_2 \). 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

\[
A = \varepsilon \times \delta \times c
\]

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

### 5.2.3.1.3 Catalase activity (CAT) (EC 1.11.1.6):

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, (1972). 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.

### 5.2.3.1.4 Glutathione reductase activity (GR) (EC 1.8.1.7):

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, (1978). 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, $\varepsilon$ is the molar extinction coefficient, $\ell$ is the distance that the light travels through the solution, and $c$ is the concentration of the absorbing species.

### 5.2.3.1.5 Peroxidase activity (POX) (EC 1.11.1.7):

Peroxidase is non-specific in nature. It utilize different compounds as substrates to metabolize H$_2$O$_2$ 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. (1966). 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 5 ml 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.

### 5.2.3.1.6 Polyphenol oxidase (PPO) (E.C. 1.10.3.1):

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, (1974). 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.

5.2.3.2 Antioxidative metabolites:

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

5.2.3.2.1 Estimation of 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, (1985).

**Extraction:**

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

**Procedure:**

Total glutathione (GSH + GSSG), was determined by adding 0.1 ml 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, 0.1 ml of 2 mM NADPH, 0.1 ml 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 1.5 ml potassium phosphate buffer (0.5M, pH 7.5) and 0.2 ml 4-vinylpyridine to 1 ml 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.

5.2.3.2.2 Proline content:
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. (1973).

**Extraction**

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.

**Procedure:**

The extract was filtered through Whatman No. 2 filter paper. Two mL of filtrate along with 2 mL of glacial acetic acid and 2 mL 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.

\[
\text{Proline content (µmoles per g tissue)} = \frac{\mu g \text{ proline per ml} \times \text{ml toluene} \times 5}{115.5 \times g \text{ sample}}
\]

Where 115.5 is the molecular weight of proline.

**5.2.3.2.3 Estimation of 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, (1979).

**Extraction:**

One g of the plant tissue was homogenised in 6 ml of ice-cold 0.8 N HClO₄ and centrifuged at 4⁰C, 10000 rpm for 30 minutes. The supernatant was collected and neutralized with 5M K₂CO₃. It was centrifuged again at same conditions (4⁰C temperature, 10000 rpm for 30 minutes). Thus a clear supernatant was obtained, which were used for estimation of ascorbic acid content.

**Procedure:** 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$_2$SO$_4$ was added to the tubes at 4°C 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.

5.2.3.2.4 Estimation of 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, (2000).

Procedure:

A homogeneous suspension was made by dispersing 10 g of 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:

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

5.2.4 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.

5.2.4.1 Estimation of hydrogen peroxide (H$_2$O$_2$):

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 5 ml 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$_2$O$_2$ content (Sinha, 1972).

Procedure:
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 H₂O₂ were calculated from the standard calibration curve (10 to 160 µmole of H₂O₂).

**5.2.4.2 Estimation of 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, (1968).

**Procedure:**

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 95°C 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 (Dipierro and Leonardis, 1997).

**5.2.5 Grain yield:** 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.

**5.2.6 Statistical analysis:**

All the results were analysed by following a three-factorial (the first factor included varieties “3 varieties as HJ 541, HJ513 and SSG 59 – 3”, second factor included Cr levels as control, 2 and 4 ppm, and the third factor included treatments of AMF with glycine betaine administration at control, 50 & 100 mM) analysis of variance (ANOVA) by using IBM SPSS Statistics 23 software along with post hoc Tukey test (George et al. 2016). On the basis of CD values obtained after this analysis for each parameter at both 35 and 95 DAS, differences between the treatment doses were evaluated. Based on the ANOVA test, the interactions were found to be significant.

**6. List Of Abbreviations**

DAS
Days after sowing
GB
Glycine betaine
AMF
Arbuscular mycorrhizal fungi
SOD
Superoxide dismutase
APX
Ascorbate peroxidase
CAT
Catalase

7. Declarations

- Ethics approval and consent to participate

The author provides ethics approval and consents to participate in the publication of this manuscript in the Journal BMC Plant Biology.

- Consent for publication

The author declares consent for publication of this manuscript to BMC Plant Biology.

- Availability of data and material

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

- Competing interests

The authors declare no competing interests.

- Funding

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- Authors’ contributions

P.K. performed the experiments, analysed the data, drafted the manuscript and prepared all the Figures 1–33 and Tables 1-2. P.K. reviewed the manuscript. Thereafter, all authors approved the final manuscript.

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