International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 159-172

EFFECT OF EXOGENOUS APPLICATION OF GLYCINEBETAINE ON BRASSICA UNDER CHROMIUM STRESS

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Received Date: 01-11-2021; Accepted Date: 10-12-2021; Published Date: 14-12-2021

ABSTRACT

Various strategies may be used to induce enhanced protection against heavy metal stress. Foliar application of GB is an effective approach to impart tolerance to plants against heavy metal stress. Heavy metals including Chromium (Cr) are important source of pollution in water and soil. These negatively affect the growth and other parameters of plants. The main objective of current study was to evaluate the effect GB on rapeseed under chromium stress. For the said purpose, pot experiments were performed using completely randomized design (CRD). Therefore, two Brassica varieties (*Brassica olearaceae* var.*capitata* and *Brassica olearaceae* var. *botritis)* were used in the experiment to study its effects and role of foliar application of GB after Cr stress induction. Seeds of each variety were thoroughly washed and sown in plastic pots containing sand. When the seedlings were 21 days old, Cr treatments were applied in the form of chromium oxide at conc. (T_0=0, T_1= 40, T_2 =60) ppm. There were 5 replicates for each treatment of both the varieties. After 15 days of treatment, data regarding shoot and root length, shoot and root fresh and dry weights, chlorophyll contents and GB contents were recorded. After 40 days of Cr treatment plants were treated with foliar application of GB. Foliar application of GB has positive effect on morphological and biochemical parameters and reduced the detrimental effect of chromium metal. So, GB is useful osmo-protectant to provide tolerance to plants under heavy metal stress.

INTRODUCTION

Brassicaceae is a large family with approximately 380 genera and about 3350 species. It has a cosmopolitan distribution. It is especially found in temperate regions of the north hemisphere (Hedge *et al*., 1976). Rapeseed (*Brassica campestris* L.) commonly known as mustard. It is a cool season crop. It is also a thermo sensitive as well as photosensitive crop (Ghosh and Chatterjee, 1988). There is a great scope of increasing yield of mustard by selecting high yielding varieties and improving management practices. Time of sowing is very important for rapeseed mustard production (Rahman *et al*., 1988). It is economically most important genus in this family (Gomez-Campo, 1980).

Glycinebetaine (GB) is a fully N-methyl substituted derivative of glycine. GB is a quaternary ammonium compound. It occurs in large amount in a wide variety of plants, animals and microorganisms (Rhodes and Hanson, 1993). Naturally, it is dipolar and electrically neutral molecule at physiological pH. It is highly soluble in water (Papageorgiou and Murata, 1995). As the GB is electrically neutral, dipolar, water soluble, these characteristics allow it to protect the 3D structures of enzymes and membrane proteins. These 3D structures are protected by hydrophilic and hydrophobic interaction (Sakamoto and Murata, 2002). GB accumulates in the chloroplasts, mitochondria, and cytosol of many plant species. In these organelles GB plays a very important role in osmotic adjustment and protection of enzymes and membranes at non-physiological salt concentrations (Papageorgiou and Murata, 1995).

It is now suggested that both, exogenous application of GB and the introduction of the GB biosynthetic pathway into plants through genetic engineering that do not naturally accumulate GB increase the tolerance of such plants to various types of abiotic stresses (Sakamoto and Murata, 2004). It is evidenced that GB is synthesized in plants in two different pathways from two different substrates, i.e. choline and glycine (Hanson and Scott, 1980). Biosynthesis from choline occurs as a result of the two-step, oxidation of choline via the toxic intermediate betaine aldehyde the major biosynthetic pathway in various plants, animals and microorganisms. These reactions are catalyzed by choline monooxygenase and betaine aldehyde dehydrogenase (BADH), and soluble NAD^+ dependent enzyme that are predominantly localized in chloroplast stroma (Hanson *et al*., 1985). GB accumulates in response to salt stress in most crops including sugar beet, spinach, barley, wheat, and sorghum (Rhodes and Hanson, 1993). In some plant such as *Lycopersicum escuelentum*, *Zea maize* (Agboma *et al*., 1997), pea (Makela *et al*., 1998), rice, mustard, *Arabidopsis*, and tobacco are not able to produce GB under stress or non-stress conditions (Rhodes and Hanson, 1993).

It is evident that foliar application of glycine betaine rapidly penetrates into leaf tissue and is quickly translocated from leaves to other plant organs through vascular transport. However, GB can also be taken up via roots (Park *et al*., 2006). A number of reports are available which demonstrate positive effects of exogenous application of GB on plant growth and final crop yield under abiotic stress, e.g. tobacco, wheat, maize, barley, sorghum, soybean and common beans (Ashraf *et al*., 2007). Plants take up water and mineral nutrients through roots while leaves are meant for gaseous exchange. Leaves are covered by a cuticle, which is composed of ketones, esters

International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 159-172

of long chain fatty acids and long chain alcohol. Cuticle is an effective barrier to penetration of water and solutes (Marschner. 1995).

The primary objective of carrying out the present study was to assess that up to what extent GB applied exogenously at the adult growth stages could ameliorate the adverse effects of heavy metal stress on potential rapseed crop (*Brassica compestris*).

MATERIAL AND METHOD

Two Brassica varieties (*Brassica olearaceae* var. *capitat* and *Brassica olearaceae* var. *botrytis*) were used in the experiment to study the effects of Cr and role of foliar application of GB. Experiment was done in Botany lab of University of Gujrat, Hafiz Hayat Campus, Gujrat-Pakistan. Seeds of each variety were sown in sand in plastic pots.

15 pots for each variety were used. Irrigation was done immediately after sowing seeds. After 7 days of sowing Hoagland solution was given for better germination. Hoagland solution contain many essential nutrients require for the plants for better germination. After 21 days of old seedling, Cr treatment was given in the form of chromium oxide T_0=0 ppm, T_1=40 ppm and T_2 =60 ppm. There were 5 replicates for each treatment of both varieties. After 15 days of treatment. Following parameters were measured.

Determination of growth parameters

Root /shoot lengths were measured. Root and shoot fresh weights were also measured with help of balance. Then, they were oven-dried for one week and their dry weights were recorded. Numbers of leaves per plant were also counted.

Determination of chlorophyll contents

Chlorophyll “a”, chlorophyll “b”, and carotenoid were determined according to Arnon method (1949). 0.5g fresh weight of leaf was taken. It was grinded with 2ml alcohol, then 5 ml more alcohol was added and after keeping overnight in test tubes, OD (optical density) was measured with the help of spectrophotometer.

Chlorophyll ‘a”, Chlorophyll “b” and Carotenoid were measured by following formulas:

International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 159-172

Chlorophyll "a": 12.7 × OD (663) – 2.69 × OD (645) mM

Chlorophyll "b": 22.9 × OD (645) – 4.68 × OD (663) mM

Carotenoid: OD (480) + 0.114 × OD (663) – (0.638) × OD (645) mM

Exogenous application of GB

There were three GB levels which were applied exogenously as foliar spray. Following were the three levels of T_0=0mM, T_1=50mM, T_2=100mM. All parameters were studied again after foliar spray of GB.

Determination of GB

GB was determined following the Grieve and Grattan (1983) method. Dry leaf material (1.0 g) was ground in 10 ml of distilled water and filtered. After filtration, 1 ml of the extract was mixed with 1 ml of 2M HCl. Then 0.5 ml of this mixture was taken in a glass tube and 0.2 ml of potassium tri-iodide solution was added to it. The contents were shaken and cooled in an ice bath for 90 min with occasional shaking.

Then 2.0 ml of ice cooled distilled water and 20 ml of 1-2 dichloromethane (cooled at - 10° C) were added to the mixture. The two layers formed in the mixture were mixed by passing a continuous stream of air for 1-2 min while tubes were still in ice bath (4°C).The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm. The concentrations of the betaine were calculated against the standard curve.

Determination of Na+ and K^+

Samples were taken after digestion and got the reading from flame photometer (Jenway). Standard solutions with different grades were made in testing nutrients (Na^+, K^+). Grades were made e.g. 50,100,150,200,250 ppm. Reading of standards from flame photometer was taken. Graph of standards was made by taking reading on x- axis and solution concentration on y axis. The sample reading was multiplied with correction factor. After multiplying with CF and sample reading, value on graph paper were read and got the reading of desired nutrients.

Statistical analysis

Statistical analyses were carried out by analysis of variance (ANOVA) and Microsoft office 2003.

RESULTS

Shoot length

Data showed that effect of GB is highly significant ($p<0.001$) for shoot length for both varieties at both treatments (50 and 100mM). Foliar application of GB reduced the effect of Cr on Brassica varieties. It also showed that V2 variety more response to GB than V1.

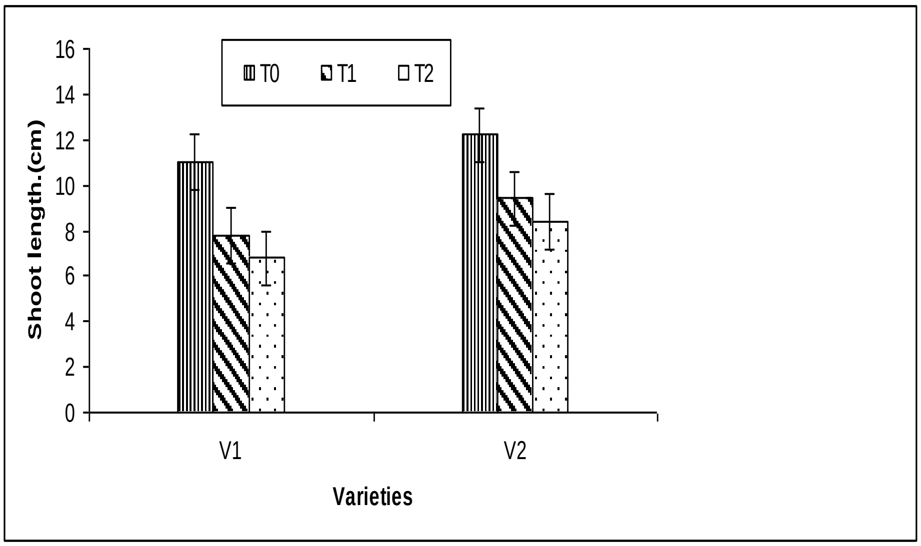

Fig. 1: Shoot length of two Brassica varieties when plants were 50 days old after GB spray under "Cr" stress. Where (T_0=0, T_1=50 and T_2=100mM).

Root length

As the concentration of chromium increases in Brassica varieties, root length decrease. Data regarding to foliar application of GB showed that GB positively (significant $p<0.01$) increases the root length in Brassica varieties at both treatments (50 and 100mM) as compared to control.

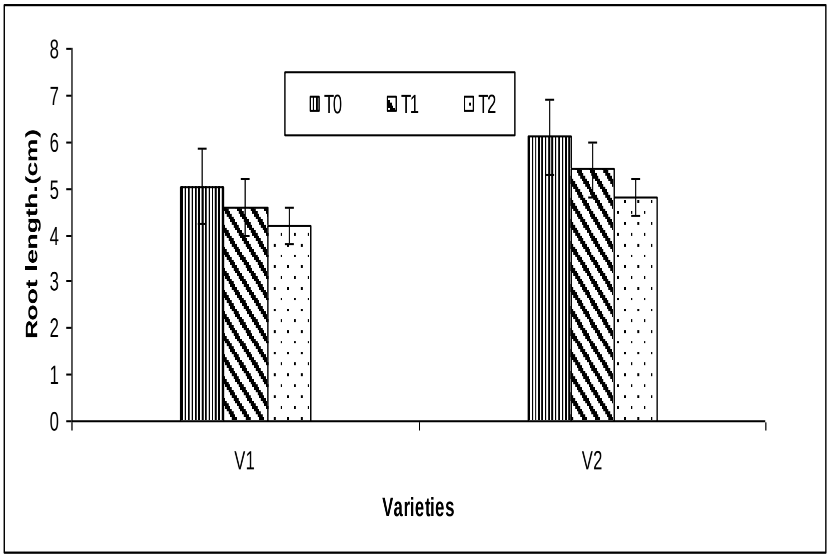

Fig. 2: Root length of Brassica varieties after GB foliar application when plants were 50 days old. Where (0, 50, and 100mM T_0, T_1, T_2 respectively).

Number of leaves

Results showed that effect of Cr on number of leaves in both varieties cause decreases the number of leaves in both varieties Data after foliar application of GB on leaves in both varieties is highly significant ($p<0.001$). The foliar application of GB on Brassica varieties cause increase number of leaves after Cr stress (Fig. 3).

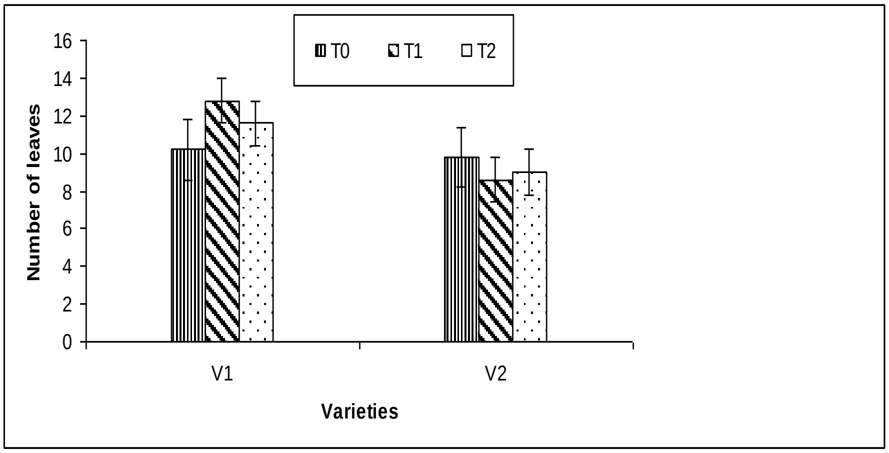

Fig. 3: Number of leaves of Brassica varieties after GB spray when plants were 37 days old where (T0=mM, T1=50mM and T2=100mM).

Chl "a" contents

Effect of GB on Brassica varieties was highly significant. The interaction between GB and chromium was also high significant. Foliar application of GB increases the chl "a" content in both varieties of Brassica (Fig. 4). Foliar application of GB is effective for the plants under different types of heavy metal stress.

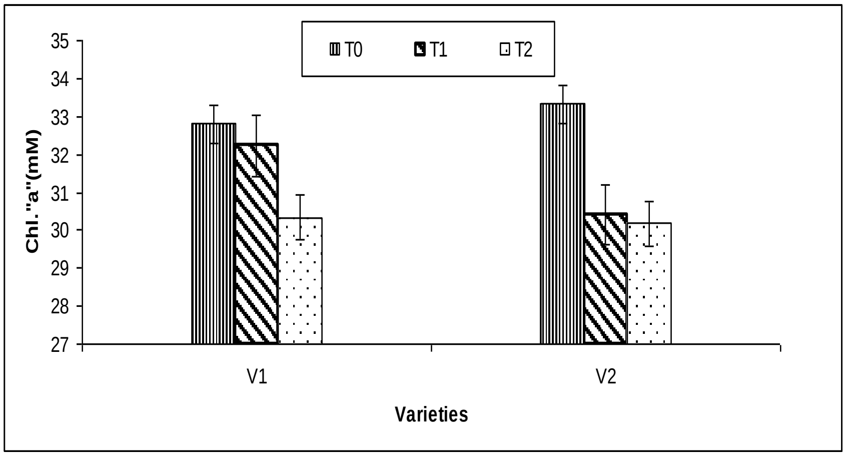

Fig. 4: Chl a contents after GB spray of Brassica varieties when plants were 49 days old Where (T0=0, T1=50 and T2=100) mM.

Chlorophyll "b" contents

Results showed that Cr concentration decreases the chl 'b' content in both cultivars Effect of foliar application of GB is non-significant. GB increases the chl "b" contents in both varieties (Fig. 5).

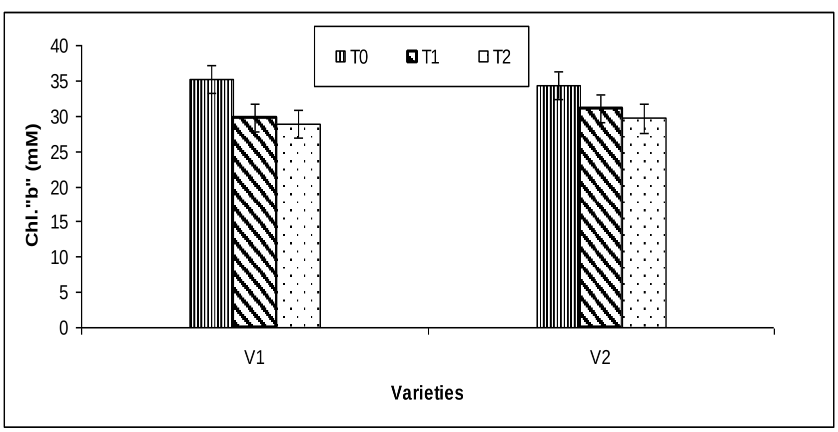

Fig. 5: Chl "b" contents after GB spray when plants were 49 days old under chromium stress. Where (T0=0, T1=50 and T2=100) mM

Carotene pigments

Effect of GB was highly significant ($p<0.001$) on carotene contents. GB increases the carotenes contents.

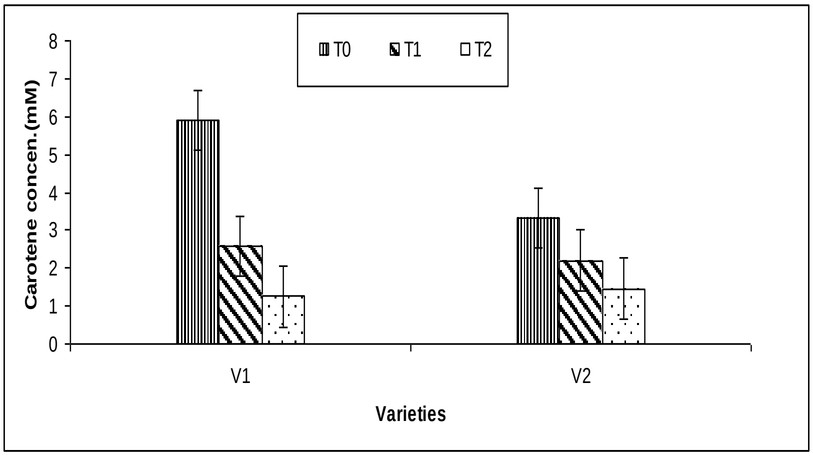

Fig. 6: Showed result when GB was applied, when plants were 49 days old.

Where, (T0=0, T1=50 and T2=100mM)

Root fresh weight

Effect of GB on variety was highly significant. Foliar application of GB reduced the effect of heavy metal and increased the root weight in both varieties of Brassica (Fig. 7).

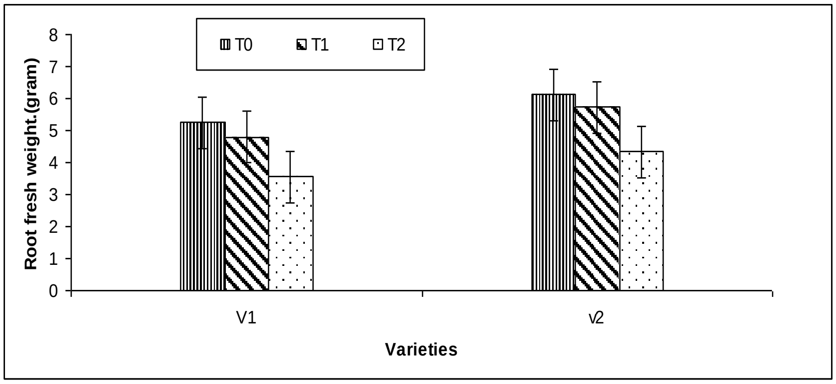

Fig. 8: Root fresh weight after GB spray in two Brassica varities under Cr stress when plants were 48 days old

Root dry weight

Effect of GB on Brassica varieties is highly significant ($p<0.001$) at both the treatments as compared to control. Data regarding to Cr stress showed that Cr cause the reduction in root dry weight and foliar application of GB minimize this adverse effect of Cr metal.

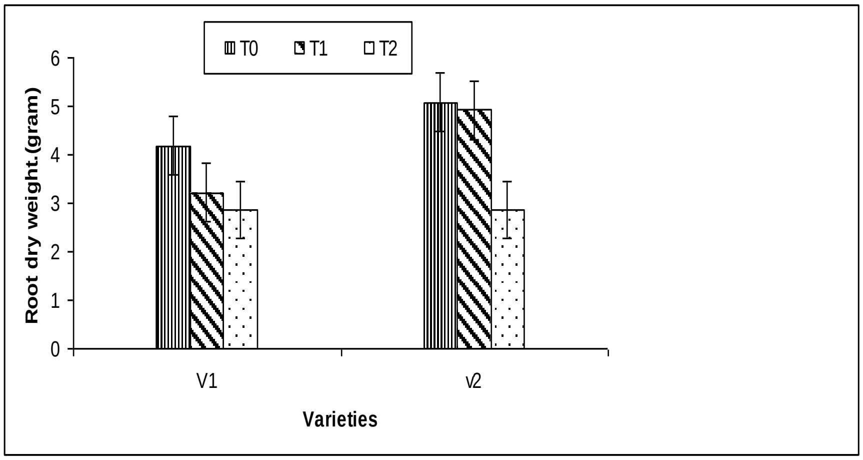

Fig. 9: Root dry weight after GB spray when plants were 50 days old in two Brassica cultivars under 0mM, 50mM and 100 mM

Shoot fresh weight

GB increases the shoot dry weight significantly ($p<0.001$) in both Brassica varieties. The foliar application of GB on Brassica varieties increased the shoot fresh weight in both varieties under different heavy metal stress conditions (Fig. 10).

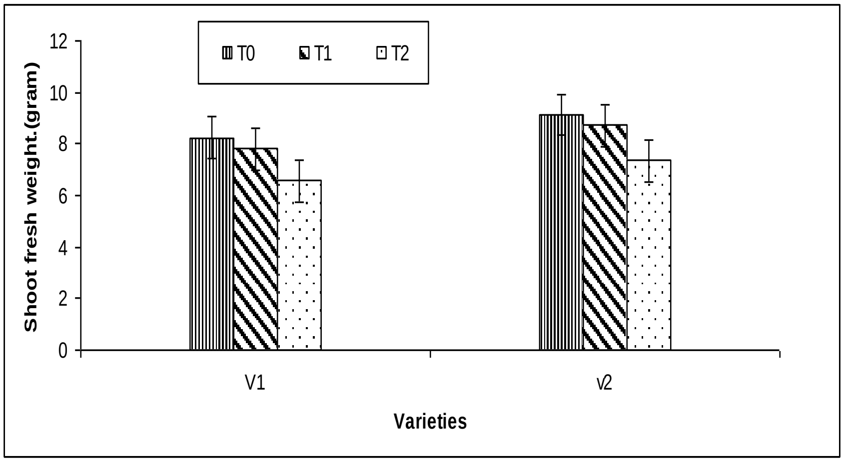

Fig. 10: Shoot fresh weight of Brassica varieties when plants were 39 days old after exogenous application of GB.

Shoot Dry weight

International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 159-172

Effect of GB on both verities is highly significant ($p<0.0001$). Data in graph showed that GB decreased the drastic effect of chromium (Fig .11).

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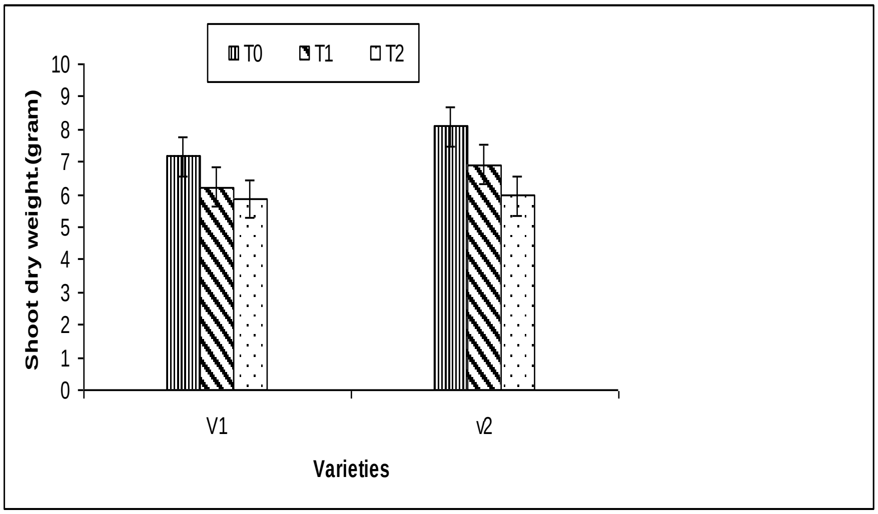

Fig. 11: Shoot dry weight of two Brassica varieties after GB application when plants were 49 days old

GB= (T0=0, T1=50, T2=100) mM.

K^+ Uptake in roots

Effect on K^+ uptake in root in both Brassica cultivars is highly significant. Exogenous application of GB increased the uptake of K^+ ions in root (Fig. 12).

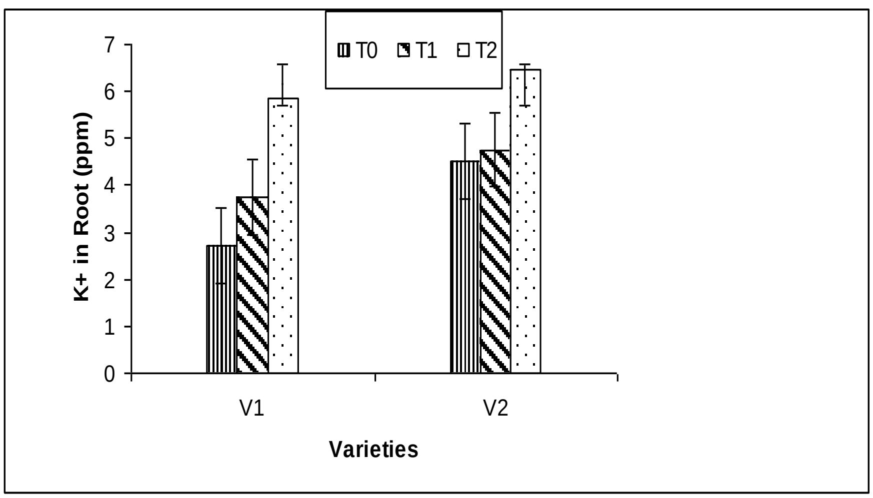

Fig. 12: K^+ ion contents after GB application when Brassica plants were 51 days old under Cr stress.
GB= T0=0mM, T1=50Mm T2=100mM

K^+ uptake in shoot

GB increased the K^+ ion in shoot significantly ($p<0.001$) at both treatment (50mM, 100mM), but 100 mM GB has more positive effect as compared to Cr stress .GB increased the uptake of K^+ ions in shoot (Fig 13).

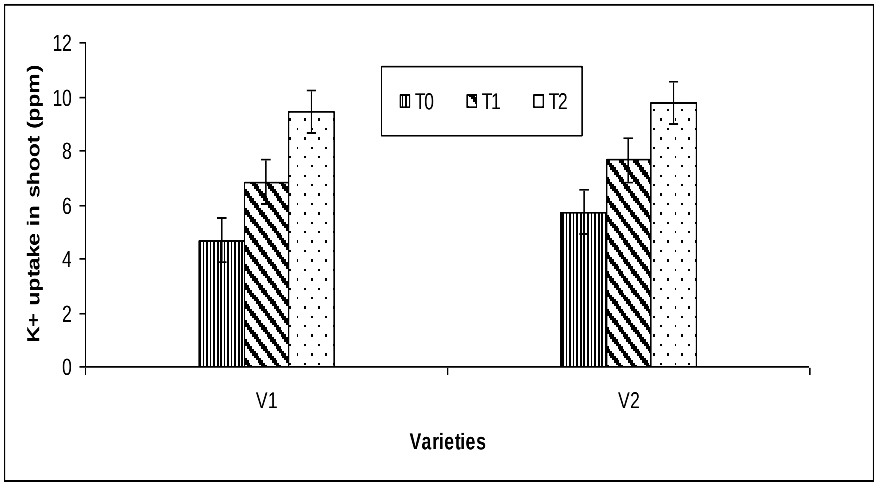

Fig. 13: K^+ ion contents after GB spray in two Brassica varieties under chromium tress
Where, GB (T0=0mM, T1=50mM, and T2=100mM).

Na+ uptake in Root

GB increased the Na^+ ion contents in root significantly ($p<0.001$). And there is no significant variation in both varieties (Fig 14).

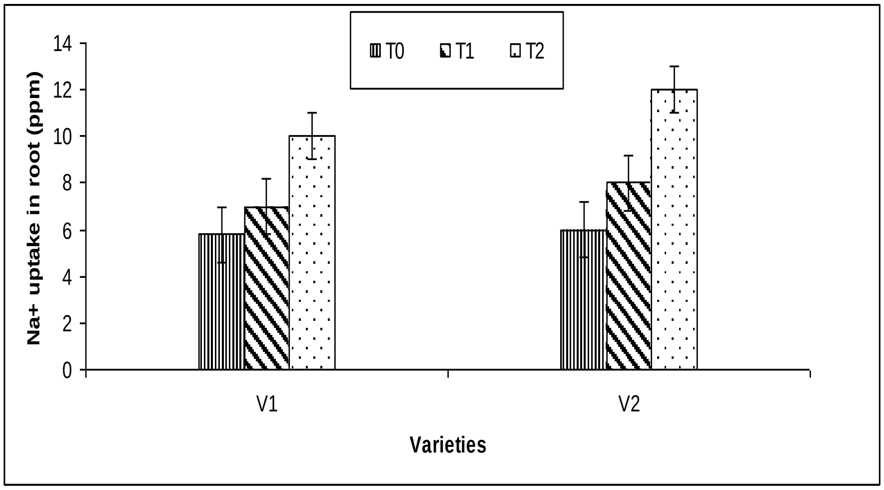

Fig. 14: Na^+ ion contents after exogenous application of GB on Na^+ uptake in root of two Brassica varieties, when plants were 53 days old.

International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 159-172

Na^+ in Shoot

Effect of GB on Na^+ contents in both varieties are highly significant ($p<0.001$). GB increased the Na^+ ion contents in Brassica at both treatment 50 and 100 mM as compared to control. There is no significant variation in both Brassica varieties (Fig. 15).

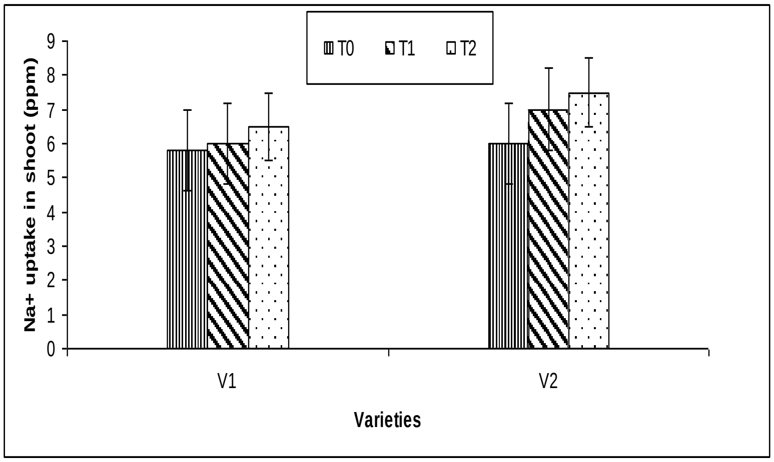

Fig. 14: Na^+ ion contents in shoot of Brassica varieties after GB spray.

Where (T0=0mM, T1=50mM, and T2=100mM).

DISCUSSION

In my pot experiment when foliar application of GB was applied to the Brassica varieties under chromium stress, then it was observed that there was increased in shoot length that showd that GB decrease the effect of Cr stress in both varieties of Brassica. My result matches with the work of Ashraf and Foolad that exogenous application of GB to plants, may help to reduce the adverse effects of environmental stress (Ashraf *et al.*, 2007). Exogenous application of GB also improves the growth, survival, and tolerance of a wide variety of accumulator/non-accumulator plants under various stress conditions (Diaz-Zorita *et al.*, 2001).

According to my result, Cr greatly influences in chl "a", chl "b"and carotene pigments. As the Cr concentration increase in both varieties, chlorophyll "a" contents decrease, ultimately leads to the yellowing of leaves. Same effect was seen with chlorophyll "b" and carotene. Data regarding to my pot experiment foliar application of GB had increased the contents of Chl 'a', Chl 'b' and carotenes. So, foliar application of GB reduces the effect of Cr on photosynthetic pigments in both varieties. Foliar application of GB increases the endogenous GB contents in both varieties that play very important role in photosynthesis and other metabolic activity. Foliar application of GB could increase its content in soybean plant, leading to an improvement in photosynthesis activity, leaf area development, and seed yield of both well irrigated and drought-stressed soybean plants

(Makela *et al.*, 1998). My result matches with the Hainaut's result in which he studies the effect of foliar application of GB in soyabean plants (Harinasut *et al.*, 1996).

CONCLUSION

It was concluded that the foliar application of GB showed positive effect on morphological and biochemical parameters and reduced the detrimental effect of chromium metal. So, GB is useful osmo-protectant to provide tolerance to plants under heavy metal stress. It is finally suggested that exogenous application of GB minimized the toxic effects of chromium acting as an osmoprotectant in Brassica varieties.

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