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AMELIORATING INFLUENCE OF SULFUR ON GERMINATION ATTRIBUTES OF CANOLA (*BRASSICA NAPUS* L.) UNDER CHROMIUM STRESS

SUMMERA JAHAN, SUMERA IQBAL*, KHAJISTA JABEEN AND SARA SADAF

Department of Botany, Lahore College for Women University, Lahore, Pakistan
**Corresponding author's e-mail: sumeraiqbal2@yahoo.com*

Abstract

An experiment was performed to evaluate the role of sulfur to induce tolerance in *Brassica napus* L. against chromium stress by estimating the changes in germination parameters. Petriplates were assembled in Randomized Complete Block Design. A total 9 sets of treatments viz., control, chromium treated (40 and 160ppm), sulfur treated (50 and 150ppm) and sulfur (50 and 150ppm) combined with chromium (40 and 160ppm) with three replicates was used. Chromium under both concentrations was responsible for significant decline in germination parameters i.e. germination percentage, germination rate, seedling vigor index, shoot and root length, fresh weight and dry weight of seedlings. Sulfur application under chromium stress resulted in improvement of germination parameters such as germination percentage, germination rate, seedling vigor index, shoot and root length, fresh weight and dry weight of seedlings in contrast to chromium treatment. So, it can be concluded that sulfur in appropriate dose can be used to ameliorate the negative effects of chromium by increasing the germination potential of canola.

Key words: Canola, Haevy metal, Seed germination, Sulfur.

Introduction

Canola (*Brassica napus* L.) is an economically important crop to improve the production of edible oil. Canola oil is among the healthiest edible oils and it is commonly used due to lower saturated fats, higher proportion of unsaturated fats with a favorable mix of mono plus polyunsaturated fats which lowers the blood cholesterol level. Farmers can get 40,000 to 50,000 rupees from an acre of canola crop. Pakistan has to spend million dollars for import of edible oil inspite of being an agricultural country (Shah, 2012). Pakistan's local production of edible oil is not sufficient to fulfill the emergent demand of population. Over the last two decades edible oil is produced at the rate of 2.6% while its requirement is 9% means its consumption rate is 2.764 million tons from which 31% is home produced while 69% is imported (Anon., 2006). Canola seeds have oil content ranging between 42-45%; also have low erusic acid as well as low glucosinolates contents (Malik *et al*., 2004).

The term "heavy metal" is often thought to be metals or their compound that are toxic. Heavy metals as Zn, Cu, Ni, Cr and Co are micronutrients because they are required by living system only in minute quantities for physiological functions, but at higher concentration they are pollutants of ecosystem (McGrath *et al*., 1995). The compounds of hexavalent chromium are used most frequently in Pakistan industry for various purposes as, Leather industry is among the major source of chromium pollution in Pakistan, which usually causes the liberation of Cr during tanning process and affect plants, animals and human life. Leather industry during tanning uses the chemicals as chromium sulfate, formic acid, sulfuric acid, sodium chloride, sodium bicarbonate, calcium hydroxide, magnesium sulfate, dyes, fat liquors etc. Textile, paints and pigment industries also uses chromium as metal alloys for different processes (Andaleeb *et al*., 2008). These wastes from various industries are disposed in to canal system, which is a major source for crop irrigation. About 32,500 ha of agricultural lands are irrigated with wastewater which have significant amount of toxic heavy metals including chromium (Qadir *et al*., 2000). Seed germination is the most critical stage in seedlings establishment and determining successful crop production. The first physiological process which is affected by excess Cr is seed germination. Consequently, the ability of a seed to germinate in surplus chromium points out towards the degree of tolerance to chromium in specific plant species (Peralta *et al*., 2001). Excessive chromium is involved in the inhibition of germination rate, germination time and uniformity in germination. At seedling stage shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight and growth tolerance index adversely affected by the enlarged chromium concentrations. Seedlings are more sensitive to chromium than that of seed germination. This is because of the impermeability of seed coats and selectivity of embryos against chromium (Akinci & Akinci, 2010). Oilseed rape and *Brassica* species require sulphur during their growth for the synthesis of protein and glucosinolates (Zhao *et al*., 1993). Sulphate assimilation is an important pathway for plant life under optimal and stressed conditions because it is a major source of reduced sulphur compounds for different cellular functions of the plants such as synthesis of various protein molecules, cysteine, methionine and glutathione. Glutathione is a very important sulfur metabolite and help the plant to tolerate both biotic and abiotic stresses (Kopriva & Renninberg, 2004). So, present investigation was aimed to investigate the potential of sulfur application in alleviating harmful effects of chromium on germination attributes of Canola seed.

Material and Methods

A petriplate experiment was conducted to check the interactive effects of chromium and sulfur on germination parameters during canola (*Brassica napus* L.) growing season 2012-2013 in the plant physiology laboratory, Lahore College for Women University, Lahore. Seeds of canola (*Brassica napus* L.) variety Pakola were obtained from National Agricultural Research Centre (NARC), Islamabad. For conducting experiment petriplates were first autoclaved and then washed afterwards they were dried in the oven at 170^{o}C for half an hour. Seeds were surface sterilized with 0.2% mercuric chloride and rinsed three times with distilled water. Then 30 seeds were placed in five rows of six seeds on filter paper in the petriplates. Petriplates were assembled in randomized complete block design (RCBD). There were total 9 sets of treatments viz., control, chromium treated (40 and 160ppm), sulfur treated (50 and 150ppm) and sulfur (50 and 150ppm) combined with chromium (40 and 160ppm) with three replicates. Germination of canola seeds was recorded in each treatment on daily basis up to seven days. After seven days root length (cm), shoot length (cm) and fresh weight (g) was noted from each of the replicate of nine treatments. For taking data about dry weight seedlings were oven dried at 70^{o}C for 72 hours until their weight becomes constant.

Close & Wilson (2002) method was used for the estimation of germination percentage:

$$\text{Germination \%age} = \frac{\text{Number of seeds germinated}}{\text{Total no. of seeds sown}} \text{X } 100$$

Data that was recorded daily up to seven days regarding the number of seeds germinated per day was used for the calculation of germination rate by the equation as given by Khan & Ungar (1984):

$$\text{Germination rate} = \sum g/t$$

where,
g = Percentage of seed germination every day
t = Total germination period

Seedling vigor Index was determined by taking the length of seedlings in centimeters and then multiplies it with germination percentage. This relation was given by Abdul Baki & Anderson (1973):

Seedling Vigor Index = seedling length × germination percentage

Results and Discussion

Interactive effects of sulfur and chromium on the germination parameters of canola (*Brassica napus* L.) are revealed as follows:

By the analysis of the germination percentage and germination rate (Figs. 1 & 2) it is clear that seeds of S_{50} and S_{150} treatments had significant ($p<0.05$) differences between them as well as from seeds of control treatment and had significantly higher germination percentage and rate in comparison to control. Both treatments $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ had significantly higher germination percentage and rate in contrast to Cr_{40}. Similarly, seeds in treatment $Cr_{160}S_{150}$ had significantly improved germination percentage and rate in comparison to Cr_{160}.

Seed germination is amongst the most important physiological processes which would be indicative of a crop to tolerate a stress and germinate in the presence of that stress such as the ability of a crop plant to germinate in the presence of chromium would state its tolerance to chromium stress (Peralta *et al*., 2001).

Similar results regarding decline in germination rate and percentage by chromium was reported earlier by Jain *et al*. (2000) in *Saccharum officinarum* L. Chromium (20 & 80ppm) resulted in decline in germination from 32 to 57%. Decline in seed germination under chromium stress could be because of harmful consequences of chromium on amylase action and result in the accumulation of sugars in the embryo axis. Similarly chromium is also involved in the enhancement of protease activity resulting in decline in proteins which are essential for germination leading to slow or loss of germination (Zeid, 2001).

Higher germination rate and %age under sulfur treatment as well as sulfur interaction with chromium might be due to the secondary metabolites of sulfur such as methionine. It plays an important role in a variety of metabolic processes for instance synthesis of protein, *S*-Adenosyl methionine, ethylene and polyamine, all of these components are crucial for germination of seeds as well as seedlings growth. Methionine synthase and *S*-adenosylmethionine synthetase are basic components during switching from a dormant to a highly active metabolic state in the seed germination (Gallardo *et al*., 2002). Similarly glutathione (GSH) is also a reduced compound of sulfur and highly active under stress conditions and it has the ability suppress the germination inhibition caused by ABA produced during abiotic stress. In the presence of both GSH and ABA germination rate of the seeds of Arabidopsis thaliana was higher over the seeds which have alone ABA (Chen *et al*., 2012).

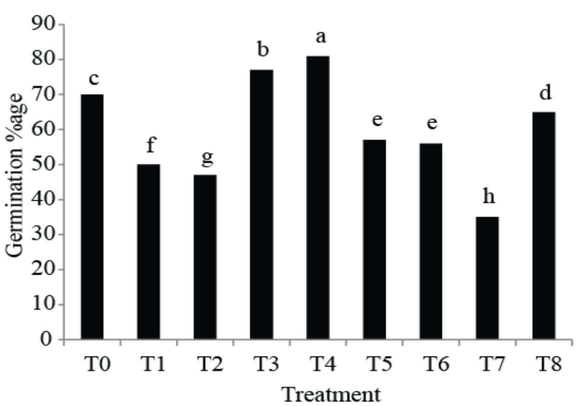

Fig. 1. Interactive effects of chromium and sulfur on germination percentage of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium, + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8 = 160ppm chromium + 50ppm sulfur

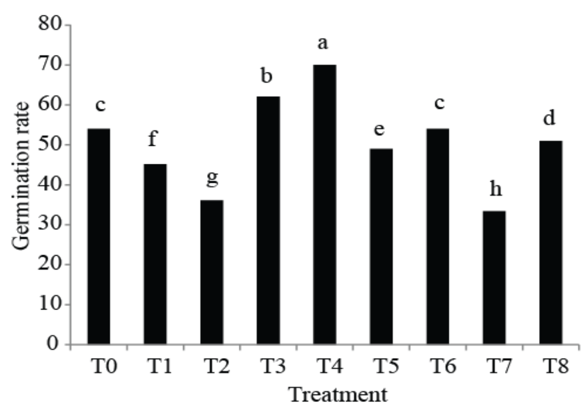

Fig. 2. Interactive effects of chromium and sulfur on germination rate of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7=160ppm chromium + 50ppm sulfur, T8=160ppm chromium + 50ppm sulfur

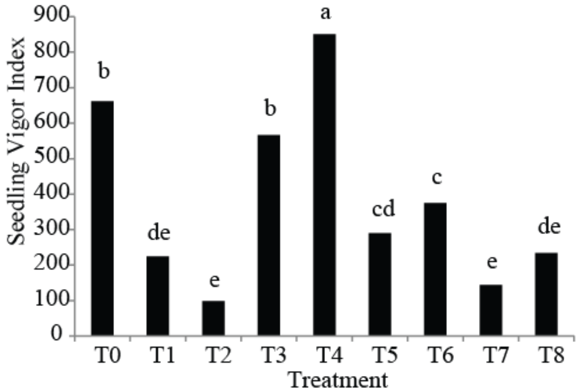

Fig. 3. Interactive effects of chromium and sulfur on seedling vigor of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8=160ppm chromium + 50ppm sulfur

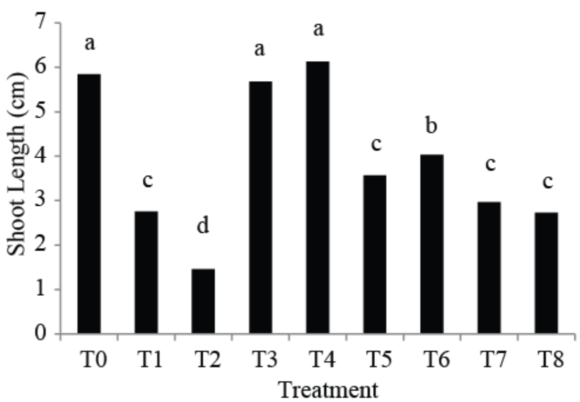

Fig. 4. Interactive effects of chromium and sulfur on shoot length (cm) of seedlings of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8 = 160ppm chromium + 50ppm sulfur

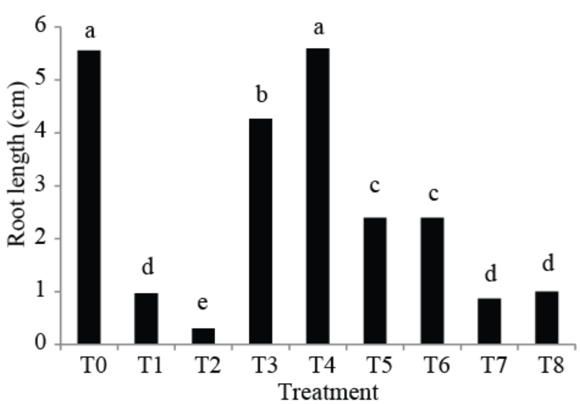

Fig. 5. Interactive Effects of chromium and sulfur on root length (cm) of seedlings of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8 = 160ppm chromium + 50ppm sulfur

In case of seedling vigor index (Fig. 3) Cr_{40} and Cr_{160} differed significantly ($p<0.05$) from control as well as sulfur treated seedlings and they had shown minimum seedling vigor index but had non-significantly differences between them. In case of $Cr_{40}S_{150}$ sulfur improved seedling vigor and it was significantly higher in comparison to Cr_{40}. $Cr_{40}S_{50}$ differed non-significantly from Cr_{40}. Maximum sulfur dose S_{150} resulted in significantly higher seedling vigor in comparison to control and it differed significantly from all other treatments. In the current work seedling vigor index decrease by chromium similar results were also reported earlier by Ganesh *et al*. (2009) that in *Glycine max* seedling vigor index decreases due to increasing chromium concentration in the nutrient solution. Seedling vigor index decreases because the growth of seedlings decreases with increasing chromium concentration. This inverse relationship between the chromium concentration and growth of seedlings could be due to the interference and destructive effects of chromium on various metabolic activities of seedlings which would finally results in slower and stunted growth (Lalitha *et al*., 1999). Increase in seedling vigor index (SVI) under alone sulfur treatment and its application under chromium stress in present work may be due to its stress mitigative secondary metabolites as methionine which is also a substrate for the synthesis of various polyamines with important roles in stress tolerance and they helps to maintain the normal plant growth and development, the most prominent polyamines among these are putrescine, spermidine and spermine (Alcázar *et al*., 2010).

Chromium stress in Cr_{40} and Cr_{160} resulted in significant ($p< 0.05$) decline in seedlings shoot length (Fig. 4) from control. Sulfur applied under chromium stress was resulted in significantly higher shoot length in $Cr_{40}S_{150}$ in contrast to Cr_{40}. Combined application of sulfur (50 and 150ppm) nutrition with Cr (160ppm) in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ had significantly increased shoot length in comparison Cr_{160}. Maximum significant decline in shoot length among all the treatments was recorded in Cr_{160} with maximum chromium with respect to control. Chromium reduced the shoot growth in canola in current research work. Similarly in *Avena sativa* chromium when present in the range of 2, 10 and 25ppm in the nutrient solution harmfully effects the shoot growth (Rout *et al*., 1997). This reduction in shoot length under chromium might be due to the decline in root growth which results in lesser water and nutrients transportation to the above ground plant parts. Likewise, transport of chromium to the above ground plant parts directly effects cellular metabolism of shoots which is mainly responsible for the decline in shoot length (Shanker *et al*., 2005).

Control and sulfur treated seedlings (Fig. 5) have non-significant differences ($p<0.05$) in root length among themselves. But chromium treatment in Cr_{40} and Cr_{160} significantly reduced the root length when compared to control. Seedlings in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ treatment had significantly improved root length in comparison to Cr_{40}. Similarly, treatments $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ had significantly higher root length of seedlings in contrast to Cr_{160}. Reduction of root growth is the most lethal effect of heavy metals in trees as well as crops (Breckle, 1991;Tang *et al*., 2001). In the current study chromium reduced the root length of canola seedlings, similar results in case of root length of chromium treatment was reported by Chen *et al*. (2001) that root length of *Triticum aestivum* was affected negatively by 20ppm chromium in soil over control plants. Chromium adverse effect on the growth of root could be as a result arrest of root cell division and consequently restriction of root elongation. Under elevated chromium levels, root growth decreases might be due to the direct exposure of roots with chromium in the medium which result in disintegration of various root regions and finally they become incapable to absorb water from the medium (Barcelo *et al*., 1986). Sulfur treatment resulted in higher root growth in the present work might be due increase in cell division in the meristematic region of the plant by sulfur and augmentation of the cell elongation. So, sulfur leads to the development of healthier root shoot system in the plants (Chandel *et al*., 2002).

A significant ($p< 0.05$) reduction in seedlings fresh weight (Fig. 6) was found under both chromium levels (40 & 160ppm) when compared with the seedlings in the control treatment. Sulfur application under chromium stress in treatment $Cr_{40}S_{50}$, $Cr_{40}S_{150}$ had significantly healthier fresh weight of seedlings when compared with the seedlings in Cr_{40}. Similar results were found in treatment $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$, they had significantly higher seedlings fresh weight in contrast to Cr_{160}. Chromium under both concentrations reduced the seedlings fresh weight (g) in *Brassica napus*. Similarly Faisal & Hasnain (2005) found that in sunflower (*Helianthus annuus* L.) seedlings significant loss of fresh weight was 52.7% by 300mg/mL chromium when compared to control seedlings. This reduction in fresh weight of seedlings under chromium stress might be as a result of decreased uptake of water. Application of sulfur in Indian mustered helps to enhance healthier roots which provides sufficient water and nutrients to the aerial parts of the plant and results in gain in fresh biomass (Diepenbrock, 2000).

Significant decline in dry wt. of chromium (40 & 160ppm) treated seedlings was found (Fig. 7) when compared to the control seedlings. There was no significant difference among control and sulfur (50 & 150ppm) treated seedlings however sulfur nutrition under elevated chromium levels in treatment $Cr_{40}S_{50}$, $Cr_{40}S_{150}$ had significantly higher dry weight over Cr_{40} and similarly $Cr_{160}S_{40}$ and $Cr_{160}S_{150}$ had significantly improved dry weight over Cr_{160}. Dry weight of canola seedlings was decreased under chromium treatment in the current research work. Similar results of decline in seedlings dry weight by hexavalent chromium were reported by Akinci & Akinci (2010). They found that root dry weight decreased by 12.4% in 10ppm and 65.1% 70ppm chromium over the control. Furthermore, shoot dry weight was 35.8% in 20ppm and 50.8% 70ppm chromium treatment which result in loss of total dry weight of seedlings. This reduction in seedlings dry weight because of limited growth in root and shoot parts of seedlings such as decrease in relative growth rate as well as in tolerance index under elevated chromium concentrations. Gain in fresh biomass as well as enhanced photosynthetic activity helps to provide more assimilates to the developing parts of plants and help in increasing total dry matter production (Singh & Singh, 1983).

The data presented here showed that both levels of chromium severely affect the germination parameters while sulfur application under chromium stress can help in improving germination potential of canola. Further optimization of proper dose of sulfur to mitigate chromium stress in canola is required under natural field conditions.

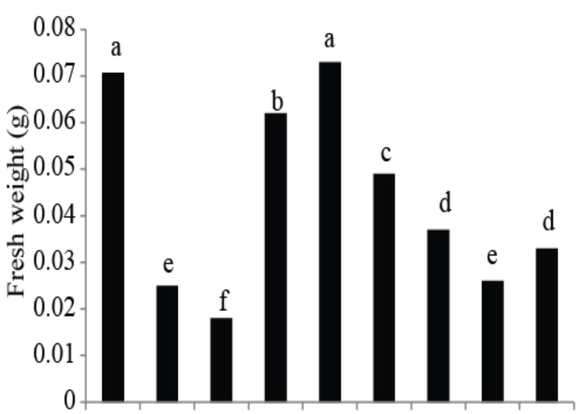

Fig. 6. Interactive Effects of chromium and sulfur on seedling fresh weight (g) of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8 = 160ppm chromium + 50ppm sulfur

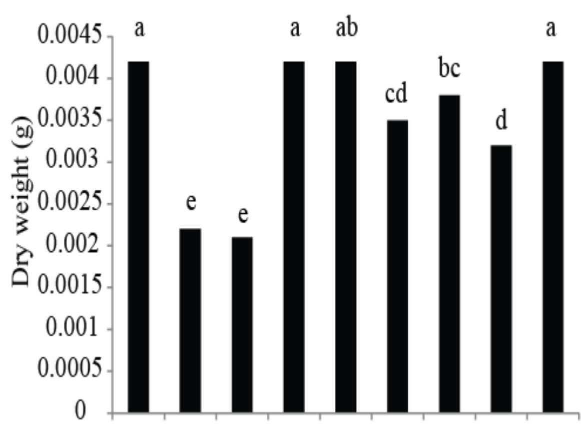

Fig. 7. Interactive effects of chromium and sulfur on seedling dry weight (g) of *Brassica napus* L.
T0 = Control, T1 = 40ppm chromium, T2 = 160ppm chromium, T3 = 50ppm sulfur, T4 = 150ppm sulfur, T5 = 40ppm chromium + 50ppm sulfur, T6 = 40ppm chromium + 150ppm sulfur, T7 = 160ppm chromium + 50ppm sulfur, T8 = 160ppm chromium + 50ppm sulfur

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