Biotechnological
Communication

BBRC
Bioscience Biotechnology
Research Communications

Biosc.Biotech.Res.Comm. Vol 13 (2) April-May-June 2020 Pp-651-660

# Escalating Rhizospheric Chromium Pollution Grades as Plant Foes or Friends?-Evaluation by Enzyme Assay, Physiological Growth and Photosynthetic Phytochemicals of Mash [*Vigna mungo* (L.) Hepper] genotypes.

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## ABSTRACT

The presence of heavy metals in soils may be toxic or beneficial to the environment. The plants may require some of these elements considered as essentials (like Fe, Cu, Zn, Cr or Mo) in trace quantities, but at higher concentrations they may be poisonous. An experiment was conducted on four mash bean (*Vigna mungo* (L.) Hepper) genotypes to evaluate the toxic or beneficial effects of chromium (III) applied in rhizosperic environment of plant. Photosynthetic phytochemicals in the form of Chlorophyll a, Chlorphyll b, total caretenoids, Plant nitrate reductase activity and physiological growth as leaf area index (LAI) were recorded against 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 mg $kg^{-1}$ chromium (III) concentrations. Seeds of four genotypes sterilized with 10% (V/V) hydrogen peroxide were sown in earthen pots filled with homogenized loamy soil. Chromium was added in soil as $CrCl_3$ solutions after twenty days of germination. Data were collected on expiry of twenty five days after chromium addition. Increasing amount of chromium (Cr) appeared to be responsible for gradual reduction in Nitrate Reductase Activity (NRA), photosynthetic pigments and physiological growth of leaf in term of Leaf Area Index (LAI). The lowest significantly effective) dose was 20 mg $kg^{-1}$ in this regard. While the most effective proven dose was 60 mg $kg^{-1}$ for each attribute. The observations were excluded from the ongoing trend when 10mg $kg^{-1}$ chromium (Cr) reflected an increase in studied characteristics at the most being 13.59% for Nitrate Reductase Activity (NRA) at this level. Of the genotypes, MASH 80 was the most sensitive while MASH 88 was the least sensitive to chromium stress

**KEY WORDS:** CHROMIUM; MASH; NITRATE REDUCTASE; CHLOROPHYLL ; CAROTENOID; LEAF AREA INDEX.

ARTICLE INFORMATION

*Corresponding Author: alibaggar@hotmail.com
*Received 15th May 2020 Accepted after revision 19th June 2020*
*Print ISSN: 0974-6455 Online ISSN: 2321-4007 CODEN: BBRCBA*

*Thomson Reuters ISI Web of Science Clarivate Analytics USA and Crossref Indexed Journal*

*NAAS Journal Score 2020 (4.31) SJIF: 2020 (7.728)*

*Online Contents Available at: http//www.bbrc.in/*
*DOI: 10.21786/bbrc/13.2/43*

## INTRODUCTION

Chromium (Cr) is an element naturally occurring in rocky soils and volcanic dust. It has been classified as a carcinogen agent according to the International Agency for Research on Cancer. Therefore, this metal needs an accurate understanding and thorough investigation in soil–plant systems. Due to its high solubility, Cr (VI) is regarded as a hazardous ion, which contaminates groundwater and is transferred to food chain through soil plant interaction system. Chromium also has negative impacts on the growth of plants by impairing their essential metabolic processes (Sharma et al. 2020). Many industries like dyeing, electroplating, leather, tanning and steel discharge effluents of chromium causing significant rise in environmental Cr contents (Joutey et al. 2015). Chromium high solubility contaminates groundwater and enters into food chain through soil plant interconnection (Joutey et al. 2015;Kumar et al. 2019;Kumar et al. 2019). The Agency for Toxic Substances and Disease Registry (CERCLA, 2019). Chromium is ranked 17th among the most hazardous metals (Agency for Toxic Substances and Disease Registry, 2019).

Its high redox potential enables chromium to change oxidation state easily (Shahid et al., 2017; Prado et al. 2016). It s most common oxidation states are hexavalent and trivalent (Ashraf et al., 2017). These oxidation forms differ in respect of their bioavailability, toxicity and translocation in plants (Shahid et al. 2017). Of these Cr (III) is the most stable form of Chromium, whereas Cr (VI) is the highest noxious one for plants. When enter into the cell, these oxidation states attack proteins, lipids and DNA (Tchounwou et al. 2012;Stambulska et al. 2018).

In plants excess Chromium concentration disrupts many biochemical and physiological phenomenon (UdDin et al. 2015;Kamran et al. 2017). Toxicity of metal rely upon its complex interactions with signal transduction, genetics and macromolecules (Santos et al. 2012; Eleftheriou et al. 2015;Kumari et al. 2016). Chromium toxicity affects growth of plant by many ways including changes in structure of cell membrane, chloroplast, pigments, disturbing water balnce, mineral nutrition, enzymes and assimilation process (Reale et al. 2016; Ali et al. 2015;Farooq et al. 2016;Cervantes and Campos-Garcia, 2007;Anjum et al. 2017). The major toxic effects of chromium are due to its role in production of reactive oxygen species (ROS), which alters the redox balance in plants (Anjum et al. 2017).

Taking all into consideration, we review the literature that addresses Cr uptake, translocation and sub-cellular distribution in plants. We also discuss different effects of Cr on plant pigments, photosynthetic parameters, enzymatic and non-enzymatic antioxidative system and various endogenous levels of plant hormones. Roots of some plant secrete organic molecules as acids which act as ligand and can modify solubility of metals in the soil (Khanna et al. 2019;Kaur et al. 2017;Kaur et al. 2018; Kohli et al. 2018). Plant uptake Cr through active and passive mechanism (Shanker et al. 2005; Cervantes2001;Appenroth et al. 2008;De Oliveira et al. 2013). Due to it has structural structural similarity with sulphate and phosphate, uptake of chromium by root cells is through sulphate or phosphate (De Oliveira et al. 2013;De Oliveira et al. 2016). After entry into plant cells, the Cr (VI) is converted to Cr (III) which by binding to the cell walls can block the further transport of Cr within plant (Kabata-Pendias and Szteke, 2015).

Mash bean (*Vigna mungo* (L.) Hepper), belonging to family Papillionaceae, is among the most important pulse crops of the world. In Pakistan, it is amongst the least researched pulse crops inspite of its high nutritive and economic value. Chemical analysis of mash bean seed indicates that it contains oil, fats, protein, carbohydrates and a fair amount of vitamin A and B (James, 1981). It fix free atmospheric $N_2$ for its consumption and enriches with N for next crop (Sen, 1996). Considering the importance of mash bean and considering the ever increasing toxicity of chromium in environment, the present experiment was devised to find out the extent of chromium concentration which could be toxic to plant.

## MATERIAL AND METHODS

To find out whether chromium metal is toxic or beneficial element for plant, an experiment was devised to evaluate the Photosynthetic pigments, Nitrate Reductase (EC 1.6.6.1) Activity (NRA) and leaf area index of four mash bean (*Vigna mungo* (L.) Hepper) genotypes under various chromium applied concentrations.After the initial survey, effluents hazards free sandy loam soil was selected. Soil was air-dried ground, passed through 2mm sieve and mixed well. Seeds of four mash genotypes i.e., MASH 80, MASH 88, MASH 97 and MASH $ES^{-1}$ were used in the experiment. The genotypes have their origin in Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan) and National Agricultural Research Centre (NARC) Islamabad (Pakistan). These were obtained from Pulse Section, Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan). For imposing metal pollution in soil, chloride of chromium of Sigma Aldrich, Japan was used.

Experiment was designed with complete randomization of treatments and genotypes to avoid unequal exposure of environmental factors. Each treatment was repeated four times by pots and plants. Pots of 30 cm diameter were filled with 10 kg sandy loam soils and lined with polyethylene bags ensuring seepage prevention. These were arranged in completely randomized design. Seeds sterilized with 0.1% (V/V) $HgCl_2$, similar in size and weight, were germinated and thinning was performed after germination to maintain one seedling in each pot in order to avoid the imbalanced uptake of nutrients by plants. Weeds were uprooted from time to time by hand weeding and hoeing in order to avoid weed crop competition. Insects and pests were control by foliar spray of Thiodon insecticides of Hoechst (Pvt) Ltd, Pakistan. Plants were irrigated with normal irrigation water.

Quantified amounts of chromium chloride were added in soil accordingly to raise the chromium levels of 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 mg $kg^{-1}$ soil. Metals salts were applied in soil as a water solution of $CrCl_3$, method similar to that used by (Stoeva and Bineva, 2003) after twenty days of sowing while the pots without the addition of metals salts acted as control.Photosynthetic phytochemicals in the form of pigments contents were measured by using the formula of Arnon, 1949) after twenty five days of metal imposition. The leaves were extracted with 80% acetone. By using spectrophotometer (Hitachi Model-U 2001 Japan), the absorbances were measured at 645nm and 663nm for Chl a and b contents respectively and at 480nm for carotenoids. Carotenoids contents were calculated after (Goodwin, 1965) and chlorophylls were calculated according to the (Lichtenthaler, 1987) formulae

Table 1. Chlorophyll a contents (mg $g^{-1}$ leaf F. wt) of 45 days old mash (*Vigna mungo* (L.) Hepper) grown in chromium (III) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) (Values represent means ± SE). Values in parentheses represent % age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

| Chromium (mg $kg^{-1}$ soil) | MASH 80 | MASH 88 | MASH 97 | MASH ES-1 | TREATMENTSMEANS (LSD=0.044 ;n=16) |
|---|---|---|---|---|---|
| | | (LSD=0.088 ;n=4) | | | |
| Control | 1.204±0.002 (A) | 1.055±0.054 (BC) | 0.979±0.031 (CD) | 0.995±0.085 (BC) | 1.058a±0.103 |
| 10 | 1.192±0.115 (0.996) (A) | 1.236±0.129 (-17.156) (A) | 1.070±0.053 (-9.295) (B) | 0.903±0.068 (9.246) (DE) | 1.100a±0.159 (-3.969) |
| 20 | 0.827±0.057 (31.312) (EF) | 0.992±0.089 (5.971) (BC) | 0.782±0.120 (20.127) (F) | 0.508±0.100 (48.944) (GH) | 0.777b±0.198 (26.559) |
| 30 | 0.436±0.061 (63.787) (H) | 0.586±0.027 (44.450) (G) | 0.449±0.090 (54.136) (H) | 0.342±0.035 (65.628) (I) | 0.453c±0.104 (57.183) |
| 40 | 0.241±0.016 (79.983) (JK) | 0.331±0.010 (68.625) (I) | 0.296±0.091 (69.765) (IJ) | 0.167±0.030 (83.216) (KL ) | 0.259d±0.077 (75.519) |
| 50 | 0.113±0.028 (90.614) (LM) | 0.161±0.02 (84.739) (KLM) | 0.153±0.040 (84.371) (LM) | 0.087±0.016 (91.250) (LM) | 0.129e±0.040 (87.807) |
| 60 | 0.078±0.013 (93.521) (M) | 0.157±0.032 (85.118) (KLM) | 0.084±0.004 (91.419) (M) | 0.075±0.009 (92.462) (M) | 0.098e±0.038 (90.737) |
| GENOTYPES MEANS → | 0.585b±0.462 | 0.645a±0.427 (-10.256) | 0.545c±0.382 (6.837) | 0.439d±0.362 (24.957) | 0.553±0.412 |
| | | (LSD=0.033 ; n=28) | | | |

Carotenoids (mg $g^{-1}$ leaf fresh weight) = (Acar/EM) × 1000. Chl a (mg $g^{-1}$ leaf fresh weight) = (12.7(OD663) - 2.69 (OD645)) × V/1000 ×W.Chl b (mg $g^{-1}$ leaf fresh weight) = (22.9(OD645) - 4.68(OD663)) × V/1000 ×W. Where Acar = OD480+0.114(OD663)-0.638(OD645); EM (100%) =2500; OD =Optical density; V = Volume of sample; W = Weight of sample.

Nitrate reductase activity was determined on the expiry of twenty five days after metal imposition using the method of (Sym, 1984).

The leaf area index (LAI) was calculated by using the following formula given by Puttaswamy et al., (1976).

LAI = L × W × N × K Where, L= length of the leaf (cm);W= maximum width of the leaf in cm;N= number of leaves per plant;K= constant (0.65 for legume crops).

The data collected were analyzed for analysis of variance using COSTAT computer package (CoHort Software, Berkeley, CA). Duncan's New Multiple Range test at 5% level of probability (Duncan, 1955) was used to compare means. Significant F values were tested for mean differences by LSD tests at 0.05% significance level, by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989)

## RESULTS AND DISCUSSION

According to Duncan's Multiple Range Test (Table: 1), increasing intensity of metal stress by its escalating levels appeared to be responsible for gradual reduction in chlorophyll a contents. Index of variability in chlorophyll

a revealed that chromium (III) application above the limit of 10mg kg-1 caused statistically marked reduction in the pigment concentration. Maximum effect in all the genotypes was predominantly observed by 60mg $kg^{-1}$. A deviation from the ongoing role of metal was noted when 10mg $kg^{-1}$ metal was supplemented to soil medium of MASH 88 and MASH 97 plants which exhibited an increase in chlorophyll a contents by 17.156% and 9.295% respectively from control. Among the genotypes, MASH 88 revealed maximum (0.645) and MASH ES-1 revealed minimum (0.439) values. MASH 97 differed from MASH 80 by a value of 6.837%.

Table 2. Chlorophyll b contents (mg $g^{-1}$ leaf F. wt) of 45 days old mash (*Vigna mungo* (L.) Hepper) grown in chromium (III) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) (Values represent means ± SE). Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

| Chromium (mg $kg^{-1}$ soil) | MASH 80 | MASH 88 (LSD=0.089 ;n=4) | MASH 97 | MASH ES-1 | TREATMENTSMEANS (LSD=0.049 ;n=16) |
|---|---|---|---|---|---|
| Control | 0.639±0.043 (F) | 0.935±0.135 (BC) | 0.915±0.126 (C) | 0.860±0.170 (CD) | 0.837a±0.166 |
| 10 | 0.624±0.101 (2.347) (F) | 1.039±0.061 (-11.122) (A) | 1.008±0.050 (-10.163) (AB) | 0.830±0.063 (3.488) (D) | 0.875a±0.182 (-4.540) |
| 20 | 0.402±0.0289 (37.089) (H) | 0.896±0.082 (3.660) (CD) | 0.733±0.114 (19.890) (E) | 0.461±0.093 (46.395) (GH) | 0.623b±0.221 (25.567) |
| 30 | 0.205±0.031 (67.918) (JK) | 0.520±0.024 (44.385) (G) | 0.416±0.086 (54.535) (H) | 0.305±0.032 (64.534) (I) | 0.361c±0.129 (56.869) |
| 40 | 0.107±0.007 (83.255) (LM) | 0.290±0.009 (68.983) (IJ) | 0.269±0.087 (70.601) (IJ) | 0.142±0.028 (83.488) (KL) | 0.202d±0.091 (75.866) |
| 50 | 0.042±0.014 (93.427) (MN) | 0.134±0.022 (85.668) (KL) | 0.133±0.039 (85.464) (KL) | 0.063±0.023 (91.970) (LMN) | 0.093e±0.048 (88.888) |
| 60 | 0.012±0.013 (98.122) (N) | 0.060±0.029 (35.187) (LMN) | 0.032±0.014 (96.502) (MN) | 0.027±0.009 (96.860) (MN) | 0.033f±0.024 (96.057) |
| GENOTYPES MEANS → | 0.290d±0.254 | 0.553a±0.387 (-90.689) | 0.501b±0.372 (-72.758) | 0.384 c±0.335 (-32.413) | 0.432±0.359 |
| (LSD=0.037 ; n=28) | | | | | |

Chromium (III) stress imposition induced a reduction in chlorophyll b contents and established an inverse correlation between the two (Table: 2). A remarkable and statistically non significant effect of chromium (III) in decreasing pigment concentration was when supplied above 10mg $kg^{-1}$ concentration. Maximum effect in all the genotypes was by 60mg kg-1 but in MASH 88 was by 50mg $kg^{-1}$. Metal level of 10mg $kg^{-1}$ in MASH 88 and MASH 97 provided an opposite index of its action and increased the chlorophyll b contents by 11.122% and 10.163% from untreated control plants. Among the genotypes, MASH 88 revealed maximum (0.553) and MASH 80 revealed minimum (0.590) values. Differences of 72.758% and 90.689% were statistically obvious for MASH 97 and MASH 88 respectively from MASH 80. Higher concentrations of chromium (III) induced reduction in carotenoids contents corresponded to its levels of application (Table: 7).

Carotenoids were affected significantly by chromium (III) concentrations of not less than 20mg $kg^{-1}$. Maximum effect in all the genotypes was by 60mg $kg^{-1}$. Though irregularly, chromium (III) at lower level of its concentration, exhibited enhancement effects as 10mg $kg^{-1}$ revealed an increase of 16.760 % and 10.606% for plants of MASH 88 and MASH 97 respectively. Similarly 20mg $kg^{-1}$ chromium (III) increased the carotenoids contents of MASH 88 by 21.092%. Differences of 69.587% and 78.350% were statistically obvious for MASH 97 and MASH 88 respectively from MASH 80.Genotypes, MASH 88 revealed maximum (0.346) and MASH 80 revealed minimum (0.194) values for total carotenoids contents.

From the data for mean values, it could be inferred that the chromium (III) supply impaired the activity of nitrate reductase for its reduction potential. The decrease in Nitrate Reductase Activity (NRA) established an inverse correlation with metal concentration (Table: 4). Nitrate Reductase Activity (NRA) value, when measured under the influence of more than 10mg $kg^{-1}$ chromium (III), was found to be statistically lower than that of untreated control. Maximum effect (42.382%) was conceived by metal toxicity of 60mg $kg^{-1}$. A similar pattern of metal stress was extendable to all the genotypes. However, the application of 10mg $kg^{-1}$ chromium (III) to the plants of MASH 97 and MASH ES-1 stimulated the Nitrate Reductase Activity (NRA) by 1.433 % and 13.593% respectively. Different sensitivity range for chromium (III) was found in genotypes. Among the genotypes, MASH 88 revealed maximum (0.732) and MASH 80 revealed minimum (0.626) values.

**Table 3. Total Carotenoids contents (mg $g^{-1}$ leaf F. wt) of 45 days old mash (*Vigna mungo* (L.) Hepper) grown in chromium (III) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) (Values represent means ± SE). Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).**

| Chromium (mg $kg^{-1}$ soil) | MASH 80 | MASH 88 (LSD=0.077 ;n=4) | MASH 97 (LSD=0.077 ;n=4) | MASH ES-1 | TREATMENTSMEANS (LSD=0.041 ;n=16) |
|---|---|---|---|---|---|
| Control | 0.432±0.57 (FG) | 0.531±0.119 (DE) | 0.594±0.017 (CD) | 0.773±0.164 (A) | 0.582a±0.159 |
| 10 | 0.403±0.102 (6.712) (G) | 0.672±0.044 (-16.760) (B) | 0.657±0.067 (-10.606) (BC) | 0.545±0.039 (29.495) (DE) | 0.569a±0.126 (2.233) |
| 20 | 0.197±0.033 (54.398) (I) | 0.643±0.076 (-21.092) (BC) | 0.480±0.059 (19.191) (EF) | 0.275±0.056 (64.424) (H) | 0.399 b±0.188 (31.443) |
| 30 | 0.130±0.023 (69.907) (IJ) | 0.285±0.052 (46.327) (H) | 0.298±0.062 (49.831) (H) | 0.177±0.024 (77.102) (I) | 0.222 c±0.083 (61.855) |
| 40 | 0.059±0.008 (86.342) (JK) | 0.186±0.007 (64.971) (I) | 0.180±0.068 (69.696) (I) | 0.072±0.018 (90.685) (JK) | 0.125d±0.069 (78.522) |
| 50 | 0.069±0.056 (84.027) (JK) | 0.079±0.014 (85.122) (JK) | 0.081±0.017 (86.363) (JK) | 0.036±0.010 (95.342) (K) | 0.066 e±0.033 (88.659) |
| 60 | 0.068±0.064 (84.259) (JK) | 0.027±0.017 (94.915) (K) | 0.016±0.005 (97.306) (K) | 0.019±0.004 (97.542) (K) | 0.032 e±0.037 (94.501) |
| GENOTYPES MEANS → | 0.194 c±0.159 | 0.346 a±0.258 (-78.350) | 0.329 a±0.242 (-69.587) | 0.271 b±0.277 (-39.690) | 0.285±0.242 |
| (LSD=0.031 ; n=28) | | | | | |

From the data for mean values, it could be inferred that the chromium (III) supply reduced the leaf area index (Table: 8). The decrease in leaf area index (LAI) established an inverse correlation with metal concentration. Leaf area index (LAI) value, when measured under the influence of more than 10mg $kg^{-1}$ chromium (III), was found to be statistically lower than that of untreated control. Maximum effect (42.036%) was conceived by metal toxicity of 60mg $kg^{-1}$. A similar pattern of metal stress was extendable to all the genotypes. However, the application of 10mg $kg^{-1}$ chromium (III) stimulated the leaf area index (LAI) in all genotypes Different sensitivity range for chromium (III) was found in genotypes. Among the genotypes, MASH ES-1 revealed maximum (340.992) leaf area index (LAI) and MASH 88 revealed minimum (297.288) values.

Our results revealed that chlorophyll concentration was decreased by chromium application in a concentration dependent manner (Table and 2). A significant reduction of chlorophyll in chromium treated plant is reported by many researchers (Rai et al. 2014; Rajendran et al. 2019;Sinha et al. 2004;Balal et al. 2017;Islam et al. 2016;Zou et al. 2009; Amin et al. 2013;Tang et al. 2012;Amin et al. 2014). Chlorophyll reduction might be either due to inhibiting biosynthesis of chlorophyll

(Lushchak, 2010; Sharma et al. 2019; Chandra and Kulshreshtha, 2004) or destruction of chlorophyll molecule in Cr treated plants (Valko et al. 2006). Reduction in chlorophyll contents may be due to the increased activity chlorophyllase enzyme and nutrients deficiency because of higher concentration of metal translocation toward shoots (Khan et al. 2016;Shakoor et al. 2014). Plants exposed to Cr stress showed depleted chlorophyll contents that might be due to the disrupted chlorophyll biosynthesis (Chandra and Kulshreshtha, 2004). Chromium reduces chlorophyll contents by inhibiting activity of δ-aminolevulinic acid dehydratase (ALAD) enzyme which is involved in chlorophyll synthesis (Hayat et al. 2012).

**Table 4. Nitrate Reductase (EC 1.6.6.1) Activity (NRA) of 45 days old mash (*Vigna mungo* (L.) Hepper) grown in chromium (III) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) (Values represent means ± SE). Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).**

| Chromium (mg $kg^{-1}$ soil) | MASH 80 | MASH 88 (LSD=0.0444 ;n=4) | MASH 97 | MASH ES-1 | TREATMENTSMEANS (LSD=0.026 ;n=16) |
|---|---|---|---|---|---|
| Control | 0.743±0.023 (E) | 0.963±0.039 (A) | 0.837±0.030 (D) | 0.951±0.037 (AB) | 0.873a±0.097 |
| 10 | 0.844±0.029 (-13.593) (D) | 0.902±0.026 (6.334) (C) | 0.849±0.036 (-1.433) (D) | 0.910±0.025 (4.3112) (BC) | 0.876a±0.040 (-0.343) |
| 20 | 0.609±0.012 (18.034) (JK) | 0.837±0.030 (13.084) (D) | 0.763±0.010 (8.841) (E) | 0.721±0.052 (24.185) (EF) | 0.733b±0.089 (16.036) |
| 30 | 0.583±0.013 (21.534) (KL) | 0.658±0.043 (31.671) (GHI) | 0.726±0.007 (13.261) (EF) | 0.616±0.014 (35.226) (IJK) | 0.646c±0.059 (26.002) |
| 40 | 0.551±0.014 (25.841) (LM) | 0.670±0.017 (30.425) (GH) | 0.692±0.024 (17.323) (FG) | 0.579±0.009 (42.481) (KL) | 0.623c±0.063 (28.636) |
| 50 | 0.522±0.002 (29.744) (MIN) | 0.634±0.018 (34.164) ( HIJ) | 0.640±0.011 (23.536) (HIJ) | 0.547±0.022 (43.112) (LM) | 0.586d±0.055 (32.875) |
| 60 | 0.533±0.027 (28.263) (MN) | 0.462±0.060 (52.024) (O) | 0.490±0.032 (42.457) (NO) | 0.528±0.135 (44.479) (MIN) | 0.503e±0.0753 (42.382) |
| GENOTYPES MEANS → | 0.626c±0.115 (9.668) | 0.732a±0.168 (-5.627) | 0.714a±0.118 (-3.030) | 0.693b±0.171 | 0.691±0.149 |
| (LSD=0.020 ; n=28) | | | | | |

Metal stress reduced carotenoids contents (Table 3). Reduction in carotenoids contents might be attributed to activation of osmotic stress leading the biosynthesis of abscisic acid (ABA) by carotenoid cleavage catalyzed by a 9-cis epoxycarotenoid dioxygenase (NCED). Another possible reason for reduction in carotenoids contents might be biosynthesis of anthocyanins. The anthocyanins are synthesized during stress and interfere with carotenoids (Burger and Edwards, 1996). The experimental results revealed, as a general trend, reduction in nitrate reductase activity by metal stress (Table 4). Inhibition of NRA by metal might be caused either by reduction of biosynthesis enzyme or by suppression of activity of existing enzyme. Depolarization of NR thiol or SH groups by metal also reduces enzyme activity (Jones and Mhuimhneachain 1995). It has been suggested that NR activity depends upon active photosynthesis or products of photosynthesis as it requires photosynthetically generated reductant (NADH) and energy (Raghuram and Sopory 1995).

Reduced NRA may be attributed to reduced N contents availability to plant either due to shortage in soil or consumption by plant (Campbell 1999). Stress mediated decreased cytokinin levels might cause a reduction in nitrate reductase activity (Bueno et al 1994). Through Phosphorus limitation (Gniazdowska and Rychter 2000). Another reason for NRA might be due to reduced chlorophyll contents or reduced rate of photosynthesis (Rai et al 1992; Li et al. 2012; Zhang et al 2018). The experimental results revealed a gradual reduction in leaf area index with increasing concentration of metal (Table 5). Leaf area reduction can be due to growth inhibition

in metal treated plants (Ouariti and Ghorbal, 1997). Leaf growth reduction might be the result of low water potential due to very negative solute potential in the soil solution (Hayward and Spurr, 1944).

Table 5. Leaf Area Index (LAI) of 45 days old mash (*Vigna mungo* (L.) Hepper) grown in chromium (III) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) (Values represent means ± SE). Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

| Chromium (mg $kg^{-1}$ soil) | MASH 80 | MASH 88 | MASH 97 | MASH ES-1 | TREATMENTSMEANS (LSD=17.393 ;n=16) |
|---|---|---|---|---|---|
| | | (LSD=34.79 ;n=4) | | | |
| Control | 401.957±12.967 (ABC) | 362.462±13.717 (CDE) | 447.702±6.819 (AB) | 429.642±6.003 (ABC) | 410.441b±34.487 |
| 10 | 381.375±10.975 (5.369) (BCD) | 421.377±14.754 (-16.254) (ABC) | 449.850±23.829 (-0.479) (AB) | 463.490±6.982 (-7.878) (A) | 429.023a±35.276 (-4.527) |
| 20 | 301.787±34.864 (25.030) (EFGH) | 307.422±13.670 (15.185) (CDE) | 309.630±19.615 (30.840) (EFG) | 418.030±13.275 (2.702) (ABC) | 334.217c±53.842 (18.571) |
| 30 | 290.52±21.424 (24.920) (FGHI) | 363.897±33.022 (-0.384) (CDE) | 287.690±27.565 (35.740) (FGHI) | 313.842±28.829 (26.952) (DEF) | 288.987d±31.012 (29.591) |
| 40 | 267.355±28.422 (7.723) (FGHI) | 249.967±28.425 (31.036) (FGHI) | 263.34±11.829 (41.101) (FGHI) | 253.887±24.070 (40.907) (FGHI) | 258.637e±22.787 (36.985) |
| 50 | 245.197±14.083 (33.480) (FGHI) | 242.267±19.941 (33.160) (JHI) | 251.845±19.081 (43.747) (FGHI) | 260.230±28.350 (39.430) (FGHI) | 249.885ef±20.091 (39.117) |
| 60 | 243.377±77.637 (38.999) (GHI) | 233.627±9.060 (35.544) (HI) | 226.802±16.358 (49.340) (I) | 247.827±12.767 (42.317) (FGHI) | 237.908f±37.148 (42.036) |
| GENOTYPES MEANS → | 304.510c±67.758 | 297.288c±69.293 (2.371) | 319.551b±88.408 (-4.939) | 340.992a±89.694 (-11.980) | 315.585±80.156 |
| | (LSD=13.148 ; n=28) | | | | |

Reduced cytokinin contents by metal might be responsible for growth reduction by inhibition of cell division and cell elongation.The results of the experiment indicated that plants could tolerate chromium up to 10 mg/kg soil as no toxicity was recorded at this concentration. These effects of chromium may simply be due to a dose dependent response of the seedlings where the low dose stimulates the growth while high dose suppresses the growth (Shah et al. 2008). The absence of effect at low concentration could be attributed to the fact that low dose of metal accumulates in roots than in the shoot and the effect is restricted to the root but not in the shoot (Selvam and Wong, 2008).

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