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EFFECTS OF CHROMIUM STRESS ON SEED GERMINATION AND EARLY SEEDLING GROWTH PERFORMANCES OF PEARL MILLET *PENNISETUM GLAUCUM* (L.) R. BR. (POACEAE)

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Abstract: Heavy metals are widely available in the environment due to the natural processes, industrial, anthropogenic activities and ultimately in the results contaminate the immediate environment. The presence of the heavy metals such as Pb, Cd, Cr, Ni, Fe and Cu in environment disturb the quality of ecosystems, soils, water, air and vegetation. The chromium metal at higher level in ecosystem is an alarming signal for both developed and developing countries. *Pennisetum glaucum* is an annual grass which is widely cultivated in drought, rain fed and high temperature areas. The limited amount of literature available on the impact of chromium stress on *P. glaucum.* This study was conducted to investigate the beneficial or harmful effect of chromium stress on seed germination and seedling growth performances of *P. glaucum* in *in vitro* conditions. The different concentration of chromium 0, 25, 50, 75 and 100 ppm was applied. In present study, the overall results suggests the variable response on the rate of seed germination percentage and plant growth of *P. glaucum* to chromium stress was recorded. Results showed that the chromium treatment at 25 ppm significantly ($p<0.05$) reduced root growth of *P. glaucum*. The chromium at 50 ppm level significantly decreased the rate of percentage of seed germination of *P. glaucum*. The seedling dry weight of *P. glaucum* seedling was decreased highly at 75 ppm chromium. Root / shoot ratio also decreased due to gradual increase in chromium from low (25 ppm) to higher (100 ppm) levels. Similarly, the chromium at 25 to 100 ppm gradually decreased the percentage of tolerance and seedling vigor index of *P. glaucum*. An effective efforts for minimize the chromium toxicity and tolerance in plants are required.

Keywords: heavy metal, pearl millet, root, shoot, toxicity.

Introduction

Chromium is an important environmental pollutant and commonly used in leather tanning industries [CICATELLI & al. 2017; GOMES & al. 2017] chrome plating and stainless steel industries. Chromium with atomic number 24 and atomic weight of 52 is known to cause carcinogen [KUMAR & CHOPRA, 2015]. The addition of metals in the environment due by human and industrial activities is a serious concern for germination and growth of flora and fauna. Metals at cellular level produced oxidative stress on plants [SMEETS & al. 2009; KAPOOR & al. 2022] and uptake at higher concentrations become toxic for plant growth. The leaching of metals via food chain caused human health hazards [KUMAR & al. 2013] and environment [KIMBROUGH & al. 1999; KOTAS & STASICKA, 2000].

It is recognized that heavy metals due to their toxicity, long persistence in nature can accumulate in the trophic chain and cause organism dysfunction [CHEN & al. 2021]. The harmful effects of chromium on photosynthesis, and water relations capabilities in plant was reported [SHANKER & al. 2005]. The hexavalent chromium toxicity under 10 and 50 µM stress for *Amaranthus viridis* and *Amaranthus cruentus* in hydroponic system was recorded [BASHRI

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& al. 2016]. The excess level of chromium produced harmful impact on germination and seedling growth of field Pea (*Pisum sativum* Malviya Matar-15 (HUDP-15), Pusa Prabhat (DDR-23), *Arabidopsis thaliana* and uptake of certain nutrients in *Citrullus*, rice, castor bean, food crops and some vegetables [PANDEY & PANDEY, 2008; DUBE & al. 2003; DU & al. 2003; de SILVA & al. 2021; ABDELGAWAD & al. 2022; ALI & al. 2022; BASIT & al. 2022; CHEN & al. 2022]. The research work on the risk assessment of chromium in date palm (*Phoenix dactylifera*) leaves as use in livestock feed recorded [PILLAY & al. 2003]. The chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.), okra (*Abelmoschus esculentus*); *Sesbania sesban* L. (Merrill) and tolerance limit in *Hibiscus cannabinus* L., *Cannabis sativa* L., *Zea mays* L., wheat and alfalfa seeds was recorded [AKINCI & AKINCI, 2010; MOHANTY & al. 2015; DING & al. 2016; ANJUM & al. 2017; CITTERIO & al. 2003; KNEŽEVIĆ & al. 2021; LEI & al. 2021; MUSHTAQ & al. 2021, 2022; MURTHY & al. 2022]. Effect of chromium (VI) phytotoxicity on morpho-physiological characteristics, yield, and yield components of two chickpea (*Cicer arietinum* L.) varieties, *Brassica oleracea* L. var. *acephala* DC., *Brassica juncea* and *Vigna radiata*, peas, sunflower, tumble weed, wheat, rice seedlings, regulation of cell death, chromium uptake and proline metabolism in wheat, and pulses observed [NODELKOSKA & DORAN, 2000; FAISAL & HASNAIN, 2005; TORRESDEY & al. 2005; FOZIA & al. 2008; JUN & al. 2009; DIWAN & al. 2010; OZDENER & al. 2011; WAKEEL & XU, 2020; SINGH & al. 2020; 2022a; 2022b; WANI & al. 2022]. MUKHERJI & ROY (1977) studied the effect of $K_2Cr_2O_7$ solutions between 10-5 and 10-3 M on germination and seedling elongation of rice and on the content of reducing sugars and amino acids in potato tuber slices. There was considerable water loss, and the effect of chromium on the changes in permeability became intensified gradually with increasing time of exposure.

Pennisetum glaucum L. R. Br. is member to family Poaceae and known as Bajra. Pearl millet is found in Africa and in the sub-continent. This purple majesty species is the most widely grown millet in plain and hilly areas. *P. glaucum* consider as a multiple cereal grain crop food, fodder, fuels and prefer to grow in well-drained soil in full sun light. *P. glaucum* is naturally rich in nutrients, gluten free and is sold in products likewise whole as raw grain, breads, biscuits and pasta grown at more than 27 million hectares worldwide [SATURNI & al. 2010; NAMBIAR & al. 2011; MANWARING & al. 2016].

An ever increase of chromium metal pollution is affecting growth of agricultural crops in regional and global level. *P. glaucum* is economically an important grain crop of Pakistan and successfully growing in drought area. There seems little literature available on chromium toxicity on germination and early seedling growth of millet cereal as compared to wheat, rice and maize in Pakistan. This investigation was carried out to determine the influence of chromium on early seedling growth performances of an important agricultural cereal crop *P. glaucum* of growing for grain and fodder production.

Materials and methods

The legume seeds of *P. glaucum* were obtained from super store. The percentage of germination was first checked. The surface of seeds were sterilized with 1N NaOCl solution for one minute to avoid any fungal type of action and washed with double distilled water. Ten seeds were kept on filter paper in each Petri dishes having 90 mm diameter in equal distance. The Petri dishes were washed to drop off the chances of further fungal infectivity. Chromium salt was used as potassium chromate with five 0, 25, 50, 75 and 100 ppm concentrations and initially, five ml of chromium solution was applied. Old solution from each petri dish was changed after two days and two ml of prepared chromium solutions were added in each set of treatment. The Petri dishes were placed at room temperature (32±2 °C) along with 240 Lux light intensity. The experiment was completely randomized with three replicates and terminated after ten days. Seed germination, root, shoot, seedling lengths and root / shoot ratios were noted. The treated three tallest seedlings were oven dried at 80 °C for 24 hours to get dry weight. The seedling vigor and tolerance indices was determined [BEWLY & BLACK, 1982; IQBAL & REHMATII, 1992].

T.I. = (Mean root length in metal solution / Mean root length in distilled water) x 100

Statistical analysis

The recorded data was analyzed for ANOVA and Duncan's Multiple Range Test on personnel computer using COSTAT version 3. The level of significance was found at $p < 0.05$ level.

Results and discussion

The abiotic stress conditions likewise addition of heavy metals (Cr) in the immediate environment affect plant growth and development. Chromium is a toxic heavy metal and higher level suppress the seed germination and plant growth. Increasing heavy metal concentrations in the soil, non-biodegradable nature, long biological life in ecosystem have become a significant problem in the modern industrialized world due to several anthropogenic activities which, ultimately pose a threat on human life also [THAKUR & al. 2016].

The damage in plant growth due to metal may result at any stage of growth of plant [PRODGERS & INSKEEP, 1981]. The biotic and abiotic factors influence on crop yield and abiotic stress such as drought, salinity, extreme temperatures and heavy metal (HM) contamination are the common factors reported in the scientific literature worldwide. GANGAIAH & al. (2013) demonstrated the effects of different heavy metals such as chromium (Cr), cobalt (Co) and lead (Pb) on pearl millet (*Pennisetum glaucum* (L.) R.Br.) seed germination and seedling growth using doses of 1, 10, 20, 50 and 100 ppm along with control cultures i.e. tap and distilled water media. This present study proved the decreased in rate of seed germination percentage as well as seedling growth of *P. glaucum* as the increased concentrations in the substrate. The treatment of chromium at higher concentration 100 ppm decreased growth characteristics of *P. glaucum*. Statistically analyzed data showed that seed germination, seedling growth and seedling dry weight of *P. glaucum* seedlings were reduced significantly ($p<0.05$) with increased concentrations of chromium (Table 1). Chromium treatment at 75 and 100 ppm significantly ($p<0.05$) affected seed germination percentage (76.66) and (73.33%) against control (100%). The crop yield production depends upon the prevailing conditions of biotic and abiotic stress. Chromium concentrations at 1, 10, 20, 50 and 100 ppm decreased seed germination of *P. glaucum* by in vitro conditions [GANGAIAH & al. 2013]. It was determined that inhibitory effects of Cr were more on all seedling growth variables of *P. glaucum* as compared to control treatment. Similarly, KABIR & al. (2011) studied the tolerance of *Samanea saman* (Jacq.) Merr. for Cu, Fe, Pb and Zn under laboratory conditions and showed that with increasing concentrations of metals reduced seed germination.

The use of genetic engineering to modify plants for metal uptake, transport and sequestration may open up new avenues for enhancing efficiency of plant [EAPEN & D'SOUZA, 2005]. The rapid industrialization, and modern agricultural practices have resulted in increased heavy metal contamination in the environment, which causes toxicity to the living organisms [KAVAMURA & ESPOSITO, 2010; MIRANSARI, 2011]. Heavy metal stress adversely reduce growth and productivity of plant. The protective efficacy of seed priming with SiO_2 NPs (400 mg/L) in relieving the Cr (200 µM) phytotoxicity mainly in *Brassica napus* L. seedlings recorded. In this study an evidence was established about the Cr-detoxification by seed primed SiO_2 NPs in *B. napus*, indicated the potential of SiO_2 NPs as stress reducing agent for crops grown in Cr contaminated areas. The different Cr levels (200, 300, and 400 mg/kg soil) affected the growth of mung bean seedlings with the use of Azospirillum brasilense and salicylic acid [ALI & al. 2023]. Furthermore, the Cr treatment decreased shoot and root length, plant height, dry weight, and chlorophyll content of mung bean. 37.15% plant height, 71.85% root length, 57.09% chlorophyll contents, 82.34% crop growth rate was decreased when Cr toxicity was @ 50 µM. Furthermore, Mung bean seedlings reported severely damaged by Cr contamination, which limits their growth and physiological characteristics.

Table 1. Effects of chromium on germination and growth of Pearl millet

TRT (ppm)	**SG** (%)	**Root length** (cm)	**Shoot length** (cm)	**Seedling size** (cm)	**Seedling dry weight** (g)	**Root / shoot length Ratio**
00	100.00±0.0a	12.94±1.1a	6.32±0.4a	19.26±1.3a	0.026±0.004a	2.03±0.1a
25	100.00±0.0a	3.95±0.3b	2.15±0.3b	6.04±0.6b	0.026±0.004a	2.03±0.2a
50	83.33±3.3b	3.53±0.2b	1.47±0.1bc	5.01±0.3bc	0.020±0.004ab	2.53±0.3ab
75	6.60±3.3bc	2.91±0.4b	1.01±0.3c	3.40±1.6cd	0.013±0.001ab	3.21±1.0ab
100	73.33±3.3c	0.94±0.2c	1.55±0.2bc	2.48±0.4d	0.010±0.004b	0.63±0.1b
L.S.D. $p<0.05$	8.13	1.62	0.876	2.33	0.014	1.405

Symbol used: TRT = treatment; SG = Seed germination; Number followed by the same letters in the same column are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Potassium dichromate at concentrations above 0.5 mM suppressed growth of radicle and plumule of pea significantly. Its deleterious effect was more pronounced on the growth of roots than on shoots. In addition chromium treated plants a larger proportion of pods failed to set seeds and the average number of seeds per pod was lower [BISHNOI & al. 1993]. The root growth performances of *P. glaucum* was strongly affected by all concentration of chromium. The chromium at 25 ppm level produced a negative and significant impact on root and shoot growth of *P. glaucum*. Similarly, ISLAM & al. (2016) also found decrease in growth of *Zea mays* L. under chromium. Chromium is non-essential element for plant growth. The seedling size of *P. glaucum* showed similar trend of decline as recorded for root and shoot growth. The highest seedling size of *P. glaucum* was recorded for control (19.26 cm) and lowest (2.48 cm) at 100 ppm of chromium. The high uptake of chromium from the medium could be important reason of reduction in seedling height of *P. glaucum*. Seedlings of *P. glaucum* significantly decreased its dry weight with 100 ppm of chromium. Heavy metals produced toxic effects on the plant biomass [SINGH & al. 2015]. Phytotoxic effects of chromium on germination and seedling growth of different plant species investigated [JOUTEY & al. 2013; NAZ & al. 2013], root growth and environmental contamination [PRADAS & al. 2014; PRADAS & al. 2021].

Chromium stress influence on growth, nutritional quality and tolerance in plant [MOHAMMED & al. 2021; LÓPEZ-BUCIO & al. 2022]. The metal tolerance character found in well adapted plant species. The seedlings of *P. glaucum* also tested for tolerance to different level of chromium. The seedlings of *P. glaucum* responded differently for tolerance to chromium (Figure 1). Chromium at 25 ppm showed high percentage of tolerance for *P. glaucum* and lowest to chromium at 100 ppm. The chromium pollution treatment showed the order of phytotoxicity tolerance 25>50>75>100 ppm in seedlings of *P. glaucum*.

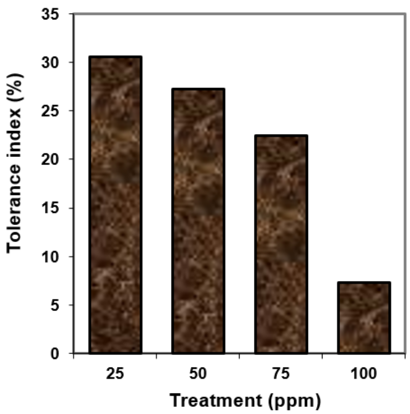

Figure 1. Percentage of tolerance in *P. glaucum* using different concentration of chromium (25, 50, 75, 100 ppm) as compared to control.

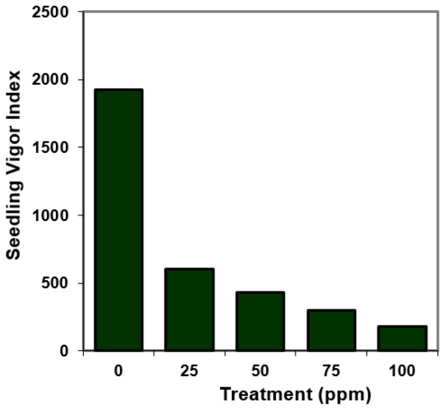

Figure 2. Seedling vigor index for *P. glaucum* using different concentration 0, 25, 50, 75, 100 ppm of chromium.

An association between seedling vigor index percentages in chromium was also recorded with similar trends (Figure 2). The results suggests that an increase in chromium concentration decreased seedling vigor index. Seedling vigor of *P. glaucum* was prominently decreased at 100 ppm of chromium and agreed with the findings of AMIN & al. (2013) who has been found that chromium treatment at 100 mg kg^{-1} adversely affected seedling vigor index of *Hibiscus esculentum* L.

Conclusion

It was concluded that excess level of chromium produced phytotoxic effects on seedling growth of *P. glaucum*. The chromium at 25 ppm significantly ($p<0.05$) affected root, shoot and seedling length of *P. glaucum*. The seedlings *P. glaucum* showed lowest tolerance and seedling vigor indices to chromium at 100 ppm level which might be due to disturbances in metabolic system. It is suggested that such types of ecotoxicological studies can be useful for cultivation of vegetation in chromium polluted areas based on tolerance index.

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