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INTERACTIVE EFFECTS BETWEEN WATER STRESS AND HEAVY METALS ON SEED GERMINATION AND SEEDLING GROWTH OF TWO GREEN GRAM (*VIGNA RADIATA* L.WILZEC) CULTIVARS

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ABSTRACT

Plants are sessile and sensitive organisms that inevitably encounter a variety of abiotic stresses in nature. Abiotic stresses such as salinity, drought, heavy metal toxicity and extreme temperatures are critical factors that reduce crop yields by more than 50% worldwide. Sustainable agriculture in harsh environments requires an understanding of the ways that crop plants respond to both biotic and abiotic factors. The aim of the study was to determine the influence of water stress and heavy metals (Cd and Cr) singly and in combination on germination and seedling growth of two Green gram (*Vignaradiata*L.Wilzec) cultivars.Seeds of two Green gram (*Vignaradiata* L. Wilczek) cv. ML – 267, TM 96-2 cultivars were subjected to water stress by using PEG – 6000 (-0.3 MPa , -0.6 MPa , -0.9 MPa and -1.2 MPa); Cadmium and Chromium(20,60,100,200,400ppm). The seed germination percentage decreased significantly with increasing concentration of Cd and Cr. Decreased osmotic potential caused a decrease in germination percentage. In comparison with the control treatment, the lowest germination rate values determined at -0.9MPa, 400 ppm Cd and 400 ppm Cr. Shoot and root length was reduced significantly with a rise in PEG and heavy metal levels. Cd concentration had a more depressing effect on the root than on the shoot growth. Interactive effects between heavy metal and water stress on dry weight and root length was significant. According to this study the interactive effect of both stresses were however, less than additive.

Key words : Cadmium, Chromium, Water stress, Seed germination.

INTRODUCTION

Seed germination and growth are of vital importance for continuation of plant life. The seed as well as the seedlings are extremely vulnerable to environmental stress. Environmental stresses such as salinity, drought, cold, heat adversely affects the plant growth by making a series of morphological, physiological and biochemical changes. Seed germination, seedling growth and responses of plants to water stress are considered critical for the establishment of a crop (Gelmond, 1978) and consequently constitute important criteria for the evaluation of tolerance of germplasm. The presence of polluting agents in the environment changes the percent germination of seeds and subsequent seedling growth of crops (Misra and Pandey, 2002). Heavy metal and drought stresses are likely to co-occur, as metal contaminated soils tend to have poor water holding capacity and evaporation rates are high due to sparse vegetation cover. It is important to understand the interactions between these stresses as they can potentially reduce the prospects of establishing plant cover on disturbed area.

In view of the above, an assessment of multiple stress effects is very important in obtaining a realistic view of the impact of current changes in the environment on crop plants. The present study was aimed to evaluate the interactive effects of two potential stress factors i.e. water stress and heavy metals (Cd / Cr) applied individually and in combination on the seed germination and early seedling growth of two green gram cultivars.

MATERIAL AND METHODS

Seed Germination and Induction of Stress:
Seeds of two Green gram (*Vignaradiata* L. Wilczek) cv. ML – 267, TM 96-2 cultivars were surface sterilized using 0.1% $HgCl_2$ and washed repeatedly with sterile distilled water to remove the remnants of adsorbed sterilants. The seeds were then transferred to the germination boxes lined with sterile filter papers for germination and subjected to water stress by using PEG – 6000 according to Michel and Kaufmann (1973). PEG - 6000 was used in four concentrations to maintain four levels of osmotic potentials of PEG solution namely -0.3 MPa (Ws1), -0.6 MPa (Ws2), -0.9 MPa (Ws3) and -1.2 MPa (Ws4).

Seeds placed on sterilized filter papers were exposed to varying concentrations of cadmium solutions (20, 60, 100, 200 and 400 ppm) made using anhydrous $CdCl_2$ and chromium (20, 60, 100, 200 and 400 ppm) supplied as solutions of CrO_3. The concentrations though rather high, were based on dose response curves plotted from pilot experiments conducted prior to the study, using a wide range of cadmium levels (10 μM to 3 mM) and chromium levels (100 μM to 4.0 mM). Water stress and cadmium application; water stress and chromium application were simultaneously given to study the combined effect of both the stresses on seedling growth. Water stress + cadmium combinations i.e. -0.6 MPa + 20 ppm Cd (Ws2 + Cd 20), -0.6 MPa + 100 ppm Cd (Ws2 + Cd 100), -0.9 MPa + 20 ppm Cd (Ws3 + Cd 20), -0.9MPa + 100 ppm Cd (Ws3 + Cd 100) and Water stress + chromium combinations [-0.6 MPa + 20 ppm Cr (Ws2 + Cr 20), -0.6MPa + 100 ppm Cr (Ws2 + Cr 100), -0.9 MPa + 20 ppm Cr (Ws3 + Cr 20), -0.9 MPa + 100 ppm Cr (Ws3 + Cr 100)] were studied. Distilled water was used in place of PEG solution or cadmium or chromium to maintain the control. A completely randomized design was adopted for the experiment with three replications of fifteen seeds each. All the experiments were repeated twice.

Growth Parameters:
For growth analysis, samples were collected at 2 day intervals from the 2nd day after sowing, up to the 10^{th} day and growth parameters like % seed germination, root length, shoot length, fresh and dry weights were measured. Emergence of the radicle was taken as an index for the purpose of identifying seed germination. The root length of the seedlings was measured to the nearest mm with the help of cotton thread and a cm ruler. Fresh weight of the seedlings was recorded to the nearest mg using an electronic balance. The seedlings were oven dried at 80^0C in a hot air oven to a constant dry weight and the data was recorded to the nearest mg using sensitive electronic balance. All the observations are means of three replications.

Seed Germination Percentage (G %):
Percentage of seed germination (G %) was calculated according to AOSA, 1990. It was calculated by using the formula:
G% = 100 × A / N, where
A = Number of seeds found germinated
N = Total number of seeds used in the germination test.

RESULTS

Seed Germination Percentage:
Percentage of seed germination increased from second day to sixth day after sowing and germination is stabilized by sixth day in control as well as in the treatments. Percent seed Germination decreased as the osmotic potential decreased. ML-267 cultivar of Green gram was

sensitive at all the levels of water stress studied. But, TM 96-2 cultivar was tolerant at lower level of water stress showing 95% seed germination. None of the cultivars germinated at the highest level of stress i.e. -1.2MPa osmotic potential. Our results confirmed the findings of Almansouri*et al.*, (2001) and Kaydan and Yagmur, (2008) who reported that moderate stress only delayed germination, whereas the highest concentration of PEG reduced final germination percentages.

Figure-1: Effect of Water stress on percentage of seed germination in two Green gram cultivars

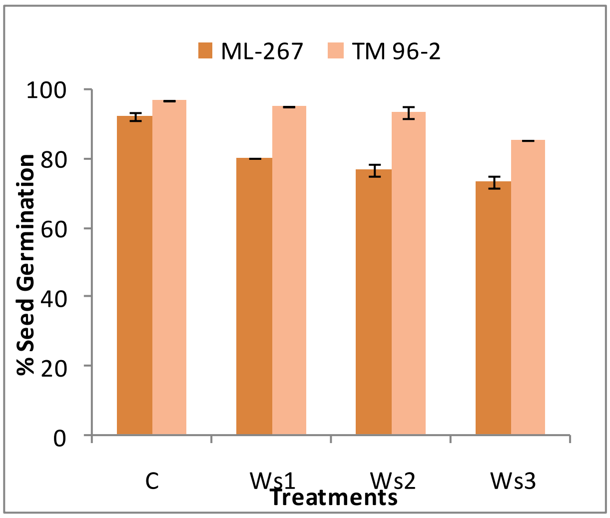
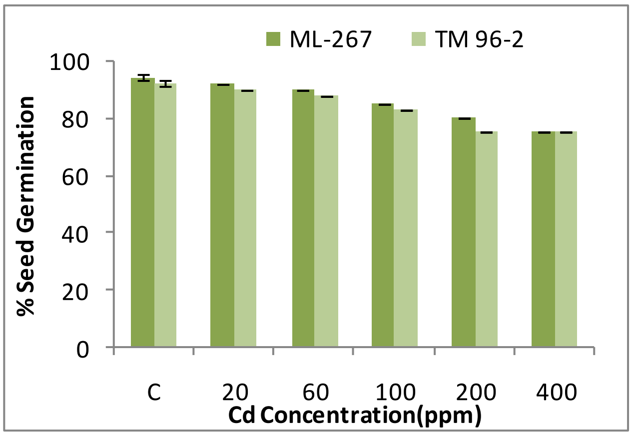
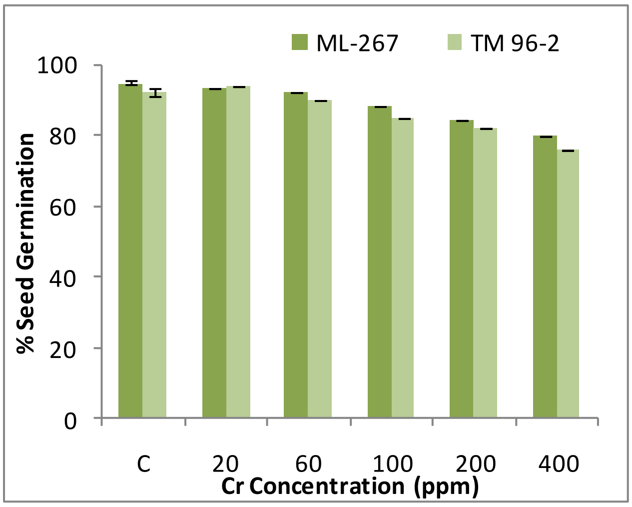


Figure-2: Effect of Cadmium on percentage of seed germination in two Green gram cultivars



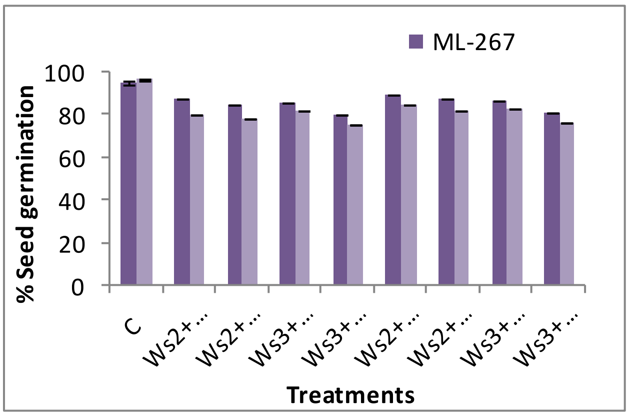
The progressive fall in the germination percentage observed in this experiment, with the decrease in water potential, probably was caused by the low velocity of water absorption and consequently the hydrolysis of carbohydrates (Taizand Zeiger, 1998). Similar types of reduction in germination of different plants under moisture stress has been reported earlier (Masoud sinaki *et al.*, 2004 and Lobato *et al.*, 2008).

Figure-3:Impact of Chromium on percentage of seed germination in two Green gram cultivars



Repeated Measures ANOVA (RM ANOVA) is used to compare the effect of treatment, duration and variety. Both treatment and cultivar had significant effect on the seed germination percent. Dunnet's test shows that there is significant difference between seedlings of control and other treatments in Green gram cultivars.

Figure-4: Effect of Water stress+Cd and Water stress+Cr on percentage of seed germination in two Green gram cultivars



In general cadmium and chromium reduced the seed germination of both the cultivars studied and greater reduction was noticed at higher concentrations (Fig 2,3). TM 96-2 cultivar of Green gram was more sensitive to chromium at 400 ppm. Though a general decrease in germination occurred at all the levels of cadmium stress, significant differences were observed only at concentrations above 100 ppm. No germination was found on second day in Cd treated seedlings at 200 ppm and 400 ppm in both the cultivars of Green gram.The inhibition of germination of seeds may be due to the accumulation of heavy metals in seeds.

A negative correlation was observed between heavy metal doses and the germination percentage of seeds. Inhibition of seed germination by the heavy metals has often been reported (Munzuroglu and Geckil, 2002; Neogy*et al*., 2002;cavusoglu and EmineYalcin, 2010) in many studies. Peralta *et al.*, (2001) found that 40 ppm of Cr (VI) reduced by 23% the ability of seeds of Lucerne to germinate and grow in the contaminated medium. The reduced germination of seeds under chromium stress could be a depressive effect of chromium on the activity of amylases, which is one of the important factors for germination inhibiton in many plants in view of the subsequent impaired transport of sugars to the embryo axes (Zeid, 2001).

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Table 1: Effect of Water stress on Root and shoot lengths, dry weight in Green gram cultivars

Cultivar		Root length (Cm)		Shoot length(Cm)		Dry Weight(g/10seedlings)	
		ML - 267	**TM 96 -2**	**ML - 267**	**TM 96 -2**	**ML - 267**	**TM 96 -2**
Treatment	DAS	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
C	2	1.77 ± 0.03	1.67 ± 0.03	-	-	0.39 ± 0.003	0.36 ± 0.003
	4	5.43 ± 0.03	4.77 ± 0.03	5.93 ± 0.07	5.37 ± 0.03	0.47 ± 0.000	0.43 ± 0.010
	6	6.87 ± 0.03	5.47 ± 0.03	8.33 ± 0.09	8.07 ± 0.03	0.53 ± 0.010	0.47 ± 0.007
	8	7.83 ± 0.03	6.17 ± 0.03	11.10 ± 0.06	10.50 ± 0.06	0.57 ± 0.003	0.51 ± 0.007
	10	8.40 ± 0.06	7.53 ± 0.03	14.20 ± 0.06	13.80 ± 0.06	0.61 ± 0.007	0.53 ± 0.007
Ws1	2	2.57 ± 0.03	2.40 ± 0.06	-	-	0.30 ± 0.003	0.27 ± 0.003
	4	5.73 ± 0.07	5.17 ± 0.07	5.43 ± 0.03	5.07 ± 0.03	0.34 ± 0.003	0.32 ± 0.003
	6	7.10 ± 0.06	5.63 ± 0.03	7.60 ± 0.00	7.10 ± 0.06	0.38 ± 0.003	0.36 ± 0.003
	8	8.03 ± 0.03	6.33 ± 0.03	10.20 ± 0.06	9.77 ± 0.03	0.42 ± 0.003	0.40 ± 0.003
	10	8.67 ± 0.03	7.73 ± 0.03	13.13 ± 0.03	12.27 ± 0.09	0.45 ± 0.003	0.42 ± 0.003
Ws2	2	1.37 ± 0.03	1.07 ± 0.03	-	-	0.25 ± 0.003	0.24 ± 0.007
	4	4.30 ± 0.06	2.87 ± 0.03	2.47 ± 0.03	2.27 ± 0.03	0.31 ± 0.007	0.30 ± 0.007
	6	4.83 ± 0.03	3.37 ± 0.09	2.80 ± 0.06	2.40 ± 0.06	0.36 ± 0.007	0.34 ± 0.003
	8	5.17 ± 0.03	4.20 ± 0.06	3.37 ± 0.03	2.97 ± 0.03	0.40 ± 0.007	0.37 ± 0.007
	10	5.17 ± 0.03	4.20 ± 0.06	3.67 ± 0.03	3.17 ± 0.03	0.43 ± 0.007	0.39 ± 0.010
Ws3	2	0.87 ± 0.03	0.77 ± 0.03	-	-	0.21 ± 0.007	0.19 ± 0.007
	4	3.40 ± 0.06	2.67 ± 0.03	-	-	0.26 ± 0.007	0.21 ± 0.003
	6	4.00 ± 0.06	3.07 ± 0.03	0.30 ± 0.00	----	0.30 ± 0.003	0.26 ± 0.003
	8	5.03 ± 0.03	3.77 ± 0.03	0.30 ± 0.00	----	0.34 ± 0.003	0.31 ± 0.006
	10	5.03 ± 0.03	4.03 ± 0.03	0.30 ± 0.00	----	0.36 ± 0.003	0.32 ± 0.003
Parameter				ANOVA Results			
Treatment		F = 17694.393 ; p = 0.001*		F= 37316.606, p < .0001		F = 636.686, p < 0.0001	
Cultivar		F = 5890.988; p = 0.001*		F = 279.273, P = 0.001		F = 93.657, p = 0.0001	
Duration		F =2835.324 ; p =0.001 *		F = 27709.905, P = 0.001		F = 5226.884, p = 0.001.	

Table 2: Effect of Ws+Cd and Ws+Cr on the parameters in Green gram cultivars

			Root Length(Cm)		Shoot Length(Cm)		Dry Weight(g/10 seedlings)	
		D	**ML-267**	**TM96-2**	**ML-267**	**TM96-2**	**ML-267**	**TM96-2**
			Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
WS + Cd	**C**	4	5.43 ± 0.033	4.77 ± 0.033	5.93 ± 0.067	5.37 ± 0.033	0.46 ± 0.003	0.43 ± 0.003
		6	6.87 ± 0.033	5.47 ± 0.033	8.33 ± 0.088	8.07 ± 0.033	0.51 ± 0.003	0.49 ± 0.007
		8	7.83 ± 0.033	6.17 ± 0.033	11.10 ± 0.058	10.50 ± 0.058	0.56 ± 0.009	0.52 ± 0.003
		10	8.40 ± 0.058	7.53 ± 0.033	14.20 ± 0.058	13.80 ± 0.058	0.57 ± 0.003	0.54 ± 0.000
	Ws2+20	4	0.97 ± 0.033	0.80 ± 0.058	1.10 ± 0.058	0.83 ± 0.033	0.41 ± 0.003	0.39 ± 0.003
		6	1.37 ± 0.033	1.20 ± 0.058	1.43 ± 0.033	1.03 ± 0.033	0.46 ± 0.003	0.44 ± 0.003
		8	1.57 ± 0.033	1.37 ± 0.033	2.23 ± 0.033	1.67 ± 0.033	0.51 ± 0.003	0.48 ± 0.003
		10	1.77 ± 0.033	1.47 ± 0.033	2.93 ± 0.033	2.17 ± 0.033	0.54 ± 0.003	0.50 ± 0.003
	Ws2+100	4	0.10 ± 0.000	0.10 ± 0.000	0.30 ± 0.000	0.10 ± 0.000	0.37 ± 0.003	0.36 ± 0.003
		6	0.10 ± 0.000	0.10 ± 0.000	0.50 ± 0.000	0.30 ± 0.000	0.43 ± 0.003	0.42 ± 0.003
		8	0.10 ± 0.000	0.10 ± 0.000	0.60 ± 0.000	0.40 ± 0.000	0.47 ± 0.003	0.46 ± 0.003
		10	0.10 ± 0.000	0.10 ± 0.000	0.70 ± 0.000	0.50 ± 0.000	0.50 ± 0.003	0.48 ± 0.003
	Ws3+20	4	0.47 ± 0.033	0.30 ± 0.000	0.60 ± 0.000	0.40 ± 0.000	0.34 ± 0.003	0.31 ± 0.003
		6	0.97 ± 0.033	0.77 ± 0.033	1.17 ± 0.033	0.93 ± 0.033	0.40 ± 0.003	0.37 ± 0.003
		8	1.07 ± 0.033	0.97 ± 0.033	1.37 ± 0.033	1.03 ± 0.033	0.45 ± 0.003	0.42 ± 0.003
		10	1.17 ± 0.033	1.07 ± 0.033	1.50 ± 0.058	1.23 ± 0.033	0.47 ± 0.003	0.44 ± 0.003
	Ws3+100	4	0.10 ± 0.000	0.10 ± 0.000	0.43 ± 0.033	0.30 ± 0.000	0.30 ± 0.003	0.28 ± 0.003
		6	0.10 ± 0.000	0.10 ± 0.000	0.50 ± 0.000	0.30 ± 0.000	0.36 ± 0.003	0.34 ± 0.003
		8	0.10 ± 0.000	0.10 ± 0.000	0.60 ± 0.000	0.40 ± 0.000	0.41 ± 0.003	0.39 ± 0.003
		10	0.10 ± 0.000	0.10 ± 0.000	0.60 ± 0.000	0.40 ± 0.000	0.43 ± 0.000	0.41 ± 0.003
WS + Cr	**C**	4	5.43 ± 0.033	4.77 ± 0.033	5.93 ± 0.067	5.37 ± 0.033	0.46 ± 0.003	0.43 ± 0.003
		6	6.87 ± 0.033	5.47 ± 0.033	8.33 ± 0.088	8.07 ± 0.033	0.51 ± 0.003	0.49 ± 0.007
		8	7.83 ± 0.033	6.17 ± 0.033	11.10 ± 0.058	10.50 ± 0.058	0.56 ± 0.009	0.52 ± 0.003
		10	8.40 ± 0.058	7.53 ± 0.033	14.20 ± 0.058	13.80 ± 0.058	0.57 ± 0.003	0.54 ± 0.000
	Ws2+20	4	1.17 ± 0.033	0.97 ± 0.033	4.83 ± 0.033	1.93 ± 0.033	0.43 ± 0.003	0.41 ± 0.003
		6	1.57 ± 0.033	1.37 ± 0.033	6.03 ± 0.033	2.43 ± 0.033	0.49 ± 0.003	0.47 ± 0.003
		8	1.73 ± 0.067	1.57 ± 0.033	8.23 ± 0.033	3.57 ± 0.067	0.54 ± 0.003	0.52 ± 0.003
		10	2.03 ± 0.033	1.63 ± 0.067	9.40 ± 0.058	4.63 ± 0.033	0.56 ± 0.003	0.54 ± 0.003
	Ws2+100	4	0.60 ± 0.000	0.40 ± 0.000	1.60 ± 0.000	0.80 ± 0.000	0.39 ± 0.000	0.37 ± 0.003
		6	0.70 ± 0.000	0.50 ± 0.000	2.47 ± 0.033	1.63 ± 0.033	0.45 ± 0.003	0.43 ± 0.003
		8	0.70 ± 0.000	0.50 ± 0.000	2.90 ± 0.000	2.13 ± 0.033	0.50 ± 0.003	0.47 ± 0.003
		10	0.70 ± 0.000	0.50 ± 0.000	3.30 ± 0.058	2.87 ± 0.033	0.53 ± 0.003	0.49 ± 0.000
	Ws3+20	4	0.97 ± 0.033	0.83 ± 0.067	0.90 ± 0.000	0.50 ± 0.000	0.35 ± 0.003	0.31 ± 0.003
		6	1.17 ± 0.033	1.07 ± 0.033	1.33 ± 0.033	1.03 ± 0.033	0.40 ± 0.003	0.36 ± 0.003
		8	1.37 ± 0.033	1.27 ± 0.033	1.47 ± 0.033	1.27 ± 0.033	0.45 ± 0.003	0.41 ± 0.007
		10	1.57 ± 0.033	1.47 ± 0.033	2.07 ± 0.033	1.87 ± 0.033	0.47 ± 0.003	0.43 ± 0.007
	Ws3+100	4	0.50 ± 0.000	0.30 ± 0.000	0.60 ± 0.000	0.30 ± 0.000	0.32 ± 0.003	0.30 ± 0.000
		6	0.50 ± 0.000	1.20 ± 0.900	1.03 ± 0.033	0.60 ± 0.000	0.37 ± 0.003	0.35 ± 0.003
		8	0.50 ± 0.000	0.30 ± 0.000	1.13 ± 0.033	0.70 ± 0.000	0.41 ± 0.003	0.39 ± 0.003
		10	0.50 ± 0.000	0.30 ± 0.000	1.23 ± 0.033	0.70 ± 0.000	0.43 ± 0.003	0.41 ± 0.003

could be a depressive effect of chromium on the activity of amylases, which is one of the important factors for germination inhibiton in many plants in view of the subsequent impaired transport of sugars to the embryo axes (Zeid, 2001).

Figure 4 revealed that cultivars of *Vignaradiata* showed significant decrease in percent germination with increase in level of combination of stresses i.e. Ws + Cd and Ws + Cr. The mean and S.E. values on the sixth day has been presented. TM 96-2 cultivar of Green gram was found to be sensitive with Ws + Cd 100 and Ws + Cr 100. As the severity of water stress increased, the reduction in seed germination percentage also increased in Ws3 + Cd 100 or Ws3 + Cr 100 which showed more reduction than other doses.

In Green gram, at Ws1 the root length increased when compared to control by 3.2% in ML-267 and 2.6% in TM 96-2 while, Ws3 caused 40.12% and 46.5% reduction in root length in the same plants (Table 1).Shoot and root length was reduced significantly with a rise in PEG and heavy metal levels. The present observations are in agreement with the report of Subin and Steffy, 2013. Cd concentration had a more depressing effect on the root than on the shoot growth. Interactive effects between heavy metal and water stress on dry weight and root length was significant (Table 2).

CONCLUSION

The present study showed that water stress and heavy metals both caused reduction in seedling growth. Seed germination was less sensitive than seedling growth to both the stresses. Cadmium caused more damage than Chromium and their effect in combination with water stress was less than additive in both the cultivars of Green gram. Ws+Cd treatment triggered more stress than Ws+Cr.

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