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Germination and early growth responses of Vigna mungo L. Hepper cultivars to water stress and heavy metals

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Abstract
Plants exhibit various responses to environmental stresses. A laboratory experiment was conducted to determine the influence of heavy metals(Cd, Cr) and water stress induced by polyethylene glycol(PEG) on germination and early seedling growth of two black gram (*Vigna mungo*L.Hepper) cultivars. Seeds of two black gram (*Vigna mungo* L.Hepper) cv. LBG-623 and LBG-752 cultivars were subjected to water stress by using PEG – 6000 (-0.3MPa, -0.6MPa,-0.9MPa and -1.2MPa) heavy metal stress by Cadmium and Chromium(20,60,100,200,400ppm). Results indicated that a significant inhibitory effect was observed at all levels of single and combination of stresses.

Key words: Cadmium, Chromium, *Vigna mungo*, germination, water stress

INTRODUCTION

Plants are often simultaneously exposed to abiotic stresses that limit crop yields. Plant responses to stress depend on an interrelated network of morphological, physiological and molecular mechanisms. The molecular mechanisms underlying abiotic stress tolerance have been intensely studied with much emphasis on individual stresses (Suzuki et al., 2005). Recent studies have revealed that the response of plants to combinations of two or more stress conditions is unique and cannot be directly extrapolated from the response of plants to each of the different stresses applied individually.

Water stress was one of the limiting factors for crop productivity. Cadmium and chromium are the toxic heavy metals accumulated in soil and water due to pollution. Excessive level of heavy metals in the soil environment adversely affect the germination of seeds, plant growth, alter the level of biomolecules in the cells and interfere with the activities of many key enzymes related to normal metabolic and developmental processes (Zhang et al., 2009; Rahoui et al., 2010; Hema et al., 2013).

Plants are well protected against various stresses when they are in the seed stage. However, during germination and subsequent seedling stage they become stress sensitive (Sunanda Sarkar, 2012). The early stage of seedling growth determines the establishment of crop stand.

As black gram is stress sensitive legume, the present study was undertaken to analyze whether water stress and heavy metals and their combination has effect on germination and early seedling growth.

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MATERIALS AND METHODS

Seeds of two black gram (*Vigna mungoL.*Hepper) cv. LBG-623 and LBG-752 cultivars were surface sterilized using 0.1% $HgCl_2$ and washed repeatedly with sterile distilled water. 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 100 ppm of cadmium solution made using anhydrous

$CdCl_2$ and 100 ppm chromium 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.

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.

Growth Parameters:

For growth analysis, samples were collected at 2 day intervals from the 4^{th} day after sowing, up to the 10^{th} day and growth parameters like % seed germination, root length, shoot length 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.

RESULTS & DISCUSSION

Water stress, heavy metals and their combination resulted in general delay in seed germination of the two cultivars. However, the cultivars showed varied responses to water stress and heavy metals. *Vigna mungo* L.Hepper cv.LBG-623 was sensitive to water stress than LBG-752 (Fig.1). LBG-752 was sensitive to cadmium and chromium toxicity and their combination with water stress at germination phase (Fig.2, 3, 4). Elevated water stress decreases water uptake by seeds there by inhibiting their imbibition and germination. Reduction in germination percentage may be due to degradation and inactivation of the essential hydrolytic enzymes. These studies support the previous findings which show reduction in germination percentage with increasing stress levels (Wafa'a A. Al-Taisan, 2010; Dahanayake Nilanthi, 2014).

Figure-1. Effect of water stress on germination percentage of black gram cultivars.

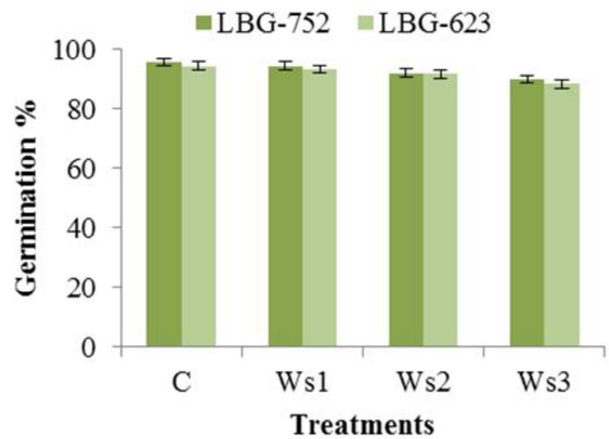
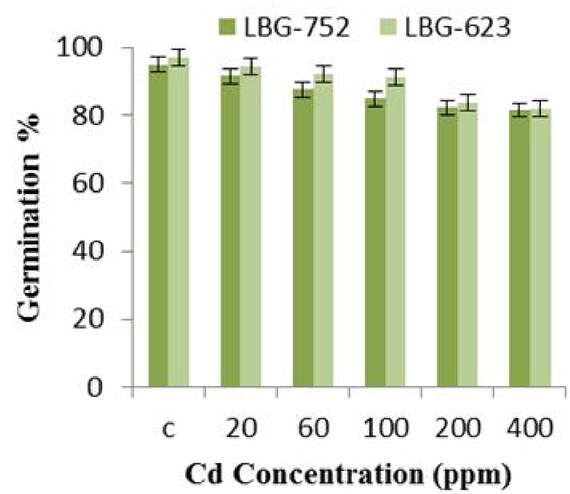


Figure-2. Effect of cadmium on germination of Black gram cultivars



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 black gram cultivars.

Higher concentration of PEG showed concomitant decrease in root and shoot length (Table 1). Root length is also a vital trait against water stress in plant cultivars. Under water stress condition, the root develops faster than the hypocotyls to acclimatize.

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

Cultivar		Root length (Cm)		Shoot length (Cm)		Dry weight (g/10seedlings)	
		LBG-752	LBG-623	LBG-752	LBG-623	LBG-752	LBG-623
Treatment	DAS	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
C	4	4.97 ± 0.09	4.63 ± 0.22	7.00 ± 0.06	6.83 ± 0.07	0.52 ± 0.003	0.47 ± 0.003
	6	6.23 ± 0.09	5.27 ± 0.09	12.37 ± 0.09	11.73 ± 0.03	0.58 ± 0.003	0.53 ± 0.003
	8	7.00 ± 0.06	5.77 ± 0.09	15.97 ± 0.09	14.77 ± 0.09	0.63 ± 0.003	0.56 ± 0.006
	10	8.30 ± 0.12	7.37 ± 0.18	17.37 ± 0.07	16.20 ± 0.12	0.65 ± 0.003	0.59 ± 0.003
Ws1	4	4.87 ± 0.03	4.40 ± 0.06	5.57 ± 0.03	3.40 ± 0.06	0.43 ± 0.003	0.40 ± 0.003
	6	6.13 ± 0.03	5.17 ± 0.03	10.03 ± 0.03	9.43 ± 0.03	0.48 ± 0.003	0.45 ± 0.003
	8	6.90 ± 0.06	6.17 ± 0.17	12.17 ± 0.03	11.70 ± 0.06	0.53 ± 0.003	0.50 ± 0.003
	10	7.83 ± 0.03	6.90 ± 0.06	12.70 ± 0.06	11.93 ± 0.07	0.56 ± 0.003	0.53 ± 0.003
Ws2	4	2.03 ± 0.07	1.83 ± 0.07	---	---	0.35 ± 0.003	0.32 ± 0.003
	6	3.07 ± 0.09	2.70 ± 0.06	0.30 ± 0.00		0.41 ± 0.003	0.38 ± 0.003
	8	4.00 ± 0.06	3.80 ± 0.06	0.50 ± 0.00	0.30 ± 0.00	0.46 ± 0.003	0.42 ± 0.003
	10	4.87 ± 0.03	4.30 ± 0.12	0.50 ± 0.00	0.40 ± 0.00	0.49 ± 0.000	0.44 ± 0.003
Ws3	4	0.87 ± 0.03	0.47 ± 0.03	---	----	0.31 ± 0.003	0.27 ± 0.003
	6	1.70 ± 0.06	1.27 ± 0.09	----	---	0.37 ± 0.003	0.33 ± 0.007
	8	2.60 ± 0.10	2.27 ± 0.03	---	----	0.42 ± 0.003	0.38 ± 0.007
	10	3.43 ± 0.12	3.07 ± 0.03	---	---	0.44 ± 0.000	0.41 ± 0.000
Parameter		ANOVA Results					
Treatment		F = 4094.05; p = 0.001*		F = 2735.405; p = 0.001*		F = 1900.85; p = 0.001*	
Cultivar		F = 116.827 ; p = 0.001*		F = 648 ; p = 0.001*		F = 361.23 ; p = 0.001*	
Duration		F = 33120.85 ; p = 0.001*		F = 2161.011 ; p = 0.001*		F = 9068.63 ; p = 0.001*	

Therefore, the growth of radicle and hypocotyls should reflect the adaptability of plant to drought stress (Zhu et al; 2006). Similar results were observed by Ravi Ranjan kumar et al., 2011. Root length, Shoot length and dry weight showed significant reduction with the increase in stress levels with combination of stresses (Table 2).The reduction in root and shoot length in combination of stresses may be due to the synergistic effect of the stresses. The results are in accordance with de Silva et al.,2012; Santala and Ryser, 2009.

Figure-3. Effect of chromium on germination percentage of Black gram cultivars

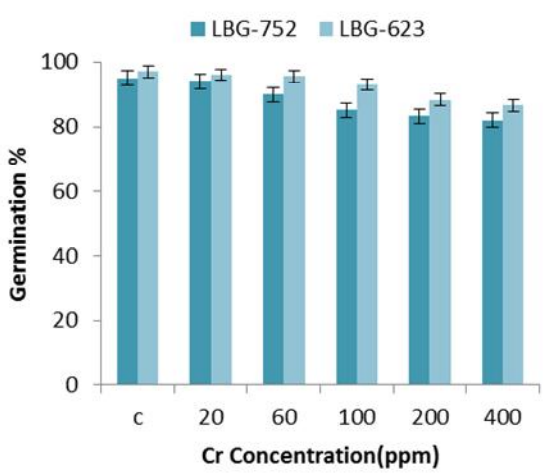
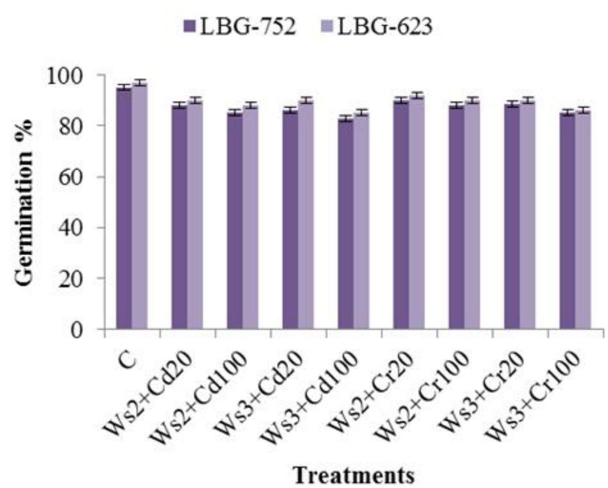


Figure-4. Effect of combination of water stress and heavy metals on germination of Black gram cultivars



Conclusion

Early seedling growth is more sensitive to combination of water stress and heavy metals than germination phase in *Vigna mungo* L.Hepper cv.LBG-752 and LBG-623.

Table-2. Effect of Ws+Cd and Ws+Cr on the early seedling growth in Black gram cultivars

			Root length (Cm)		Shoot length (Cm)		Dry weight (g/10seedlings)	
Cultivar			LBG-752	LBG-623	LBG-752	LBG-623	LBG-752	LBG-623
Treatment		DAS	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
WS + Cd	C	4	4.97 ± 0.088	4.63 ± 0.219	7.00 ± 0.058	6.83 ± 0.067	0.52 ± 0.003	0.47 ± 0.003
		6	6.23 ± 0.088	5.27 ± 0.088	12.37 ± 0.088	11.73 ± 0.033	0.58 ± 0.003	0.53 ± 0.003
		8	7.00 ± 0.058	5.77 ± 0.088	15.97 ± 0.088	14.77 ± 0.088	0.63 ± 0.003	0.56 ± 0.006
		10	8.30 ± 0.115	7.37 ± 0.176	17.37 ± 0.067	16.20 ± 0.115	0.65 ± 0.003	0.59 ± 0.003
	Ws2+20	4	0.77 ± 0.033	0.53 ± 0.033	1.37 ± 0.033	0.87 ± 0.033	0.42 ± 0.017	0.37 ± 0.003
		6	1.07 ± 0.033	0.80 ± 0.000	2.47 ± 0.033	1.27 ± 0.033	0.46 ± 0.003	0.43 ± 0.003
		8	1.23 ± 0.067	1.00 ± 0.000	3.13 ± 0.067	1.53 ± 0.067	0.50 ± 0.003	0.47 ± 0.003
		10	1.67 ± 0.033	1.17 ± 0.033	3.37 ± 0.088	1.80 ± 0.058	0.53 ± 0.003	0.50 ± 0.003
	Ws2+100	4	0.20 ± 0.000	0.17 ± 0.033	0.50 ± 0.000	0.30 ± 0.000	0.36 ± 0.003	0.32 ± 0.003
		6	0.27 ± 0.033	0.20 ± 0.000	0.97 ± 0.033	0.80 ± 0.000	0.42 ± 0.003	0.38 ± 0.003
		8	0.30 ± 0.000	0.23 ± 0.033	2.17 ± 0.033	1.97 ± 0.033	0.46 ± 0.003	0.43 ± 0.003
		10	0.37 ± 0.033	0.30 ± 0.000	2.60 ± 0.058	2.37 ± 0.033	0.49 ± 0.003	0.46 ± 0.003
	Ws3+20	4	0.47 ± 0.033	0.27 ± 0.033	0.40 ± 0.000	0.20 ± 0.000	0.38 ± 0.003	0.34 ± 0.003
		6	0.97 ± 0.033	0.47 ± 0.033	1.57 ± 0.033	0.70 ± 0.000	0.44 ± 0.003	0.40 ± 0.003
		8	1.10 ± 0.058	0.77 ± 0.033	1.77 ± 0.033	0.80 ± 0.000	0.49 ± 0.007	0.43 ± 0.007
		10	1.27 ± 0.033	0.87 ± 0.033	1.87 ± 0.033	1.00 ± 0.000	0.51 ± 0.003	0.46 ± 0.007
	Ws3+100	4	0.00 ± 0.000	0.00 ± 0.000	---	---	0.33 ± 0.003	0.31 ± 0.007
		6	0.20 ± 0.000	0.10 ± 0.000	0.47 ± 0.033	0.40 ± 0.000	0.39 ± 0.003	0.37 ± 0.007
		8	0.20 ± 0.000	0.10 ± 0.000	0.57 ± 0.033	0.50 ± 0.000	0.44 ± 0.003	0.41 ± 0.007
		10	0.30 ± 0.000	0.20 ± 0.000	0.70 ± 0.000	0.60 ± 0.000	0.48 ± 0.003	0.44 ± 0.007
	C	4	4.97 ± 0.088	4.63 ± 0.219	7.00 ± 0.058	6.83 ± 0.067	0.52 ± 0.003	0.47 ± 0.003
		6	6.23 ± 0.088	5.27 ± 0.088	12.37 ± 0.088	11.73 ± 0.033	0.58 ± 0.003	0.53 ± 0.003
		8	7.00 ± 0.058	5.77 ± 0.088	15.97 ± 0.088	14.77 ± 0.088	0.63 ± 0.003	0.56 ± 0.006
		10	8.30 ± 0.115	7.37 ± 0.176	17.37 ± 0.067	16.20 ± 0.115	0.65 ± 0.003	0.59 ± 0.003
	Ws2+20	4	4.83 ± 0.033	4.63 ± 0.033	3.47 ± 0.033	3.03 ± 0.033	0.46 ± 0.003	0.43 ± 0.003
		6	6.20 ± 0.058	5.47 ± 0.033	5.37 ± 0.033	5.20 ± 0.058	0.52 ± 0.003	0.49 ± 0.003

……Table-2. Effect of Ws+Cd and Ws+Cr on the early seedling growth in Black gram cultivars

		Root length (Cm)		Shoot length (Cm)		Dry weight (g/10seedlings)	
Cultivar		**LBG-752**	**LBG-623**	**LBG-752**	**LBG-623**	**LBG-752**	**LBG-623**
Treatment	DAS	Mean± S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean± S.E
	8	7.03 ± 0.033	5.93 ± 0.033	6.43 ± 0.067	6.17 ± 0.033	0.57 ± 0.003	0.54 ± 0.003
	10	8.40 ± 0.058	7.73 ± 0.088	7.17 ± 0.033	6.87 ± 0.033	0.60 ± 0.003	0.57 ± 0.003
Ws2+100	4	0.57 ± 0.033	0.47 ± 0.033	2.77 ± 0.033	2.50 ± 0.058	0.45 ± 0.003	0.40 ± 0.003
	6	0.70 ± 0.000	0.60 ± 0.000	4.77 ± 0.033	4.37 ± 0.067	0.51 ± 0.003	0.46 ± 0.003
	8	0.80 ± 0.000	0.67 ± 0.033	5.33 ± 0.033	5.03 ± 0.033	0.56 ± 0.006	0.51 ± 0.006
	10	0.87 ± 0.033	0.70 ± 0.000	5.87 ± 0.033	5.60 ± 0.000	0.58 ± 0.003	0.53 ± 0.003
Ws3+20	4	0.60 ± 0.000	0.43 ± 0.033	2.47 ± 0.033	2.10 ± 0.058	0.36 ± 0.003	0.32 ± 0.003
	6	0.87 ± 0.033	0.77 ± 0.033	3.57 ± 0.033	2.70 ± 0.058	0.42 ± 0.003	0.38 ± 0.003
	8	1.47 ± 0.033	1.13 ± 0.033	4.77 ± 0.033	4.50 ± 0.058	0.46 ± 0.003	0.42 ± 0.003
	10	2.47 ± 0.033	2.03 ± 0.033	5.10 ± 0.058	5.00 ± 0.000	0.49 ± 0.003	0.44 ± 0.003
Ws3+100	4	0.57 ± 0.033	0.40 ± 0.000	---	---	0.30 ± 0.003	0.27 ± 0.003
	6	0.60 ± 0.000	0.43 ± 0.033	1.40 ± 0.000	1.10 ± 0.000	0.36 ± 0.003	0.33 ± 0.003
	8	0.67 ± 0.033	0.50 ± 0.000	1.50 ± 0.000	1.20 ± 0.000	0.40 ± 0.003	0.36 ± 0.003
	10	0.70 ± 0.000	0.60 ± 0.000	1.60 ± 0.000	1.30 ± 0.000	0.42 ± 0.003	0.38 ± 0.003

Competing interests

The authors have declared that no competing interests exist.

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