Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
3(4):917-921
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

Impact of combination of water stress and heavy metals on germination and seedling growth of two Pigeon Pea (*Cajanus Cajan* L.millspaugh) cultivars

Swapna, B[1]* and Rama Gopal, G[2].

[1]*Department of Botany, Vikrama Simhapuri University P.G.Centre, Kavali, SPSR Nellore District, Andhra Pradesh – 524201.*
[2]*Department of Botany, Sri Venkateswara University, Tirupati-517502*

**Email:* swapnaivsr@gmail.com

ABSTRACT

The ever changing climatic factors increase the chances of occurrence of abiotic stresses. Plants, in nature are frequently exposed to single or combination of abiotic stresses simultaneously that limit crop yields. The aim of the study was 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 pigeon pea (*Cajanus cajan* L.Millspaugh) cultivars. Seeds of two Pigeon pea (*Cajanus cajan* L.Millspaugh) cv. LRG-41 and Yashoda-45 cultivars were subjected to water stress by using PEG – 6000 (-0.3 MPa , -0.6 MPa , -0.9 MPa and -1.2 MPa); heavy metal stress by Cadmium and Chromium(20,60,100,200,400ppm). In comparison with the control, the lowest germination rate values were observed 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 was additive.

Key Words: Water stress, heavy metals, germination, interactive effects

INTRODUCTION

Plants are often exposed to various abiotic stresses. Abiotic stresses such as water stress, salinity, UV, heat stress, cold stress, heavy metals have profound effect on germination, growth and reproduction (Giovanni Dal Corso et al., 2010; Nafiseh Nematshahi et al., 2012). The South Asian summer monsoon is an annual wind-driven weather pattern that is responsible for 85% of India's annual precipitation and is vital for the country's agricultural sector. The Indian monsoon is changing, with less rainfall overall in more intense bursts, and more frequent dry spells in between (Deepthi et al., 2014). Increasing aridity due to scanty rainfall and higher temperatures leads to water stress. Water stress is one of the abiotic stresses which accounts for 50% yield loss in crop plants. Release of industrial effluents in to arable lands is increasing heavy metal pollution in agricultural lands. Cadmium and chromium are the toxic heavy metals which induce physiological changes in plants. Simultaneous occurrence of both water and heavy metal stress may be deleterious to plant life.

Although the molecular mechanisms underlying individual abiotic stress tolerance have been studied enormously in the past decade, the morphological, physiological and molecular responses in plants exposed to a combination of simultaneous abiotic stresses remain subtle. These studies provide avenues for identification of crop plants that are able to combat multiple stresses and also development of

How to cite this article:

Swapna, B and Rama Gopal, G. (2015). Impact of combination of water stress and heavy metals on germination and seedling growth of two Pigeon Pea (*Cajanus Cajan* L.millspaugh) cultivars . Biolife, 3(4), pp 917-921. DOI :10.17812/blj.2015.3427

Published online: 24 December 2015

crop varieties which have simultaneous multiple stress tolerance.

Pigeon pea is one of the pulses which is a protein source in Indian diet. Though the responses to stress in plants are species specific, there is variability in cultivars response also. Hence an attempt has been made to study the germination and early seedling growth in two Pigeon pea cultivars under water stress and heavy metal stress, as well as their combination.

Material and Methods

Seeds of two Pigeon pea (*Cajanus cajan* L.Millspaugh) cv. LRG-41 and Yashoda-45 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 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.

Seed Germination Percentage (G %):

Percentage of seed germination (G %) was calculated according to AOSA, 1990. It was calculated by using the formula:

$G\% = 100 \times A / N$, where

A = Number of seeds found germinated

N = Total number of seeds used in the germination test.

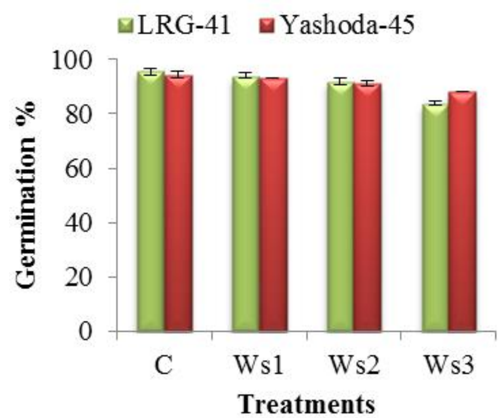
Growth Parameters:

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 & Discussions

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. None of the cultivars germinated at the highest level of stress i.e. -1.2MPa osmotic potential (Table 1). Both cultivars of pigeon pea studied, was tolerant for lower level of water stress at germination phase. But, Yashoda-45 cultivar was tolerant at Ws3 level (Figure-1).

Figure-1. Effect of Water stress on percentage of seed germination in two Pigeon pea cultivars



Our results confirmed the findings of Kaydan and Yagmur (2008) and Swapna and Rama Gopal (2014, 2015) who reported that low and moderate stress delayed germination, whereas the severe water stress reduced final germination percentages. The reduction in germination percentages might be due to decrease in the activity of hydrolyzing enzymes (Ravi Ranjan Kumar et al., 2011).

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 Pigeon pea cultivars. Heavy metals cadmium and chromium reduced the seed germination of both the cultivars studied and greater reduction was noticed at 400ppm concentration (Fig.2,3). Though reduction in germination occurred at all the levels of cadmium and chromium stress studied, significant differences were observed only above 100ppm.The inhibition of germination of seeds may be due to the accumulation of heavy metals in seeds.

Figure 4 revealed that mild and moderate water stresses with 100pmm cadmium /100ppm chromium reduced the germination percentage. No germination was found till 6th day under Ws3+Cd100 treatment in LRG-41. After that also, slight emergence of radicle was noticed which has not shown any further growth.

Figure-2. Effect of Cadmium on percentage of seed germination in two Pigeon pea cultivars

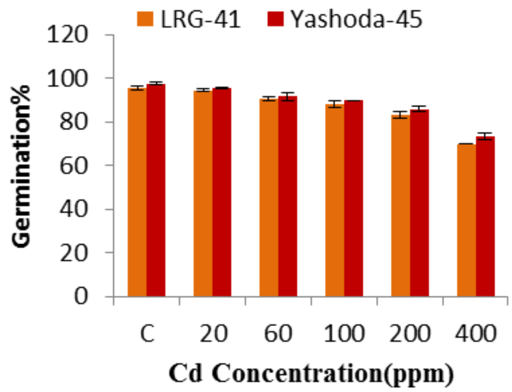


Table-1. Effect of Water stress on Root and shoot lengths, dry weight in Pigeon pea cultivars

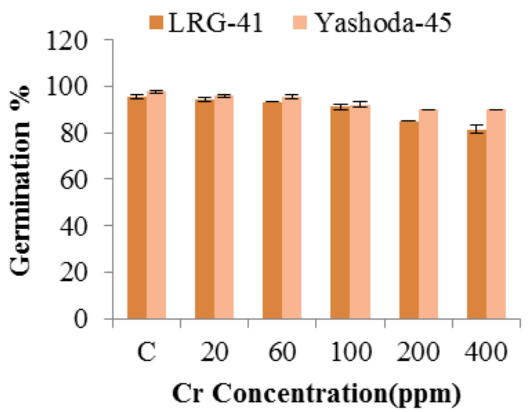
Cultivar		Root length (Cm)		Shoot length (Cm)		Dry weight (g/10seedlings)	
		LRG-41	Yashoda-45	LRG-41	Yashoda-45	LRG-41	Yashoda-45
Treatment	DAS	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
C	4	3.10 ± 0.06	3.37 ± 0.03	1.07 ± 0.07	1.53 ± 0.07	1.10 ± 0.003	1.16 ± 0.003
	6	6.43 ± 0.03	6.57 ± 0.07	5.00 ± 0.15	6.90 ± 0.23	1.15 ± 0.003	1.20 ± 0.000
	8	8.03 ± 0.03	8.17 ± 0.03	8.97 ± 0.12	9.93 ± 0.50	1.19 ± 0.003	1.25 ± 0.003
	10	8.03 ± 0.03	8.17 ± 0.03	11.07 ± 0.09	13.10 ± 0.46	1.21 ± 0.003	1.27 ± 0.003
Ws1	4	1.70 ± 0.06	1.80 ± 0.06	0.27 ± 0.03	0.37 ± 0.03	1.06 ± 0.003	1.10 ± 0.003
	6	4.47 ± 0.03	4.73 ± 0.07	3.80 ± 0.06	5.10 ± 0.06	1.11 ± 0.003	1.14 ± 0.017
	8	5.40 ± 0.00	5.77 ± 0.03	5.03 ± 0.03	6.80 ± 0.06	1.15 ± 0.003	1.21 ± 0.003
	10	5.63 ± 0.03	6.27 ± 0.03	6.87 ± 0.03	7.37 ± 0.09	1.17 ± 0.003	1.23 ± 0.003
Ws2	4	1.40 ± 0.06	1.63 ± 0.03	0.10 ± 0.00	0.23 ± 0.03	0.87 ± 0.003	0.91 ± 0.003
	6	3.23 ± 0.03	3.47 ± 0.03	3.47 ± 0.03	4.30 ± 0.06	0.91 ± 0.000	0.95 ± 0.003
	8	4.70 ± 0.06	5.00 ± 0.06	4.60 ± 0.06	5.43 ± 0.12	0.96 ± 0.003	1.01 ± 0.007
	10	4.70 ± 0.06	5.00 ± 0.06	5.80 ± 0.06	6.97 ± 0.12	1.01 ± 0.010	1.04 ± 0.003
Ws3	4	0.67 ± 0.03	0.90 ± 0.00	---	---	0.78 ± 0.003	0.82 ± 0.003
	6	2.20 ± 0.06	2.50 ± 0.06	---	----	0.81 ± 0.003	0.85 ± 0.003
	8	3.40 ± 0.06	3.80 ± 0.06	---	---	0.84 ± 0.003	0.89 ± 0.003
	10	4.17 ± 0.07	4.07 ± 0.03	---	----	0.88 ± 0.003	0.93 ± 0.003
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*	

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

			Root length (Cm)		Shoot length (Cm)		Dry weight (g/10seedlings)	
Cultivar			LRG-41	Yashoda-45	LRG-41	Yashoda-45	LRG-41	Yashoda-45
Treatment		DAS	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
WS + Cd	C	4	1.43 ± 0.03	2.07 ± 0.03	1.27 ± 0.033	1.53 ± 0.067	1.12 ± 0.006	1.19 ± 0.006
		6	3.73 ± 0.03	4.13 ± 0.03	5.00 ± 0.153	6.90 ± 0.231	1.18 ± 0.007	1.25 ± 0.006
		8	5.03 ± 0.03	6.13 ± 0.03	8.97 ± 0.120	9.93 ± 0.504	1.22 ± 0.010	1.28 ± 0.003
		10	5.57 ± 0.03	6.87 ± 0.03	11.07 ± 0.088	13.1 ± 0.462	1.24 ± 0.010	1.30 ± 0.003
	Ws2+20	4	0.53 ± 0.03	0.73 ± 0.03	---	---	1.00 ± 0.003	1.04 ± 0.003
		6	0.57 ± 0.03	0.93 ± 0.03	---	0.53 ± 0.033	1.06 ± 0.003	1.10 ± 0.003
		8	0.77 ± 0.03	1.27 ± 0.03	0.40 ± 0.000	1.27 ± 0.033	1.11 ± 0.003	1.15 ± 0.003
		10	0.80 ± 0.00	1.27 ± 0.03	0.60 ± 0.000	1.43 ± 0.033	1.13 ± 0.003	1.17 ± 0.003
	Ws2+100	4	0.23 ± 0.03	0.33 ± 0.03	---	---	0.96 ± 0.003	0.99 ± 0.003
		6	0.27 ± 0.03	0.37 ± 0.03	-	0.30 ± 0.000	1.02 ± 0.003	1.05 ± 0.003
		8	0.37 ± 0.03	0.53 ± 0.03	0.30 ± 0.000	0.50 ± 0.000	1.06 ± 0.003	1.09 ± 0.003
		10	0.40 ± 0.00	0.60 ± 0.00	0.40 ± 0.000	0.60 ± 0.000	1.08 ± 0.003	1.12 ± 0.007
	Ws3+20	4	0.23 ± 0.03	0.27 ± 0.03	---	---	0.91 ± 0.003	0.94 ± 0.003
		6	0.27 ± 0.03	0.30 ± 0.00	---	---	0.96 ± 0.003	1.00 ± 0.003
		8	0.33 ± 0.03	0.37 ± 0.03	---	---	1.00 ± 0.003	1.04 ± 0.003
		10	0.33 ± 0.03	0.37 ± 0.03	---	---	1.02 ± 0.003	1.06 ± 0.003
	Ws3+100	4	0.00 ± 0.00	0.10 ± 0.00	---	---	0.80 ± 0.003	0.84 ± 0.000
		6	0.00 ± 0.00	0.17 ± 0.03	---	---	0.84 ± 0.003	0.88 ± 0.003
		8	0.10 ± 0.00	0.20 ± 0.00	---	---	0.87 ± 0.003	0.91 ± 0.003
		10	0.10 ± 0.00	0.20 ± 0.00	---	---	0.89 ± 0.003	0.93 ± 0.003
WS + Cr	C	4	1.43 ± 0.03	2.07 ± 0.03	1.27 ± 0.033	1.53 ± 0.067	1.12 ± 0.006	1.19 ± 0.006
		6	3.73 ± 0.03	4.13 ± 0.03	5.00 ± 0.153	6.90 ± 0.231	1.18 ± 0.007	1.25 ± 0.006
		8	5.03 ± 0.03	6.13 ± 0.03	8.97 ± 0.120	9.93 ± 0.504	1.22 ± 0.010	1.28 ± 0.003
		10	5.57 ± 0.03	6.87 ± 0.03	11.07 ± 0.088	13.1 ± 0.462	1.24 ± 0.010	1.30 ± 0.003
	Ws2+20	4	0.57 ± 0.03	0.77 ± 0.03	0.57 ± 0.033	0.77 ± 0.033	1.02 ± 0.003	1.06 ± 0.000
		6	0.70 ± 0.06	0.90 ± 0.06	1.17 ± 0.033	1.47 ± 0.033	1.08 ± 0.003	1.13 ± 0.007
		8	0.90 ± 0.06	1.07 ± 0.07	2.47 ± 0.033	2.97 ± 0.033	1.12 ± 0.003	1.16 ± 0.003
		10	1.00 ± 0.06	1.07 ± 0.07	2.87 ± 0.033	3.17 ± 0.033	1.14 ± 0.003	1.18 ± 0.003
	Ws2+100	4	0.53 ± 0.03	0.67 ± 0.03	0.20 ± 0.000	0.30 ± 0.000	1.09 ± 0.003	1.11 ± 0.007
		6	0.67 ± 0.03	0.83 ± 0.07	0.40 ± 0.000	0.60 ± 0.000	1.16 ± 0.003	1.18 ± 0.006
		8	0.77 ± 0.03	0.83 ± 0.07	1.00 ± 0.000	1.60 ± 0.000	1.20 ± 0.003	1.22 ± 0.009
		10	0.77 ± 0.03	0.83 ± 0.07	1.17 ± 0.033	1.67 ± 0.033	1.22 ± 0.003	1.24 ± 0.009
	Ws3+20	4	0.27 ± 0.03	0.43 ± 0.03	---	---	0.97 ± 0.007	1.00 ± 0.003
		6	0.37 ± 0.03	0.53 ± 0.03	---	0.50 ± 0.000	1.02 ± 0.007	1.05 ± 0.003
		8	0.47 ± 0.03	0.67 ± 0.03	0.30 ± 0.000	0.80 ± 0.000	1.06 ± 0.007	1.09 ± 0.003
		10	0.47 ± 0.03	0.67 ± 0.03	0.50 ± 0.000	0.90 ± 0.000	1.08 ± 0.007	1.11 ± 0.003
	Ws3+100	4	0.13 ± 0.03	0.23 ± 0.03	---	---	0.84 ± 0.003	0.87 ± 0.007
		6	0.23 ± 0.03	0.33 ± 0.03	---	0.40 ± 0.000	0.89 ± 0.003	0.92 ± 0.007
		8	0.23 ± 0.03	0.33 ± 0.03	0.20 ± 0.000	0.60 ± 0.000	0.93 ± 0.003	0.96 ± 0.007
		10	0.23 ± 0.03	0.33 ± 0.03	0.30 ± 0.000	0.67 ± 0.033	0.95 ± 0.003	0.98 ± 0.007

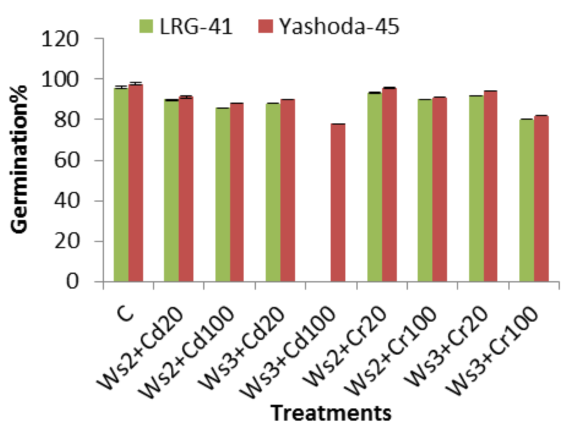
Cultivar LRG-41 of pigeon pea was found to be sensitive with Ws+Cd and Ws+Cr. depicting the severe effect of combination of stress on early seedling growth.

Figure-3. Impact of Chromium on percentage of seed germination in two Pigeon pea cultivars



In both Pigeon pea cultivars, root and shoot length; dry weight was decreased significantly with a rise in PEG, heavy metal levels and their combinations (Table 2).The present observations are in agreement with the report of de silva et al., 2012.

Figure-4. Effect of Water stress + Cd and Water stress + Cr on percentage of seed germination in two Pigeon pea cultivars



Conclusion

The observed germination and early seedling growth responses to metal stress were mostly identical to the response to water stress. The effects were less than additive with simultaneous exposure to both stresses resulting in more reduction of plants compared with exposure to each of the stresses alone. Ws+Cd triggered more stress.

Conflict of Interests:

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. AOSA (1990). Rules for Testing Seeds, USA. J Seed Technol 12:1-112.
2. Deepti Singh, Michael Tsiang, Bala Rajaratnam and Noah S. Diffenbaugh. 2014. Observed changes in extreme wet and dry spells during the South Asian summer monsoon season. Nature Climate Change, **4**,456–461.
3. De Silva N.D.G, Ewa Cholewa and Peter Ryser. 2012. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple (*Acer rubrum* L.) Journal of Experimental Botany. 2-10 .
4. Giovanni DalCorso, Silvia Farinati and Antonella Furini. 2010. Regulatory networks of cadmium stress in plants. Plant Signaling & Behavior 5:6, 663-667.
5. Kaydan, D. and Yagmur, M. 2008. Germination, Seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl. Afr.J. Biotechnol. 7: 2862-2868.
6. Michel, B.E. and Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology 51, 914-916.
7. Nafiseh Nematshahi, Mehrdad Lahouti and Ali Ganjeali. Accumulation of chromium and its effect on growth of (*Allium cepa cv. Hybrid)*. 2012. European Journal of Experimental Biology, 2 (4):969-974.
8. Ravi Ranjan Kumar, Krishna Karjol and G. R. Naik Variation of sensitivity to drought stress in pigeon pea (*cajanus cajan* [I.]Millsp) cultivars during seed germination and early seedling growth. 2011. World Journal of Science and Technology. 1(1): 11-18.
9. Swapna B and RamaGopal G. Interactive effects between water stress and heavy metals on seed germination and seedling growth of two green gram (*Vigna radiata* L.wilzec) cultivars. 2014. Biolife. 2(1):291-296.
10. Swapna, B and Rama Gopal, G (2015). Germination and early growth responses of Vigna mungo L. Hepper cultivars to water stress and heavy metals. The Ame J Sci & Med Res, 1(2):169-174. doi:10.17812/ajsmr123.

****