SHORT COMMUNICATION

Rapid Screening of Wheat Cultivars Suitable for Cultivation in Cd Contaminated Soils under Mountain Ecosystem

Shakuntala Khatri[1], Rajesh Kumar Sharma[1, 3*], Vijay Shridhar[2]

DOI: 10.18811/ijpen.v5i02.10

Abstract

Four wheat cultivars (*Triticum aestivum* L. cv. HPW-184, HPW-236, HPW-155, VL-829) have been evaluated concerning plant growth parameters under the influence of varying Cd concentrations (0-20 mg Cd L^{-1}) to identify the potential Cd tolerant cultivar. Early response of test cultivars evaluated in terms of mean germination rate, lengths, and biomass of plants was found to decrease significantly with the increasing Cd concentration ($p \leq 0.05$). Results of 2-way ANOVA test further showed that Cd concentrations, cultivars, and their interactions affected these parameters significantly except the number of leaves. The tolerance to excess Cd among the four wheat cultivars was found to be in order HPW-236 > HPW-184 > HPW-155 > VL-829. The present study suggests the suitability of *T. aestivum* cv. HPW-236 for cultivation in a Cd polluted soil under mountain ecosystem. However, the Cd accumulation potential of these cultivars exposed to the Cd contaminated soil seems a future research topic.

Keywords: Cadmium, Mountain ecosystem, Plant growth, Seed germination, *Triticum aestivum*.

International Journal of Plant and Environment (2019)

Introduction

Soil, often contaminated with heavy metals resulting from the frequent use of pesticides, phosphate fertilizers, sewage sludge, wastewater, etc., poses a risk to both the environment and human health. Consumption of heavy metal contaminated foods leads to major health risks in human beings due to their persistent nature and high accumulation potential in soft organs such as kidney, liver, spleen, etc. Heavy metals, cadmium (Cd) in particular, has high mobility in the soil-plant ecosystem and gets accumulated easily into vegetables, cereals, fruits, etc., and thus in the food chain. Cd belongs to toxic and non-essential heavy metals which can cause adverse effects on the plants such as lady's finger (*Abelmoschus esculentus* L.), carrot (*Dacus carota* L), palak (*Beta vulgaris* L.), alfalfa (*Medicago sativa* L.) plants even at low concentrations (Lehoczky *et al.,* 1996, 1998; Peralta *et al.,* 2008; Sharma *et al.,* 2010, 2014). Heavy metal stress as a major adverse factor can lower the crop productivity in contaminated fields through reducing water uptake and causing nutrient disorders and ion toxicity in the plant system. Suppression in growth and yield of crops due to heavy metal content in soil is well documented by many researchers both at National and International levels (Schutzendubel and Polle, 2002; Singh *et al.,* 2008; Sharma *et al.,* 2010; Pena *et al.,* 2015).

In Indian Himalayan Region, climate change has shown its prominent effects on different ecosystems in terms of loss of biodiversity, water scarcity, heavy rainfall and nutrient loss (Maikhuri *et al.,* 2001; Samant *et al.,* 2013; Sharma and Sharma, 2015). Local farmers use heavy doses of fertilizers to meet out the nutrient demand of plants leading to Cd contamination of soil. Wheat (*Triticum aestivum* L.) is one of the major food crops grown in northern India over 26.4 million hectare land including high altitudes of Indian Himalaya. A preliminary study carried out in Kullu Valley of Himachal Pradesh, India showed that Cd concentration in soil exceeded the safe limit set by Prevention of Food and Adulteration act 1954, whereas edible parts of radish and cauliflower had exceeded the safe limit set by the European Union (unpublished records). Therefore, the present study aims to assess the influence of Cd exposure on germination and growth performance of wheat cultivars commonly grown in different parts of Himachal Pradesh to screen the suitable cultivar for the region.

[1]G. B. Pant National Institute of Himalayan Environment and Sustainable Development, Himachal Regional Center, Mohal-Kullu (H.P.)-175126, India.

[2]School of Environment and Natural Resources, Doon University, Dehradun (U.K)-246001, India.

[3]Department of Botany, Banaras Hindu University, Varanasi (U.P.)-221005, India.

Corresponding Author: Dr. Rajesh Kumar Sharma, G. B. Pant National Institute of Himalayan Environment and Sustainable Development, Himachal Regional Center, Mohal- Kullu (H.P.)-175126, India, Phone: +91-9418916445, Email: rajeshbhu78@gmail.com

How to cite this article: Khatri, S., Sharma, R.K. and Shridhar, V. (2019). Rapid Screening of Wheat Cultivars Suitable for Cultivation in Cd Contaminated Soils under Mountain Ecosystem. International Journal of Plant and Environment, 5(2): 129-132.

Source of support: Nil

Conflict of interest: None

Submitted: 20/04/2018 **Accepted:** 30/04/2019 **Published:** 30/04/2019

Seeds of four cultivars of wheat (*T. aestivum* L. cv. HPW-184, HPW-236, HPW-155, VL-829), commonly grown in Kullu Valley during the winter season, were collected from Hill Agriculture Research and Extension Centre of Chaudhary Srawan Kumar Himachal Pradesh Krishi Vishwavidyalaya, Bajaura, Himachal Pradesh. Healthy seeds of wheat cultivars were selected and exposed to Cd concentrations @ of 0, 5, 10 and 20 mg Cd L^{-1} for 6 h. Ten treated healthy seeds of each cultivar were placed on wet blotting paper in 90 mm petri dishes. Petri dishes were kept in the laboratory at room temperature (25-28°C) during September 2014. Each treatment was repeated thrice to calculate the mean and standard error.

Influence of Cd exposure on test cultivars was analyzed in terms of mean seed germination rate (MGR) at 5th day and the plant growth parameters (number of leaves and roots, length and biomass of roots, shoots and total plant length) were analyzed at 15th day of the experiment. Percent MGR and plant response to stress (PRS) for various test parameters were calculated according to formulae given by Yu *et al.* (2006) with modifications using following equations I and II, respectively.

$$MGR\,(d^{-1}) = \frac{[Gf - Gi]}{[Tf - Ti]} \quad (1)$$

$$PRS(\%) = \frac{[Ph - Pl] \times 100}{[Pl]} \qquad (2)$$

Where Gf and Gi are percent seed germination at 0 (Ti) and 5th day (Tf) after the experiment and Ph and Pl are the response of test plant at high (20 mg Cd L^{-1}) and low Cd (control i.e. 0 mg Cd L^{-1}) exposure under laboratory conditions at the 15th day of incubation experiment. All the statistical analyses such as mean, two-way ANOVA, etc., were performed using the standard version of SPSS software. Treatment means were separated from each other using Duncan's Multiple Range Test and differences were considered significant at p <0.05.

The data corresponding to mean germination rate, and growth performance and biomass accumulation of four cultivars of wheat crops to corresponding doses of Cd, obtained in the present study, are presented in Fig. 1 and Table 1, respectively. Mean germination rate decreased significantly (p≤0.05) with increasing Cd concentrations (Fig. 1). The maximum reduction in mean germination rate was recorded in cv. VL-289 at 20 mg Cd L^{-1} concentration. The two-way ANOVA test conducted to assess the effects of different test variables such as treatments, cultivars, and treatments × cultivars showed their significant effects on MGR of test cultivars of *T. aestivum* (Fig. 1). Similar trends on the influence of increasing Cd concentration on many roots, number of leaves, root, shoot and total plant length and biomass of four test cultivars

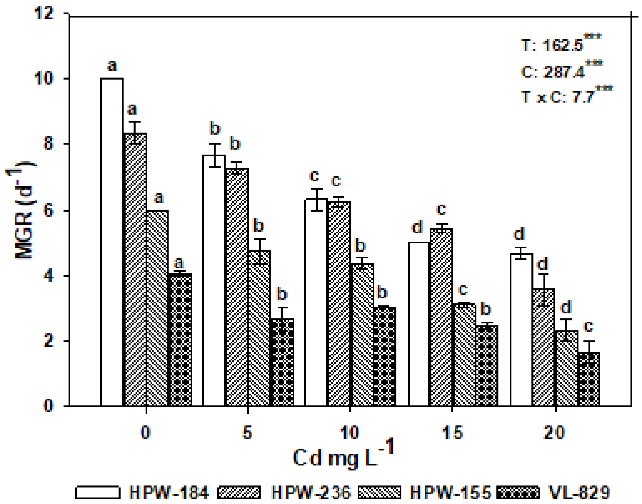

Fig. 1: Mean germination rate (MGR) of four cultivars of *T. aestivum* exposed to Cd concentrations. Bars are mean ± SE of three replicates. Bars with different letters in each cultivar are significantly different from each other at p<0.05 (Duncan's Multiple Range Test). T: Treatments, C: Cultivars, Level of significance: ***p < 0.001.

Table 1: Early growth response and biomass accumulation in roots and shoots of four cultivars of wheat (*T. aestivum*) under cadmium stress.

		Growth					*Biomass accumulation (g plant^{-1})*		
		Number (plant^{-1})		*Plant height (cm plant^{-1})*					
Cultivars (C)	*Treatment (T) (mg Cd L^{-1})*	*Root*	*Leave*	*Root*	*Shoot*	*Total*	*Root*	*Shoot*	*Total*
HPW-184	0	5.80^{a}	1.80^{a}	18.95^{a}	16.55^{a}	35.50^{a}	0.15^{a}	0.25^{a}	0.39^{a}
	5	5.70^{b}	1.70^{ab}	15.38^{b}	13.78^{b}	29.16^{b}	0.12^{b}	0.19^{b}	0.32^{b}
	10	5.40^{ab}	1.40^{ab}	14.68^{b}	12.86^{c}	27.54^{c}	0.10^{c}	0.17^{c}	0.27^{c}
	15	5.30^{ab}	1.20^{ab}	12.79^{c}	10.49^{d}	23.28^{d}	0.08^{d}	0.13^{d}	0.22^{d}
	20	5.10^{b}	1.00^{b}	8.98^{d}	9.62^{e}	18.60^{e}	0.06^{e}	0.11^{e}	0.17^{e}
HPW-236	0	6.90^{a}	2.80^{a}	25.63^{a}	18.65^{a}	44.28^{a}	0.32^{a}	0.37^{a}	0.69^{a}
	5	6.70^{b}	2.50^{a}	25.02^{b}	16.56^{b}	41.58^{b}	0.29^{b}	0.35^{b}	0.64^{b}
	10	6.60^{b}	2.30^{a}	22.06^{c}	16.32^{c}	38.38^{c}	0.24^{c}	0.30^{c}	0.54^{c}
	15	6.40^{b}	2.00^{a}	20.36^{d}	15.26^{d}	35.62^{d}	0.18^{d}	0.24^{d}	0.42^{d}
	20	5.80^{b}	2.60^{a}	18.98^{e}	13.23^{e}	32.21^{e}	0.16^{d}	0.12^{e}	0.28^{e}
HPW-155	0	5.80^{a}	2.00^{ab}	17.45^{a}	10.68^{a}	28.13^{a}	0.09^{b}	0.12^{a}	0.21^{a}
	5	5.40^{b}	1.80^{b}	14.55^{b}	8.53^{b}	23.08^{b}	0.08^{a}	0.10^{b}	0.17^{a}
	10	4.70^{b}	1.60^{a}	11.70^{c}	7.64^{c}	19.34^{c}	0.05^{b}	0.08^{c}	0.13^{b}
	15	4.60^{a}	1.40^{ab}	10.29^{d}	6.04^{d}	16.33^{d}	0.04^{bc}	0.07^{d}	0.10^{c}
	20	4.30^{b}	1.00^{b}	7.38^{e}	4.80^{e}	12.18^{e}	0.02^{c}	0.05^{e}	0.07^{d}
VL-829	0	5.70^{a}	1.60^{a}	16.22^{a}	11.98^{a}	28.20^{a}	0.08^{a}	0.13^{a}	0.21^{a}
	5	5.50^{a}	1.20^{a}	14.95^{b}	10.60^{b}	25.55^{b}	0.06^{ab}	0.10^{b}	0.16^{b}
	10	5.30^{a}	1.10^{a}	11.00^{c}	8.98^{c}	19.98^{c}	0.06^{a}	0.08^{c}	0.15^{b}
	15	5.00^{a}	1.00^{ab}	9.40^{d}	7.40^{d}	16.80^{d}	0.03^{bc}	0.06^{d}	0.08^{c}
	20	4.80^{b}	1.00^{b}	5.60^{e}	4.80^{e}	10.40^{e}	0.01^{c}	0.05^{e}	0.06^{c}
Results of two-way ANOVA test									
	T	17.9***	2.3*	440.2***	436.1***	850.5***	48.0***	1.4***	317.3***
	C	27.71***	1.0^{NS}	85.8***	542.4***	406.8***	73.3***	3.2***	567.4***
	T × C	2.9**	1.9*	3.8***	3.8***	5.8***	4.1***	38.3***	10.3***

Values are mean of three replicates.
Values followed by different letters are significantly different from each other at p<0.05 (Duncan's Multiple Range test).
Level of significance: * *p* <0.05; ** *p* <0.01; ***p < 0.001; NS: not significant. Two-way ANOVA for different growth parameters of wheat cultivars exposed to Cd stress.

of *T. aestivum* were also recorded at p≤0.05 (Table 1). The two-way ANOVA test conducted to assess the effects of different test variables such as treatments, cultivars, and treatment × cultivars showed their significant effects on test cultivars of *T. aestivum,* whereas cultivar did not show significant effects on many leaves (Table 1).

The adverse effect of Cd treatments on four cultivars of *T. aestivum* evaluated as plant response to Cd stress (%) ranged between 53.3 to 61.2, 12.1 to 25.9, 15.8 to 27.8, 49.2 to 65.5, 38.8 to 59.9, 44.6 to 63.1, 50 to 85.7, 50 to 69.2, 53,1 to 71.4 for MGR, number of roots, number of leaves; root, shoot and total plant length and biomass, respectively with median value of 49.2 to 63.1 (Fig. 2). Plant response to Cd stress (PRS) index shows that all higher Cd doses had greater reductions in the early response of all the test cultivars as compared to control (Fig. 2).

Reduction in biomass of the spinach plants treated with 10, 20, and 40 mg Cd kg^{-1} (0-40 mg Cd kg^{-1} soil) has been reported by Dube *et al.* (2002) and in carrot plants due to the treatment of Cd and Zn, alone and in combination by Sharma and Agrawal (2006). Reduced biomass in plants due to heavy metal accumulation may be ascribed to a reduction in protein synthesis during germination, interference with the uptake of macro and micronutrients or different steps of the Calvin cycle and inhibition of photosynthetic CO_2 fixation (Jackson *et al.,* 1990; Moreno-Casellas *et al.,* 2002). Reduction in root, stem, and leaf dry weights in cucumber plants treated with Cd ranging between 10-20 mg Cd L^{-1} has been ascribed to the inhibitory effect of heavy metals on cell division, cell elongation, and enzyme activity (Balsber, 1989). Growth inhibition due to metal toxicity is also found to reduce biomass accumulation (Chaoui *et al.,* 1997; Quariti *et al.,* 1997; Quazounidou *et al.,* 1997). Excessive Cd accumulation in soil resulted into reduction in root growth causing reduced mineral absorption, altered carbohydrate metabolism leading to reduced dry matter production in Indian palak (Sharma *et al.,* 2007, 2014) and rice (Moya *et al.,* 1993). Cd inhibits the growth and productivity of crops by altering chloroplast structure and disrupting the photosynthesis processes and increasing reactive oxygen species (ROS) such as O_2^{-1}, Oh and H_2O_2 (Romero-Puertas, 2004). Excess ROS results in lipid peroxidation, protein degradation and nucleotide (DNA and RNA) damage (Romero-Puertas, 2007). Cd applied @ 10 and 40 mg Cd L^{-1} concentrations reduced the seed germination in alfalfa plants significantly (p<0.01). Similar results were also reported by Claire *et al.* (1991) using Cd and other

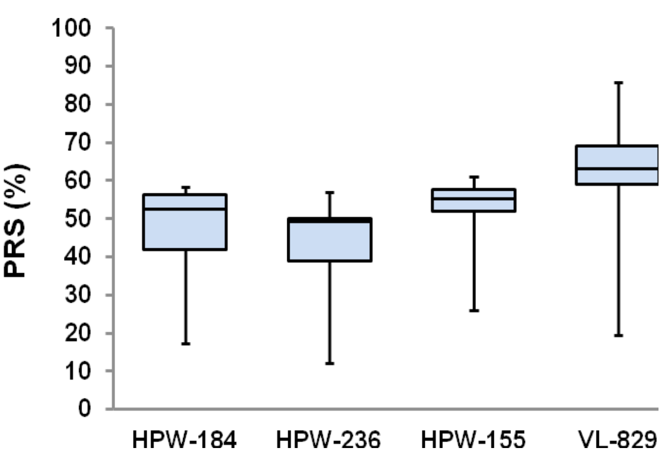

Fig. 2: Plant response to Cd stress (PRS %) for four cultivars of *T. aestivum* plants exposed to different concentrations of Cd varying between 0-20 mg Cd L^{-1}. Total numbers of test parameters are 9.

heavy metals on cabbage (*Brassica oleracea* L. var. *capitata*), lettuce (*Lactuca sativa* L.), millets (*Panicum miliaceum* L.), radish (*Raphanus sativum* L.), turnip (*Brassica rapa* L.) and wheat (*T. aestivum*).

Germination of seeds decreased significantly when exposed to Cu, Ni, Pb, and Cd at 20, 40 and 50 mg L^{-1} (Chhotu *et al.,* 2008). The metal Cd at 5 and 10 mg Cd L^{-1} had very low toxicity on seeds. Plant growth was adversely affected by heavy metal at the higher concentration of 40 and 50 mg L^{-1}, while lower concentrations (5 to 20 mg L^{-1}) stimulated shoot growth and consequently increased plant biomass (Chhotu *et al.,* 2008). Fabio *et al.* (2002) reported that maize grown in the presence of 1 mg Cd L^{-1} showed significant growth reduction of both roots and shoots. It has also been reported by Thakur and Tiwari (2012) that Cd varying between 0-20 mg Cd L^{-1} had less adverse effects on *Helianthus annuus* plants. Higher Cd (40 and 50 mg Cd L^{-1}) concentration delayed seed germination, resulted in stunted growth, highly effect on root and shoot growth Thakur and Tiwari (2012). Many researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metal or other toxic compounds (Banuelos *et al.,* 1997; Blaylock and Huang, 2000). According to Aydinalp and Marinova (2009), the seed germination of the alfalfa plants is seriously affected by 20 mg Cd L^{-1}.

The previous study showed that Cd alters the physiological and transcriptional levels of NO_3 uptake from the external solution (Rizzardo *et al.,* 2012). Astolfia *et al.* (2014) reported that long-term exposure to 2.3 mg Cd L^{-1} reduced shoot dry matter and also concentrated photosynthesis rate related to a decrease in chlorophyll content. Cd, even at low concentrations, influenced many important physiological processes, either directly or indirectly. The previous study also revealed that Cd may inhibit chlorophyll synthesis, may decrease the activity of enzymes and caused oxidative stress, however, results in deprived growth and also decrease shoot biomass (Astolfi *et al.,* 2012). According to Astolfi *et al.* (2012), Cd is an additional aspect causing a higher plant requirement for reduced S in the environment, due to the increased production of sulfur-containing metabolites, such as glutathione (GSH) and its derivatives such as phytochelatins (PCs), required for Cd detoxification. The accumulation of some trace metals such as Ca, Cu, Fe, Mn and Zn reduced in plants under Cd exposure (Clemens *et al.,* 2002; Astolfi *et al.,* 2012), whereas an increase was observed in sulfur accumulation (Lux *et al.,* 2011; Astolfi *et al.,* 2012). Several mechanisms involving metal detoxification and sequestration have been evolved by plants to counteract Cd toxicity. Several studies have shown that many transporters are involved in Cd uptake and transport and many genes are required for the metal balance via sequestration and chelations (Vert *et al*., 2009).

The present study clearly showed that Cd concentrations ranging between 0-20 mg Cd L^{-1} have significant and negative effects on four test cultivars of wheat (*T. aestivum* L), commonly grown in different regions of Himachal Pradesh during the winter season. The sensitivity of wheat cultivars to Cd excess was found in decreasing order as VL-829 > HPW-155 > HPW-184 > HPW-236 (Fig. 2). *T. aestivum* cv. HPW-236 was found to possess the tolerant to Cd exposure and, thus, can be recommended for cultivation on Cd polluted soil under the mountain ecosystem. However, Cd accumulation potential of these cultivars exposed to a Cd contaminated soil is a topic of future research.

Acknowledgments

Director, G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora (Uttrakhand) and

Ministry of Environment, Forest and Climate Change, Govt. of India, New Delhi are gratefully acknowledged for providing necessary facilities and financial support to carry out the present research.

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