AMELIORATION OF TOXIC EFFECTS OF LEAD (Pb) AND CHROMIUM (Cr) IN FOUR BLACK GRAM (*Vigna mungo* L.) HEPPER] CULTIVARS WITH THE APPLICATION OF KINETIN

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To examine the effect of kinetin (Kin) in alleviating the toxic effects of lead (Pb) and chromium (Cr) on four black gram cultivars this study was conducted in earthen pots lined with polyethylene bags and filled in with sterilized sand, at the University of Agriculture, Faisalabad, Pakistan during the spring season 2004. Thirty days after germination we imposed six treatments viz. control (T_0), Pb @ 20 mg kg^{-1} (T_1), Cr @ 30 mg kg^{-1} (T_2), Kin @ 100 µg/g (T_3), Pb @ 20 mg kg^{-1} & Kin &100 µg/g (T_4), Cr @ 30 mg kg^{-1} & Kin @100 µg/g (T_5). We observed significant reduction in the stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index of black gram plants treated with Pb and Cr singly. The joint application of Kin and heavy metals (Pb and Cr) showed significant improvement in all the parameters under investigation but it could not fully ameliorate the toxic effects of heavy metals. As regards the heavy metal ion uptake by different plant organs, plants treated with Pb and Cr alone showed higher sequestering of both metals in their roots. However, plants receiving joint application of Kin and heavy metals (Pb and Cr) showed higher translocation of both heavy metals towards their shoot. Cr particularly showed more translocation towards roots than Pb. Despite both heavy metals significantly reduced all the parameters, Pb application either alone or in combination with Kin proved more toxic. All the parameters showed highly significant negative correlation with Pb content in both roots and shoot. The Cr content in the roots of black gram plants also showed strong negative correlation with all these parameters. Among the black gram cultivars Mash ES1 (V_4) proved comparatively more sensitive, Mash 80 (V_1) showed more resistance, while Mash 88 (V_2) and Mash 97 (V_3) exhibited optimum sensitivity to heavy metal treatments. This study has great implication for the selection of black gram cultivars suitable for cultivation in the riverine areas of Pakistan and resembling agro-ecosystems in the world threatened with the drainage of untreated industrial effluents.
Keywords: Effective concentration, industrial effluents, nutrients, toxicity, transpiration.
Abbreviations: EC_{10} – Effective concentration (10% yield losses), Cr – Chromium, Pb – Lead; Kin – Kinetin.

INTRODUCTION

The enormous amount of pollutants released by current human activities including pesticides, noxious gasses and heavy metals, has endangered the healthy existence of biota worldwide. Among these pollutants, heavy metals especially have high potential to modify some metabolic pathways in plants (Assche and Clijester 1990), and reduce transpiration rate, as well as, nutritional uptake (Veselov *et al.* 2003) that determine severe stress on plant vigor and substantially reduce their yield (Pederson *et al.* 2002).

In many cities of Pakistan, effluents released by textile, fertilizers, tanneries, cement, vegetable oils, paints, oil refineries, soap, sugar and smelters industries having high concentration of toxic metals are being disposed into open urban drains (Chaudry *et al.* 1998). Afterwards they are commonly used either for growing vegetable crops in the urban areas or drained in to fresh water bodies (rivers) without receiving any treatment and ultimately become incorporated into Arabian sea, an international water body. Hence the uptake of these toxic heavy metals by aquatic flora and fauna including migratory birds make it a problem of international concern.

Currently these open drains are causing serious respiratory disorders, skin infection, diarrhea, dysentery and typhoid to the inhabitants residing in the vicinity of these drains (Ahmad *et al.* 1994). Additionally, they are contaminating the productive arable lands and the damage caused to the cultivated crops and natural plant communities has not been yet determined.

Chromium is commonly used in textile industry as a moderant. Its presence in agricultural soils is attributed to the use of organic wastes as fertilizer and the use of waste water for irrigation (Baxter 1983). Its higher concentration proves very toxic for human health. The toxic effects of Cr (VI) especially result in the destruction of cellular components in plants Organic matter, pH of sediments, and microorganisms, however can convert Cr (VI) to Cr (III), and decrease its mobility (Adeniji 2004). Lead, another heavy metal, released by automobiles and produced from industrial activities, can be found in all parts of the environment. Its higher concentrations also can cause damage to the brain, kidney, and anemia in both adults and children. It is comparatively less mobile in plants but drastically reduces many metabolic processes in plants (Vassil *et al.* 1998, Sayed 1999).

Black gram [*Vigna mungo* (L.) Hepper] an important pulse crop is cultivated over 45.7 thousand hectares in Pakistan (Anonymous 2001, Anonymous. 2001). It being a leguminous crop, demands less nitrogenous fertilizers and fits well in different crop rotation systems to maintain the fertility level of the soil (Nazir 1994). But the riverine areas of Pakistan suitable for its cultivation are prone to higher concentrations of toxic metals through the drainage of industrial effluents.

Heavy metals have been reported to reduce the contents of cytokinin probably as a result of hormone breakdown or by enhancing the activity of cytokinin oxidase (Kaminek *et al.* 1997, Kaminek M.; Motika 1997). Hence exogenous application of kinetin to alleviate the deleterious effects of heavy metal toxicity in plants is recently gaining importance (Hare *et al.* 1997, Brault and Maldiney 1999, Gadallah and El-Enany 1999).

This study was conducted to examine the efficacy of kinetin in ameliorating the toxic effects of lead and chromium on some morpho-physiological parameters of four black gram [*Vigna mungo* (L.) Hepper] cultivars commonly cultivated in the riverine areas of Pakistan currently receiving high concentrations of heavy metals through industrial effluent drainage.

MATERIALS AND METHODS

We conducted this study in earthen pots lined with polyethylene bags and filled in with eight kg sterilized sand, in a wire gauzed net-house in the Botanic Garden, University of Agriculture, Faisalabad, Pakistan, located at 73^0East longitude and 31^0North latitude, at an altitude of 135 meter from sea level, where the climate is semi arid and subtropical. Seeds of four black gram cultivars viz Mash 80 (V_1), Mash 88 (V_2), Mash 97 (V_3) and Mash ES-1 (V_4) used in this study were obtained from the Pulses Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. We sterilized the seeds with 10% v/v hydrogen peroxide (Vassilev *et al.* 2002) before sowing and by making 2cm deep holes with the help of a wooden stick (1.5 cm diameter) sowed eight seeds in each pot. Afterwards, each seed was covered with a small amount of soil for proper supplement of germination factors. By thinning we maintained only five seedlings in each pot in order to avoid imbalanced uptake of nutrients by plants. The experiment comprised six replicates and was laid out in a completely randomized design.

Thirty days after germination we treated black gram plants with following six treatments: control (T_0), Pb @ 20 mg kg^{-1} (T_1), Cr @ 30 mg kg^{-1} (T_2), Kin @ 100 µg/g (T_3), Pb @ 20 mg kg^{-1} & Kin &100 µg/g (T_4), Cr @ 30 mg kg^{-1} & Kin @100 µg/g (T_5). For both heavy metals we applied chloride salts i. e. $PbCl_2$l and $CrCl_3$ (Sigma Aldrich, Japan) in liquid form mixed with distilled water following the method used by (Stoeva and Bineva 2003). The concentrations of heavy metals based on the background metal content in the industrial effluents (Chaudry *et al.* 1998) being drained in to water bodies, EC_{10} (effective concentration for 10 % yield loss) determined through a screening trial, and on concomitant literature. At the same time, a well calibrated amount of Kinetin (Kin), 6-Furfuryl-aminopurine ($C_{10}H_9N_5O$) a product of Sigma Aldrich, Japan was applied to the respective plants through a knapsack sprayer keeping in view its half life, prevailing temperature and other environmental factors which could reduce its efficacy.

An open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company Ltd, Hoddeson, England) equipped with a PLCB-4 chamber was used for measuring the assimilation rate, stomatal conductance, and transpiration rate of the youngest fully developed intact leaves when crop was 45 days old (15 days after heavy metal application and Kinetin spray). Measurements were made from 8.00 a. m. to 9.30 a. m. with chamber specification i. e. leaf area $6.25cm^2$, ambient CO_2 concentration (Cref) 290.1µmole $mole^{-1}$, leaf chamber temperature (Tch) varied from 25 to 27.8 ºC, leaf chamber gas flow rate (V) 394 mL min^{-1}, ambient pressure (P) 98.9k Pa, water vapor pressure (e ref) into chamber ranged from 4.4 to 6.6 m bar, molar flow of air per unit leaf area (Us) 410.6 mol m^{-2} S^{-1} and PAR (Q leaf) at leaf surface was 1948 µmol m^{-2} S^{-1}.

Leaf area (cm^2) per plant was taken with a Li-Cor leaf area meter (model 3000, Li-Cor Inc Lincoln NE). The total leaf area/plant was determined and average was calculated. After drying four whole plants and their seeds to constant weight, dry biomass per plant (g) and seed weight per plant (g) war determined and harvest index (%) was calculated following (Morrison *et al.* 1999).

To determine the Pb and Cr content of treated plants we analyzed the digested samples under manufacturer's recommended standard conditions on a Perkin Elmer 3100 EDS Atomic Absorption/Emission Spectrophotometer, using a fuel rich air-acetylene flame; 10 cm burner head, 357.9 nm wavelength, 0.7 nm slit and 20mA lamp current. Some digests were diluted in order to fall into the linear calibration range of 0 to 5 mgL^{-1} (Yoshida *et al.* 1976).

All the parameters were computed using SPSS-12 computer package (SPSS Inc., Wacker Drive, Chicago, IL). Duncan's New Multiple Range test at 5% level of probability (Duncan 1955) was used to compare means. Using same statistical package, bivariate correlation was performed to check the relationship among different parameters.

RESULTS

Heavy metal ion uptake

Heavy metal ion uptake by the roots and shoots of black gram plants showed highly significant differences among the treatment means (Table 1). Plants treated with Pb and Cr alone showed significantly higher metal ion uptake by their roots as compared to those treated with both heavy metals (Pb & Cr) along with kinetin. However, the Pb and Cr content in the roots of black gram plants exhibited a different pattern. Plants treated with Pb and Cr in combination with kinetin caused significant increase in metal content in their shoots. Moreover, Pb showed ~50% translocation from the roots to shoots while Cr showed almost its two times more translocation.

A comparison among the black gram cultivars indicates maximum Pb content in the roots of Mash ES1 (V_4). In Mash 97 (V_3) as well, the Pb content in roots was significantly higher than that recorded for Mash 80 (V_1) and Mash 88 (V_2). However it differed non-significantly between Mash 80 (V_1) and Mash 88 (V_2). The Pb content in the shoots of black gram plants followed exactly the same trend as that examined for root Pb content.

Table 1a. Analysis of variance for heavy metal (Pb and Cr) ion uptake by root and shoot of black gram plants treated with Pb, Cr and kinetin alone or in combination (*P*< 0.05).

Source	Sum of squares	df	Mean square	F ratio	Significance.
Pb (Root)					
Treatments	1064.216	5	212.843	356.582	.000
Varities	8.997	3	2.999	5.024	.002
Treat * Var	22.813	15	1.521	2.548	.002
Pb (Shoot)					
Treatments	194.936	5	38.987	246.410	.000
Varities	4.345	3	1.448	9.154	.000
Treat * Var	9.074	15	.605	3.824	.000
Cr (Root)					
Treatments	1464.067	5	292.813	675.255	.000
Varities	19.933	3	6.644	15.322	.000
Treat * Var	44.273	15	2.952	6.806	.000
Cr (Shoot)					
Treatments	940.187	5	188.037	585.520	.000
Varities	5.134	3	1.711	5.329	.002
Treat * Var	11.558	15	.771	2.399	.004

Table 1b. Treatment means for Pb and Cr uptake by roots and shoots of heavy metal treated black gram plants treated with control (T_0), Pb @ 20 mg kg^{-1} (T_1), Kin @ 100 µg g^{-1} (T_3), Pb @ 20mg kg^{-1} & Kin @ 100 µg g^{-1} (T_4), and Cr 30 mg kg^{-1} & Kinetin @ 100 µg g^{-1} (T_5) (*P*< 0.05).

Heavy metal	Plant organ	Treatments			
		T_1 (Pb only)	T_4 (Pb & Kin)	T_2 (Cr only)	T_5 (Cr & Kin)
Pb	Root	5.48 b	4.42 a	-	-
	Shoot	1.93 a	2.32 b	-	-
Cr	Root	-	-	7.07 b	4.05 a
	Shoot	-	-	4.40 a	4.97 b

Treatment means sharing the same letter differ non-significantly.

Table 1c. Heavy metal uptake by black bean cultivars treated with Pb and Cr singly and in combination with Kinetin (*P*< 0.05)

Heavy metal	Plant organ	V_1 (Mash 80)	V_2 (Mash 88)	V_3 (Mash 97)	V_4 (Mash ES1)
Pb	Root	1.35 a	1.56 a	1.72 b	1.99 c
	Shoot	0.52 a	0.67 a	0.69 b	0.96 c
Cr	Root	1.43 a	1.97 b	1.67 a	2.36 c
	Shoot	1.39 a	1.56 a	1.43 a	1.87 b

Varity means sharing the same letter differ non-significantly.

Black gram cultivars did not exhibit a uniform pattern for Cr content in their roots and shoots. Mash ES1 (V_4) surpassed remaining cultivars for up taking significantly higher Cr by its roots. In Mash 88 (V_2) as well, root Cr content was significantly higher than Mash 80 (V_1) and Mash 97 (V_3), which differed non-significantly with one another.

As regards the Cr content in the shoots of black gram cultivars, its maximum value (1.87 mg) significantly higher than all the remaining three cultivars was recorded for Mash ES1 (V_4). Mash 80 (V_1) had minimum Cr content in its roots and varied non-significantly from the remaining two black gram cultivars i. e. Mash 88 (V_2) Mash 97 (V_3).

Stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index

Stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index of black gram plants treated with Pb and Cr either alone or in combination with Kinetin have been presented in Table 2a. Analysis of variance showed highly significant (α =0.001) differences among treatment and variety means and non-significant differences for the interaction between treatments and verities for all these parameters.

A comparison among the treatment means indicates that application of Pb alone caused maximum reduction in stomatal conductance and it was followed by Cr singly application (Table 2b). Stomatal conductance of plants treated with both heavy metals in combination with kinetin (Pb & Kin, Cr & Kin) was significantly higher than their alone application (Pb, Cr) but it was significantly lesser than that recorded for plants treated with Kin alone. The application of Kin alone slightly improved stomatal conductance that varied non-significantly from control plants. Among the varieties, Mash 80 (V_1) showed maximum stomatal conductance and its minimum value was recorded for Mash ES1 (V_4) (Table 1c). Nevertheless, Mash 88 (V_2) and Mash 97 (V_3) possessed intermediate stomatal conductance varying non-significantly with one another.

As compared with control application of Pb and Cr alone caused maximum reduction in the assimilation rate of black gram plants. The additional application of Kin to heavy metals treated plants significantly reduced these toxic effects. However, the assimilation rate for both treatments (Pb & Kin, Cr & Kin) remained significantly lesser than those recorded for control plants and Kin singly treatment. However, assimilation rate of plants treated with Kin alone differed non-

Table 2a. Analysis of variance for effect of heavy metal (Pb and Cr) on stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index of black gram plants treated with Pb, Cr and kinetin alone or in combination (*P*< 0.05).

Source of variation	Sum of squares	df	Mean square	F ratio	Significance
Stomatal conductance					
Treatments	20.743	5	4.149	12.208	.000
Varities	13.171	3	4.390	12.919	.000
Treat * Var	5.025	15	.335	.986	.472
Assimilation rate					
Treatments	296.772	5	59.354	21.837	.000
Varities	26.255	3	8.752	3.220	.024
Treat * Var	27.113	15	1.808	.665	.816
Transpiration rate					
Treatments	42.530	5	8.506	20.709	.000
Varities	10.218	3	3.406	8.292	.000
Treat * Var	8.895	15	.593	1.444	.132
Leaf area					
Treatments	14755.945	5	2951.189	11.942	.000
Varities	2117.289	3	705.763	2.856	.039
Treat * Var	5459.719	15	363.981	1.473	.120
Harvest index (%)					
Treatments	3835.465	5	767.093	10.854	.000
Varities	816.034	3	272.011	3.849	.011
Treat * Var	549.831	15	36.655	.519	.928

$P > 0.05$

Table 2b. Treatment means for stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index (%) of black gram plants treated with control (T_0), Pb @ 20 mg kg^{-1} (T_1), Kin @ 100 µg g^{-1} (T_3), Pb @ 20mg kg^{-1} & Kin @ 100 µg g^{-1} (T_4), and Cr 30 mg kg^{-1} & Kinetin @ 100 µg g^{-1} (T_5).

Parameters studies	Treatments					
	T_0 (Control)	T_1 (Pb only)	T_2 (Cr only)	T_3 (Kin only)	T_4 (Pb & Kin)	T_5 (Cr & Kin)
Stomatal conductance	1.86 bc	1.09 a	1.19 a	1.99 c	1.61 b	1.65 b
Assimilation rate	11.25 c	8.07 a	8.27 a	11.06 c	9.22 b	9.29 b
Transpiration rate	2.80 c	1.69 a	1.70 a	2.85 c	2.10 b	2.04 b
Leaf area	83.74 c	59.84 a	62.71 a	83.74 c	70.51 b	73.77 b
Harvest index	48.50 c	36.93 a	37.84 a	48.05 c	43.26 b	44.15 bc

Treatment means sharing the same letter differ non-significantly (*P*< 0.05)

Table 2c. Variety means for stomatal conductance, assimilation rate, transpiration rate, leaf area, and harvest index of black gram plants treated with Pb, Cr and kinetin alone or in combination.

Parameters studied	Varieties			
	V_1 (Mash 80)	V_2 (Mash 88)	V_3 (Mash 97)	V_4 (Mash ES1)
Stomatal conductance	1.99 c	1.59 b	1.37 ab	1.32 a
Assimilation rate	10.09 b	9.56 ab	9.43 ab	9.01 a
Transpiration rate	2.59 b	2.13 a	2.07 a	1.10 a
Leaf area	75.84 b	73.31 ab	72.43 ab	66.65 a
Harvest index	45.40 b	44.16 b	43.14 b	39.72 a

significantly from control. A comparison among the black gram cultivars indicates highest assimilation in Mash 80 (V_1) and minimum in Mash ES1 (V_4) but both differed non-significantly from Mash 88 (V_2) and Mash 97 (V_3).

Transpiration rate of plants treated with Pb and Cr without Kin application was at par with each other, and significantly less than control plants. The addition of Kin caused significant improvement in transpiration rate but could not reduce its difference from control plants to a non-significant level. Transpiration rate in plants treated with Kin alone did not vary significantly from that recorded for control plants. Among the black gram cultivars Mash 80 (V_1) had significantly higher transpiration rate than other three cultivars, which did not differ significantly with each other.

Leaf area of plants treated with Kin alone was at par with control plants. The application of Kin to Pb and Cr treated plants caused significant improvement in leaf area as compared with the singly application of Pb and Cr, but it still remained significantly less than control and Kin singly. The differences in leaf area among black gram cultivars exhibited almost the same pattern as that examined for assimilation rate.

Black gram plants proved slightly more sensitive to Pb singly treatment than the Cr singly application in respect of harvest indexes. A similar trend was examined for their combination with Kin. The application of Kin caused significant improvement in harvest index but could not fully compensate it to the extent recorded for control or Kin alone. Among the black gram cultivars, Mash ES1 (V_4) yielded to least harvest index and Mash 80 showed maximum harvest index following the same trend as that examined for other parameters.

Correlation between Pb and Cr content in the roots and shoot of black gram plants and their stomatal conductance, assimilation rate, transpiration rate, leaf area and harvest index.

Highly significant negative correlation was examined between the Pb content in the roots and shoot of black gram plants and their stomatal conductance, assimilation rate, transpiration rate, and leaf area (Table 3). The harvest index also showed highly significant negative correlation to the root Pb content but a significant negative relationship to shoot Pb content. All these parameters exhibited highly significant negative correlation with the root Cr content as well. However, the shoot Cr content showed highly significant negative correlation with assimilation and transpiration rates only. It had significant negative correlation with harvest index as well, but a non-significant negative correlation with stomatal conductance and leaf area.

Table 3. Pearson correlation coefficients for the linear correlation between heavy metal content in the roots and shoot of treated black gram plants and their stomatal conductance, assimilation rate, transpiration rate, leaf area and harvest index values.

Heavy metal	Plant organ	Pearson correlation coefficients				
		Stomatal conductance	Assimilation rate	Transpiration rate	Leaf area	Harvest-index
Pb	Root	-.253(**)	-.311(**)	-.292(**)	-.298(**)	-.253(**)
	Shoot	-.203(**)	-.321(**)	-.242(**)	-.245(**)	-.174(*)
Cr	Root	-.216(**)	-.292(**)	-.295(**)	-.190(**)	-.213(**)
	Shoot	-.135(NS)	-.231(**)	-.281(**)	-.130(NS)	-.173(*)

** = Highly significant, * = Significant, NS = Non-significant.

DISCUSSION

Heavy metal toxicity like other environmental stresses causes detrimental reduction in the transpiration rate (Clarkson 1993, Veselov *et al.* 2003) vital for the uptake of mineral nutrients and maintenance of internal temperature. Plants have evolved special mechanism for the inactivation of heavy metal toxicity by binding heavy metals with some internally released substances especially hormones (Salisbury and Marinos 1985, Sanita' di Toppi 1999) capable of very actively accumulating heavy metals (Costa and Morel 1993). However, plants differ widely in their potential to synthesize such substances and sometimes under stress conditions their induction is, also delayed (Veselov *et al.* 2003). Under such circumstances exogenous application of synthetic plant hormones or other relevant substances becomes imperative for alleviating the toxic effects of heavy metals.

In this study we examined the efficacy of kinetin in ameliorating the toxic effects of Pb and Cr on the stomatal conductance, assimilation rate, transpiration rate, leaf area, harvest index and heavy metal ion uptake by the roots and shoot of four black gram cultivars. Black gram plants treated with Pb and Cr singly showed higher accumulation of both heavy metals (Pb & Cr) in their roots as compared to shoots. This reduced translocation of Pb and Cr from the root to shoot in black gram plants receiving heavy metal treatments only is in line with the previous findings (Rivera-Becerril *et al.* 2002, Kidd *et al.* 2004, Singh *et al.* 2004).

Black gram cultivars varied significantly in their root and shoot heavy metal content that may be attributed to their genetic variation in accordance with (Belimov *et al.* 2003, Belimov *et al.* 2003). They tested ninety-nine pea genotypes for their Pb, Cr and some other heavy metals uptake potential and observed great variation among genotypes and majority of them tended to avoid excessive accumulation of heavy metals in their shoots. Similar results have been reported by (Schat and Vooijs 1997) as well, for *Silene vulgaris* (Moench) Garcke plants growing on metaliferous soils.

We examined that plant treated with Pb and Cr along with Kin application showed more heavy metal content in their shoots as compared to plants treated with Pb and Cr singly. The higher translocation of heavy metal ions from roots towards shoot indicates an improvement in the transpiration stream, with concomitant increase in stomatal conductance. Further more, leaf area and harvest index of plants treated with heavy metals (Pb & CR) along with kinetin were also significantly higher than plants treated with singly treatment of Pb and Cr. Resembling results have been reported by (Vassil *et al.* 1998) who attributed the higher translocation of Pb in Indian mustard (*Brassica juncea* (L.) Czern.) to the improvement in transpiration rate caused by EDTA application. (Sayed 1999) examined the effect of cadmium and kinetin on transpiration rate, stomatal opening and leaf relative water content in safflower plants and reached the same conclusion.

The differential accumulation of Pb and Cr in the roots and shoot of black gram plants may be regarded a specific characteristic of Cr as already examined by (Belimov *et al.* 2003). They observed that concentrations of different heavy metals (Cd, Cu, Ni, Pb, Sr and Zn) in the shoot of pea **(***Pisum sativum* L.**)** positively correlated with each other, with the exception of Cr as examined in this study. Difference genotypes also varied greatly for their tolerance to Cd, chromium, copper, nickel, lead, strontium and zinc.

The sequestering of both heavy metal in the plant roots and shoot seem well reflected in the form of a significant reduction in stomatal conductance, assimilation rate, transpiration rate, leaf area and harvest index. We also noted that heavy metal content in roots showed more strong negative correlation with all these morphological and physiological parameters than that existed for the heavy metal content in the shoot. Literature specifically relating the effect of Pb and Cr on the water relations and physiological attributes of black gram is very scanty. However, a number of reports are available relating the toxic effects of heavy metals on plant growth (Belimov *et al.* 2003) leaf area, and reduction in transpiration rate of wheat (Vassilev *et al.* 2002) and rice (Hsu and Kao 2003) as well as, about the improvement in these parameters with the application of kinetin (Gadallah and El-Enany 1999, Gadallah and El-Enany 1999). Nevertheless, (Clarkson 1993, Veselov *et al.* 2003) attributed similar reduction in transpiration rate and nutrient content in the heavy metal treated plants to the reduced root functioning resulting from the deficiency of some hormone vital for the maintenance of stomata in an open state and for cell expansion a prerequisite for root development (Bengston and Larson 1979). Studies pertaining to heavy metal uptake by different vegetable, arable, and fodder crops have also shown that the translocation of heavy metals to different plant organs depends upon the internal transport particularly transpiration stream (Albering *et al.* 1999).

CONCLUSION

This study provides an insight relating the possible role of kinetin in alleviating the toxic effects of Pb and Cr on four black gram cultivar commonly cultivated in Pakistan. Relatively higher concentration of both metals in the roots of Mash ES1, as compared to its shoot, also has an implication importance for its selection from the phytoremediation point of view. The low heavy metal content in the roots and shoots of Mash 80, also has good implication for its utilization in the future breeding programs, and for preferable cultivation as a fodder crop in the riverine areas of Pakistan and else ware in the world prone to excessive heavy metals (particularly lead and chromium) contamination through irrigation water.

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