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GENETIC APPROACH FOR ASSESSING THE ENVIRONMENTAL HAZARDS OF HEAVY METALS ON PLANT GROWTH IN COTTON

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ABSTRACT

This study was a genetically approach to investigate the genetic nature of cotton plants to heavy metals. The effect of cadmium (Cd), lead (Pb) and nickel (Ni) on growth, as measured by dry weight of root and shoot, were studied on a set of cotton parental genotypes and their F_1 progenies. Cotton genotypes reacted differently with heavy metals stresses. P_2 (Giza 90) showed the lowest reduction effect under Cd and Ni as well as P_5 (Pima S6) for Pb stresses. Estimates of genetic components of variance revealed that the effect of dominance components were much greater than the effect of additive components for all reduction effects under the three heavy metals stresses. Non-additive gene effects had a considerable role in controlling growth responses of cotton to such stresses, which confirmed by high estimates of broad-sense heritability. The parental genotypes and F_1 crosses had similar linear response to different heavy metals stresses, as environmental changes. So, some reliable predictions about the phenotypic expression of these genotypes could be made across environments, Cd, Pb and Ni stresses. Determination of induced reduction effects on root and shoot growth might serve as a simple and early indicator of heavy metals toxicity in plants.

Keywords: **Genetics, heavy metals, plant growth, heritability, reduction effect, cotton.**

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INTRODUCTION

Plant response to heavy metals stresses is a complex set of processes depending on the source, duration and severity of metal as well as genetic background of such plants and their developmental stages. Considerable researches have been conducted with heavy metals toxicity in plants. Cadmium (Cd), lead (Pb) and nickel (Ni) are considerable most toxic metals that can affect plant growth. Numerous differential responses of plants to such metals have been reported (Marchiol et al., 1996; Zhang et al., 1998; Ather and Ahmed, 2002; Seregin et al., 2003; Seregin and Kozhevnikova, 2005and He et al., 2009).

Likewise, plant genotypes of the same species differ substantially in their response to such toxic metals. Some genotypes exhibit a high threshold of response and others exhibit low threshold, i.e. plant metal response is appeared to be of genetic nature (Bauomy, 1998; Mahgoub et al., 1998; Ivanov et al., 2003; Tomas et al., 2006; Kopattke et al., 2007 and Daud et al., 2009).

However, no or very little works has been carried out on the genetic nature of these responses. As information on such important aspects are lacking, we have made an attempt to determine the nature of responses of different cotton genotypes to heavy metals stresses and to investigate the genetic nature of these responses on plant growth of cotton. Understanding such responses of cotton plants may throw the light on the possibility of re-use treated-water coming from sewage and industrial effluents in irrigating cotton plants.

MATERIALS AND METHODS

A set of cotton genotypes, ***Gossypium barbadense*** L., were screened for their response to cadmium (Cd), lead (Pb) and nickel (Ni) and to determine the critical toxic dose of each heavy metal. On the basis of the response pattern, six genotypes were chosen and used as parents for the present study. These genotypes were Giza 83 (P_1), Giza 90 (P_2), Giza 91 (P_3), Giza 70 (P_4), Pima S6 (P_5) and Giza 87 (P_6). Selfed seeds of these parental genotypes were grown in the field in the season of 2006. The parental genotypes were crossed in such a way using P_1, P_2 and P_3 as female parents and crossed each of P_4, P_5, and P_6 to obtain nine F_1 crosses. The six parents and their nine F_1 crosses were grown in the next season of 2007 in a complete randomized block design experiment with four heavy metals treatments and three replications for each one. Each replicate comprised two rows, having ten plants for each one, i.e. each genotype represented by eight rows. The treatments were one for each of Cd, Pb and Ni in addition to control treatment (no heavy metals).

Cadmium sulphate, lead acetate and nickel chloride were used as source of Cd, Pb and Ni ions in this study. This work was carried out at Cotton Res. Inst., Agric. Res. Center, Giza, Egypt.

Thirty days old plants were treated with heavy metals solutions at concentrations of 70 ppm Cd, 20 ppm Pb and 40 ppm Ni ions. One week later, three plants were taken randomly from each replicate for laboratory analysis. Plant growth was measured as dry matter of seedling parts. Dry weights of root and shoot of each sample were determined. Reduction effects of heavy metals on dry weights were estimated.

The obtained data were statistically analyzed after transforming reduction % into angular scale. A separate analysis of variance for each heavy metal treatment was done to determine the significance of the observed differences. Also, data of F_1 crosses were subjected to a further female x male analysis, for each treatment, to partition their genetic variation due to females, males and their interaction according to Singh and Chaudhary (1977) and Kearsy and Pooni (1996). Genetic components of variance were also estimated and eventually heritability estimates were calculated. The predicted responses of cotton genotypes under heavy metals stresses were determined as slope of phenotypic expression on environmental indices for heavy metals stresses using SPSS computer software (1995).

RESULTS AND DISCUSSION

Cotton plant growth was measured as dry root and shoots weight of five weeks old seedlings under heavy metals stresses. Performance of the studied cotton genotypes for growth estimates under heavy metals stresses are given in Figures (1) and (2). The data should that cotton genotypes reacted differently with heavy metals. The heavy metals induced reduction effects on dry weight of roots and shoots of cotton genotypes are presented in Table (1).

The data showed that P_4 exhibited the greatest reduction effect in root dry weight under the three heavy metals stresses, followed by P_1. However, P_2 showed the lowest reduction effect under Cd and Ni stresses and P_5 for Pb stress, reflecting some sort of tolerance.

But, P_1 and P_6 under Cd stress, P_1 and P_5 under Ni stress exhibited the greatest reduction effects in shoot dry weight. The same trend was observed for P_5 and P_6 under Pb stress. However, P_3 under Cd, P_3 and P_4 under Pb as well as P_2 under Ni manifested the lowest reduction effect in shoot dry weight. These results might indicate that mode of action of these heavy metals on cotton growth was different.

The behavior of F_1 crosses showed various trends in their growth reductions under heavy metals stresses, depending on their parental combinations. Most of F_1 crosses behaved nearly to their low root reduced parents, but this trend was reversed for shoot growth, under all studied heavy metals stresses. Also, some F_1 crosses exhibited greater reductions than their parents, either in root or shoot growth.

It is obvious that Pb caused more inherent effects on root system, but Cd and Ni caused harmful effects on shoots. This was true either on homozygous genotypes or heterozygous genotypes. Theses heavy metals might create unfavorable conditions inside plant cells exerting some disturbances in metabolic processes leading to dispersive effects on plant growth.

The analysis of variance for heavy metals induced reduction in dry weight of root and shoot of cotton genotypes are given in Table (2). Cotton genotypes showed significant variations in their reduction effects under the three heavy metals for root or shoot dry weight. These significances were also reflected on significant variations among cotton parents or most of their F_1 crosses. These results suggested the existence of genotypic variations among these entries in their reaction with heavy metals. Also, significant differences were observed for parents versus F_1 crosses for reduction effect of shoot dry weight only, which might reflect some sort of heterotic effects in these F_1 crosses.

In this regard, many investigators reported the reduction effects of Cd, Pb or Ni ions on root or shoot growth in different field crops: Kovacevic et al. (1999) on wheat growth, Ivanov et al. (2003) and

Seregin et al. (2004) on maiz roots, Tomas et al. (2006) on barely growth and Kopattke et al. (2007) on growth of cowpea.

Mean squares of cotton F_1 crosses, partitioned into females, males and their interactions, for reduction effects of dry weight of root and shoot under heavy metals stresses are given in Table (3). The data showed significant differences among all F_1 crosses for reduction effects under all heavy metals stresses, except for root dry weight under Ni which were similar in their response to Ni and eventually the genetic components were not clearly pronounced. The female parents exhibited significant difference in shoot dry weight reduction under all metals stresses, but male parents showed the same trends in root dry weight reduction under Cd and Pb only. These results might suggest that both female and male parents were differently reacted with these metals.

Moreover, the female x male interactions were also significant for reduction effects in both root and shoot dry weight, suggesting the great contribution of non-additive gene effects in controlling heavy metals responses in cotton, since these interactions were much greater than those of either females or males. The proportional contribution of female x male interactions in F_1 crosses, given in Table (4), showed the maximum scores for root or shoot dry weight reduction under all the three heavy metals and confirming the involvement of non-additive gene effects.

Estimates of genetic components of variance and heritability values for reduction effects in dry weight of roots and shoots in cotton under heavy metals stresses are given in Table (5). The data showed that dominance components of variances were much greater than the additive components for all reduction effects under the three heavy metals. These results might indicate that the non-additive gene effects playing a considerable role in controlling cotton growth responses to heavy metals stresses. Such effects might associate with non-allelic gene interactions. The high values of heritability estimates in broad sense confirmed the importance of such gene effects. Therefore, more tolerant cotton genotypes to heavy metals stresses could be achieved by repeated crossing rather than selection. Bauomy (1998) and Mahgoub et al. (1998) stated that both additive and non additive gene effects were operating in the

Table 1. Heavy metals induced reduction effects on dry weight of roots and shoots of cotton genotypes

Genotypes	Reduction % of Cd		Reduction % of Pb		Reduction % of Ni	
	Root	Shoot	Root	Shoot	Root	Shoot
P1	14.97	23.91	19.13	21.33	13.07	28.33
P2	10.74	14.76	16.98	16.64	7.93	11.32
P3	12.42	13.16	15.57	10.13	8.46	17.73
P4	18.21	17.79	20.88	10.28	17.58	19.75
P5	11.67	21.19	14.86	23.53	13.25	21.90
P6	12.48	23.57	15.94	22.39	11.94	20.06
Mean	**13.42**	**19.06**	**17.23**	**17.38**	**11.94**	**19.85**
P4xP1	12.63	17.83	16.46	15.04	13.52	18.56
P4xP2	13.55	21.60	19.71	14.79	16.17	21.47
P4xP3	11.90	20.81	15.92	19.46	12.72	23.63
P5xP1	17.95	30.19	17.87	25.25	13.35	20.14
P5xP2	11.87	16.09	13.12	14.35	15.65	20.21
P5xP3	10.53	27.67	11.93	22.06	10.44	18.38
P6xP1	15.26	24.19	18.21	21.77	12.15	26.36
P6xP2	11.18	23.29	14.48	23.54	12.30	20.55
P6xP3	15.89	21.54	15.87	21.38	14.96	24.43
Mean	**13.42**	**22.02**	**15.96**	**19.74**	**13.47**	**21.53**
LSD at 0.05	**2.724**	**2.324**	**3.612**	**4.968**	**4.895**	**3.837**

Table 2. Analysis of variance for heavy metals induced reduction effects in dry weight of roots and shoots of cotton genotypes

Source of variance	d.f	Reduction % of Cd		Reduction % of Pb		Reduction % of Ni	
		Root	Shoot	Root	Shoot	Root	Shoot
Genotypes	14	18.486*	48.255*	17.702*	71.732*	21.185*	47.828*
Parents (P)	5	22.507*	61.971*	16.196*	109.291*	37.978*	92.119*
F_1 crosses (F)	8	18.285*	48.055*	18.689*	49.733*	10.178	22.329*
P vs F	1	0.006	94.665*	17.334	59.932	25.283	30.356*
Replications	2	0.031	6.139	5.291	6.276	0.274	1.062
Error	28	2.649	1.929	4.657	8.811	8.553	5.253

* Significant at 5 %

Table 3. Analysis of variance for heavy metals induced reduction effects in dry weight of roots and shoots of F_1 crosses partitioned into females, males and their interactions in cotton

Source of variance	d.f	Reduction % of Cd		Reduction % of Pb		Reduction % of Ni	
		Root	Shoot	Root	Shoot	Root	Shoot
Genotypes	8	18.285*	48.055*	18.689*	49.734*	10.178	22.330*
Females (F)	2	8.516	50.367*	21.477*	80.114*	4.960	40.350*
Males (M)	2	29.129*	46.472*	19.923*	32.116	15.476	4.594
FxM	4	17.746*	47.690*	16.677*	43.352*	10.138	22.187*
Replications	2	0.910	1.789	0.047	2.122	2.508	1.044
Error	16	2.954	1.579	3.832	10.54	7.399	6.070

* Significant at 5 %

Table 4. Proportional contributions of females, males and their interactions in F_1 crosses for heavy metals induced reduction effects on dry weight of roots and shoots of cotton genotypes

Proportional contributions	Reduction % of Cd		Reduction % of Pb		Reduction % of Ni	
	Root	Shoot	Root	Shoot	Root	Shoot
Contribution of females	11.64	26.20	28.73	40.27	12.18	45.18
Contribution of males	39.83	24.18	26.65	16.15	38.01	5.14
Contribution of FxM	48.53	49.62	44.62	43.58	49.81	49.68

Table 5. Genetic components of variance and heritability for heavy metals induced reduction effect on dry weight of roots and shoots of cotton

Genetic components	Reduction % of Cd		Reduction % of Pb		Reduction % of Ni	
	Root	Shoot	Root	Shoot	Root	Shoot
VA	0.239	0.162	0.894	2.836	0.018	0.064
VD	5.170*	15.376*	4.282*	11.066*	0.913	5.372*
VG	5.409	15.532	5.176	13.902	0.931	5.436
VP	6.394	16.058	6.453	17.287	3.397	7.459
h^2b	84.59	98.72	80.42	80.42	27.39	72.87
h^2n	3.74	1.01	13.85	16.41	0.52	0.86

* Significant at 5 %

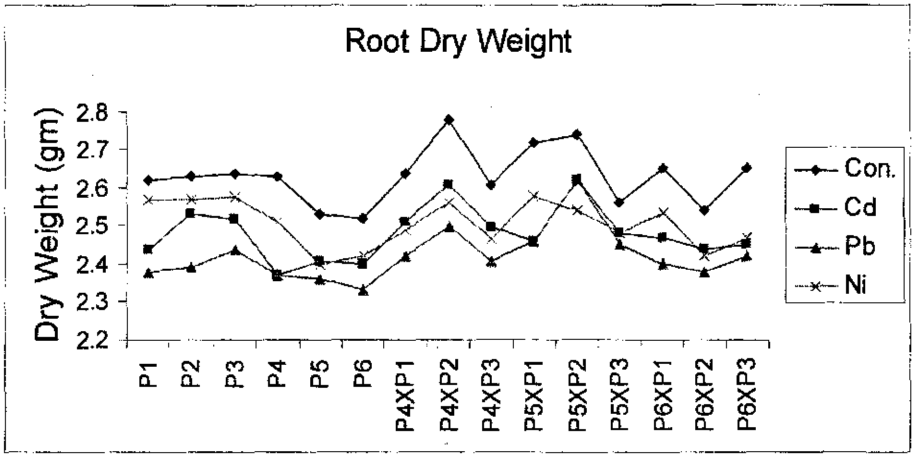

Fig. 1. Plant dry weight in grams for cotton genotypes under heavy metals stresses, measured as root dry weight.

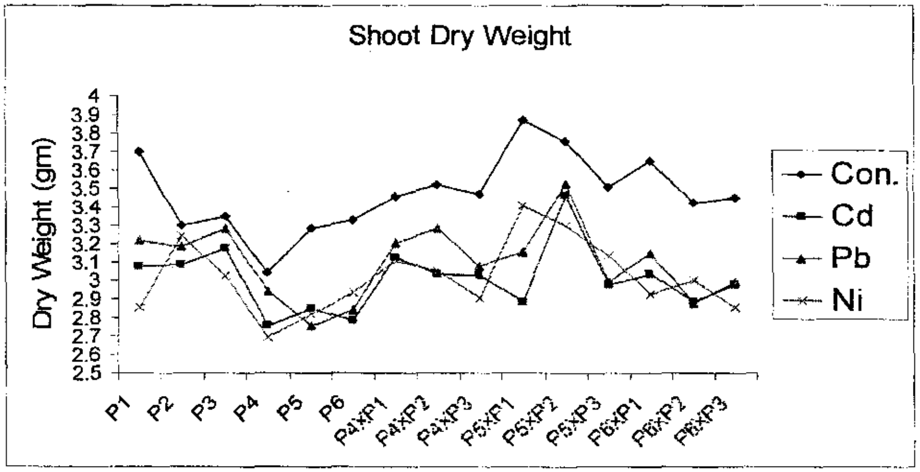

Fig. 2. Plant dry weight in grams for cotton genotypes under heavy metals stresses, measured as shoot dry weight.

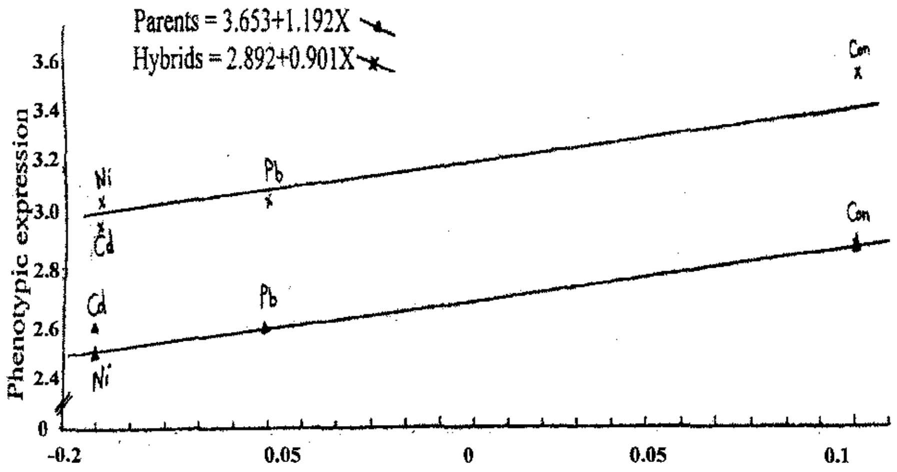

Fig. 3. Predicted response for shoot dry weight under heavy metal stress.

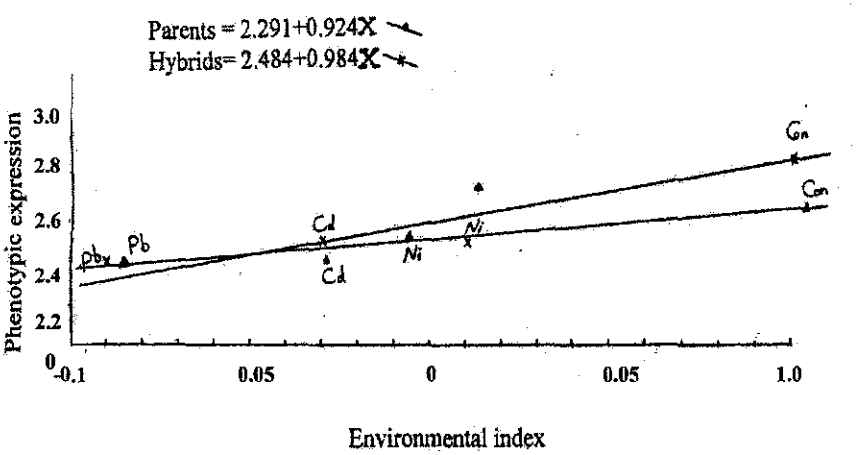

Fig. 4. Predicted response for root dry weight under heavy metal stress.

response of cucumber to heavy metals stresses.

The average phenotypic expression of parents and F_1 crosses for dry weight of roots and shoots under heavy metals stresses, which are treated as different environments, were plotted against the environmental indices (Figures 3 and 4). The linear regression of genotypes means environmental indices provided an accurate and measurable prediction of the relative response of a genotype to differences among environments. Both parents and F_1 crosses had regression slopes (b) around unity for both dry weight of roots and shoots. Therefore both parental genotypes and F_1 crosses of cotton had similar linear average response to environmental changes, heavy metals stresses, and high predictability for such attributes.

The consistency of the linear regression slopes over environment might indicate that performance of one heavy metal stress condition could be predicted from performance in another stress condition.

Finally, reduction effects of heavy metals stresses, determinated on root or shoot growth could be utilized as simple and reliable indicator of heavy metals pollution and toxicity in higher plants.

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مقاربة وراثية لتعيين الاخطار البيئيه للمعادن الثقيله على نمو نباتات القطن

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أجريت هذه الدراسة لمعرفة الطبيعة الوراثية لاستجابة نباتات القطن للعناصر الثقيله، حيث تمت دراسة تأثير كل من الكادميوم، الرصاص والنيكل علي الوزن الجاف للمجموع الخضري والجذرى لمجموعة من الأباء وهجن الجيل الأول .

أظهرت التراكيب الوراثية اختلافا فى تأثرها بالعناصر الثقيله، فكان الاب الثانى (جيزة٩٠) هو الأقل فى نسبة Reduction % تحت تأثير الكادميوم والنيكل وكذلك الأب الخامس(بيماس٦) تحت تأثير الرصاص. وبتقدير مكونات التباين الوراثى، أظهرت أن التأثير السيادى أكبر من التأثير الإضافى لكل Reduction effect تحت تأثير الثلاثه عناصر الثقيله.

ولذلك كان تأثير الجينات الغير مضيفه فعالا فى التحكم فى استجابة نباتات القطن للنمو تحت ظروف الاجهاد بالعناصرالثقيله، وهذا ما تم تأكيده بواسطة التقدير المرتفع لمعامل التوريث بالمعني الواسع. ولقد وجد أن اللأباء وهجن الجيل الأول لها نفس الاستجابة الخطية للاجهاد بالعناصر الثقيله كما فى التغيرات البيئية. لذا تم عمل تنبوءات للتعبير المظهرى لهذه التراكيب الوراثية تحت تأثير الكادميوم، الرصاص والنيكل. ويعتبر تقدير Reduction effect على نمو المجموع الخضرى والجذرى مؤشرا مبكرا لمدى سمية العناصر الثقيله على النباتات.