Identification of Genetic Variation among Bread Wheat Genotypes for Lead Tolerance Using Morpho – Physiological and Molecular Markers

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Abstract: Two field experiments were conducted with ten bread wheat genotypes (*Triticum aestivum* L.) evaluated in their sensitivity to lead pollutant under lead stress and normal conditions. The results showed that there were great differences among wheat genotypes for proline content, leaf chlorophyll content, flag leaf area as well as yield and its attributes. Wheat genotypes ACSAD 903, Sakha 94, ACSAD 939, Prl(S)/Pew(S), Tow(S)/Pew(S) and Gemmeiza 5 were classified as tolerant to lead stress as they exhibited lead sensitivity index less than unity with high values of proline content, leaf chlorophyll content, flag leaf area and yield attributes in most cases. Whereas, ACSAD 925 was ranked in the first order in sensitivity to lead stress followed by Sids 6 and Tsi/Vee(S) while, Line 1 was relatively moderate sensitive to lead stress. Heritability estimates in broad sense were high under normal and moderate under lead stress conditions for proline content, leaf chlorophyll content and flag leaf area, however it was moderately high for yield attributes and low for grain yield/fed. under normal and stress conditions. Negative and significant association was observed between lead sensitivity index of grain yield and each of proline content, leaf chlorophyll content, flag leaf area and number of productive tillers/plant. Path coefficient analysis indicated that, the maximum direct effect on lead sensitivity index of grain yield was accounted for leaf chlorophyll content (22.268%) followed by flag leaf area (12.250%), proline content (5.697%) and then number of productive tillers/plant (1.397%).The highest indirect effect was registered for proline content via leaf chlorophyll content (12.241%) followed by leaf chlorophyll content via flag leaf area (7.795%) and flag leaf area via number of productive tillers/plant(5.235%), therefore, simultaneous selection for the foregoing pair characters may have resulted in enhancement lead tolerance in wheat. The genetic variation and relationships among 10 wheat genotypes with different responses to lead tolerance were evaluated using Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) markers to establish specific DNA markers associated with lead tolerance.. A total of 40 DNA fragments were generated by 5 random primers, with an average of 8 easily detectable fragments per primer. The number of amplified fragments produced per primer ranged from 6 to 10 and size of the products ranged from 254 bp to 1930 bp. The total number of polymorphic fragments and the percentage of polymorphism were 33 and 82.5, respectively. The greatest similarity was observed between ACSAD 903 and ACSAD 939 genotypes, whereas the lowest similarity showed between ACSAD 925 and Gemmeiza 5. The dendrogram separated all genotypes into three clusters. The patterns obtained with primer OPB-10 for genotypes suggested that this primer has the ability to produce lead tolerant markers. These results will be helpful in future wheat breeding programs.
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1. Introduction:

Heavy metals such as lead (Pb) enter to the environment from natural and anthropogenic sources. The most important anthropogenic source of pollution to metals are industrial sludge sewage discharging, applying super phosphate fertilizers, burying the non-ferrous wastes in land and closing the agricultural fields to lead and zine mines or refining factories (Rowland *et al*.,1997). These metals contaminate food source and accumulate in both agricultural products and seafood through water, air and soil pollution (Lin *et al*., 2004). Lead is the most well-Known environmental intoxicants to humans. Lead is more toxic to plants, its highest uptake occurred during the heading-grain maturation period. Wheat leaf blade area, plant growth and development were reduced more markedly than the whole plant dry matter. Both water uptake and water loss are reduced by lead in wheat (Burzynski, 1987). The green tissues are more susceptible to Pb toxicity than etiolated tissues. The plastic and elastic extensibility of wheat coleopitle cell walls was

reduced and the hydration of sunflower hypocotyls segments was decreased (Gupta, 1997). Chlorophyll content of barley leaves decreased with added Pb in the Knop's solution (Kacabova and Natr, 1986). Lead inhibited apparent photosynthesis, photorespiration, CO2 uptake, stomata opening and transpiration of pea seedlings (Poskuta *et al*., 1987), and inhibits plant growth of rice cultivars (Yang YoungYell *et al.*, 2000).

Plants tolerate heavy metals through sequestration with cysteine rich peptides, proline, Chlorophyll content and other physiological and biochemical characters (Lagriffoul *et al.*, 1998; Mahgoub *et al*., 1998 and Harada *et al*., 2001). Crop tolerance to lead pollutant has been extensively investigated and genetic variation among cereal genotypes in their reaction to lead toxicity has been found in most cultivated species (Meyers *et al*.,1982; Kacabova and Natr,1986; Yang YoungYell *et al*.,2000 and Brkic *et al.*,2004).

Molecular tools facilitate the identification of genomic locations linked to traits of interest and help in indirect selection of such complex traits beside the phenotypic measurements. Identification of DNA-makers linked to a gene governing mineral stress have been applied in several studies i.e. cadmium stress in durum wheat (Penner *et al*., 1995), aluminum tolerance in rice (Wu *et al*., 2000) and manganese efficiency in barley (Pallotta *et al*., 2000 and Khobaz-Saberi *et al.*, 2002). Welsh and McClelland (1990) and Williams *et al*., (1990) developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals. The use of RAPD for identification of cultivars through DNA profiling is the current method of choice in measuring genetic variation within germplasm collections (Williams *et al*., 1990 and Hernendez *et a*l., 2001). PCR-based RAPD markers are dominant markers that are extensively used in genetic mapping (Chalmers *et al*., 2001) and identification of loci linked with different traits (Sun *et a*l., 2003). Due to technical simplicity and speed, RAPD methodology has been used for diversity analyses in several crops (Demek *et al*., 1996). Criteria for the estimation of genetic diversity can be different pedigree records, morphological traits or molecular markers (Heckenberger *et al*., 2002). Molecular markers detect variation of the DNA sequences among cultivars and therefore directly bypass problems connected with environmental effects (Maric *et al*., 1998).

The objective of this research was to determine the genetic variability, heritability for lead tolerance and the relative importance of some characters in lead tolerance variation, beside establish specific molecular markers associated with lead tolerance using RAPD-PCR to facilitate breeding of cultivars more tolerant to lead pollution.

2. Materials and methods

To study lead tolerance in bread wheat (*Triticum aestivum* L.), ten genotypes were screened and involved in this investigation (Table1). Field experiment was carried out during 2007/2008 and 2008/2009 seasons at the Experimental Farm, Faculty of Agriculture, Zagazig University, Egypt.

Table (1): Name, pedigree and origin of the studied ten bread wheat genotypes.

Name	Pedigree	Origin
Sakha 94	Opta / Rayon / Kauz	Egypt
Gemmeiza 5	Vee(S)/SWM6525 CGM4017-1GM-6GM-3GM-0GM	Egypt
Sids 6	Maya(S)/Mon(S)//CMH74 A592/3/Sakha8*2SD10002-4sd-3sd-1sd-0sd	Egypt
ACSAD 903	ACSAD529/4/C182 24/C168 3/3/Cno*2/7c//Tob Acs-W-8024-20IZ-3IZ-4IZ-0IZ	Syria
ACSAD 925	GEN/3/GOV/AZ//MUS(s)/4/Sannine/Ald(s) ACS-W-9174-10IZ-5IZ-3IZ-0IZ	Syria
ACSAD 939	Maya(S)/ON//1160.147/3/BB/GLL/4/CHAT(s)/S/Vee(s)Nac ACS-W-8163-2IZ-5IZ-0IZ	Syria
Line 1	N.S.732/Pim//Veery(S)sd735-4sd-1sd-osd/3/CM87688-02910Pm-5Y-0H-0sy-1M-0Y	Egypt
Tsi/Vee(S)	CM64335-3AP-IAp-0AP	Mex/Syr
Prl(S)/Pew(S)	CM59377-3AP-1AP-3AP-2AP-1AP-0AP	Mex/Syr
Tow(S)/Pew(S)	CM59443-4AP-1AP-4AP-1AP-0AP	Mex/Syr

Ten wheat genotypes were treated under controlled conditions carefully at beginning heading stage by spraying heavy metal lead solution. Lead acetate Pb (CH3 Coo) 2 was used as source of lead in the present study. The concentration was 30 ppm Pb ion per liter of water (200 liter/fed.). Singh (2004) investigated that selection for mineral toxicity can be carried out in a field having mineral toxicity problem.

The same 10 wheat genotypes were used as control with pure water spraying. The other agronomic practices were followed in similar conditions. A complete randomized block design with three replications was followed in a factorial arrangement. Plot size was 1.5 m^2 (4 rows, 2.5 meters length with 15 cm apart) and planted at rate of 300 seed/m 2. Date of planting was Nov., 20th and 30th in the first and second season, respectively.

At anthesis, ten guarded plants from each variety in every replicate of treated and untreated plants were used to estimate flag leaf area, also leaf chlorophyll content was estimated using SPAD- 502 apparatus(Castelli *et al*.,1996) and content of the amino acid proline (µ moles/g fresh weight) in leaves was determined using the procedure by Bates *et al*., (1973).At harvest, number of fertile spikelets/spike, number of sterile spikelets/ spike, number of productive tillers/ plant, number of grains/ spike,1000-grain weight and grain yield (ard/fed.) were determined. The wheat grains of lead treatment were discarded after yield determination.

Biometrical assessment:

Analysis of variance was carried out as described by Snedecor and Cochran (1967). The expected mean squares from the components of variance were used to estimate broad sense heritability according to Hanson, Robinson and Comstock (1956), correlation and path coefficient analyses were computed according to Svab (1973). Data of wheat grain yield were used to estimate lead tolerance measurements as follows:

1. Tolerance index (ToL)

(Rosielle and Hambling, 1981).

Tol= YP- Ys

2. Lead sensitivity index (LSI)

(Fischer and Maurer, 1978)

L.S.I= (1-YS/ YP)/SI and SI= (1- ȲS/ȲP)

Where, SI: lead stress intensity.

3. Relative performance (P)

(Abo-Elwafa and Bakeit, 1999).

P= (YS/YP)/R

Where, R= (ȲS/ȲP)

YP= Yield potential under normal conditions.

YS= Yield potential under stress conditions.

ȲS and YP= yield of all genotypes in the stress and normal conditions, respectively.

RAPD-PCR amplification

Plant material

To study lead tolerance in bread wheat (*Triticum aestivum* L.) by using molecular markers, ten genotypes were used for the present study (Table1). This work was carried out in Molecular Genetics Lab. Genetic Dept., Fac. of Agric., Zagazig Univ.

Table (2): Sequence and operon codes of the random primers used to detection of variation in 10 wheat genotypes.

Primer codes	Sequence (5^- to 3^-)
OPA-11	CAA TCG CCG T
OPB-05	TGC GCC CTT C
OPB-10	CTG CTG GGA C
OPB-18	CCA CAG CAG T
OPC-20	ACT TCG CCA C

Isolation of DNA

Total genomic DNA was extracted from frozen young leaves by the CTAB (cethyltrimethylammonium bromide) method (Doyle and Doyle, 1987) followed by an RNase-A treatment (Sigma, St. Louis, MO; R-4875) for 30 min at 37°C. The quality and quantity of DNA were checked by agarose gel electrophoresis. The final DNA concentration of each sample was adjusted to 25 ng/µl.

Primers:

A set of twenty 10-mer oligonucleotides was analyzed for RAPD-PCR. Based on the accurate amplified bands profiles and the produced polymorphic patterns of DNA fingerprinting selected five different primers were chosen (Table 2).

RAPD- PCR reactions

The RAPD amplification reactions were carried out in 25 µl containing 25 ng/µl of template DNA, 10× buffer (NH4)2SO4; 2.5mM MgCl2 (Fermentas), 10 mM dNTPs, 0.5 µM primer and 1 Unit Taq DNA polymerase (Fermentas). The RAPD amplifications occurred under the following conditions: an initial denaturation step at 94 ºC for 7 min and 30 cycles at 94 ºC for 1 min, 35 ºC for 1 min and 72 ºC for 2 min; the final elongation step was at 72 ºC for 6 min. Amplification reactions were carried out on a Perkin-Elmer Gene Amp PCR system (model 2400), and each reaction was repeated twice.

Band analysis:

The reaction products were analyzed by electrophoresis on 1.5% agarose gels in 1X TBE

buffer at 100 V, stained with ethidium bromide and photographed under UV transilluminator by digital camera with UV filter adaptor. The synthetic DNA, ladder 100 bp (Pharmacia) was employed as molecular markers for bands molecular weight. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Profiles were considered different when at least one polymorphic band was identified. Fragments were scored as 1 if present or 0 if absent based on standard marker using GelAnalyzer 3 (Egygene) software. Pairwise combinations, genetic similarity and genetic distances were estimated following Lynch (1990 and 1991). The computer package SPSS was used to construct a dendrogram based on the matrix of distance using Unweighted Pair Group Method with Arithmetic averages (UPGMA) (Sneath and Sokal 1973).

3. Results and Discussion:

A. Mean performance and heritability:

A.I Moro-physiological characters:

Mean performance for morph-physiological characters under both normal and lead stress environments are presented in Table (3). Significant differences were observed among wheat genotypes for proline content, leaf chlorophyll content and flag leaf area under normal and lead stress environments. These results indicate the presence of high degree of genetic variability among the studied wheat genotypes, valid for selecting and breeding new cultivars more tolerant to lead pollution. Similar interpretation was stated by Shutu *et al*.,(2000).

Table (3): Genetic variation and heritability of ten bread wheat genotypes for proline content, leaf chlorophyll content and flag leaf area under normal and lead stress conditions.

Character	Proline content (µmoles/g.f.w.)			Leaf chlorophyll content			Flag leaf area (cm^2)		
Genotype	Control	Lead stress	RI%	Control	Lead stress	RD %	Control	Lead stress	RD %
Sakha 94	1.450	3.350	131.034	47.260	46.700	1.185	38.116	32.808	13.926
Gemmeiza 5	1.375	4.350	216.364	46.800	42.566	9.047	41.69	39.023	6.397
Sids 6	1.616	2.150	33.044	41.500	33.900	18.313	42.600	30.528	28.338
ACSAD 903	1.600	4.625	189.063	43.560	41.300	5.188	51.707	45.623	11.766
ACSAD 925	1.584	2.500	57.828	45.360	35.200	22.398	35.268	23.86	32.347
ACSAD 939	1.715	2.300	34.111	42.830	39.200	8.475	67.760	62.566	7.665
Line 1	1.900	3.750	97.368	53.160	35.700	32.844	60.422	50.693	16.102
Tsi (S)/Vee(S)	1.825	1.900	4.109	52.400	37.900	27.672	54.200	47.100	13.099
Pr1 (S)/Pew(S)	2.050	4.200	104.878	50.260	46.200	8.078	57.233	55.486	3.052
Tow(S)/Pew(S)	1.750	3.700	111.428	45.260	39.700	12.285	49.866	48.536	2.667
Grand mean	1.687	3.283	94.606	46.839	39.836	14.951	49.886	43.622	12.556
L.S.D 0.05	0.548	1.295		4.420	5.438		8.653	10.290	
Tb%	87.200	30.090		70.130	34.170		76.800	36.500	
$GxT_{0.05}$	*			*			*		

* Significant at 0.05 level of probability.

Tb%: heritability in broad sense as percentage.

With respect to proline content, under normal conditions, Prl(S)/Pew(S), Line 1 and Tsi/Vee(S) surpassed significantly the other wheat genotypes, they exhibited higher values of proline content over the grand mean whereas, Gemmeiza 5, Sakha 94 and ACSAD 925 as group recorded the lowest values in proline content among the studied wheat genotypes, and exhibited lower values than the grand mean. The remaining wheat genotypes attained moderate values in proline content. On the other hand, under lead stress conditions, higher reaction in proline accumulation as a result of lead treatment was detected in Gemmeiza 5, ACSAD 903, Sakha 94, Tow(S)/Pew(S), Prl(S)/Pew(S) and Line 1 with relative increase percentage of 216.364, 189.063, 131.034, 111.428, 104.878 and 97.368, respectively. However, Tsi/Vee(S), Sids 6, ACSAD 939 and ACSAD 925 were the lowest one with relative increase percentage of 4.109, 33.044, 34.039 and 57.828%, respectively among the studied wheat genotypes. Relatively moderate values of proline content were detected by the rest genotypes.

For leaf chlorophyll content, under normal conditions it ranged from 42.83 (ACSAD 939) to 53.16 (Line1), with general mean of 46.839.While under lead stress conditions, leaf chlorophyll content was reduced, and the most affected wheat genotypes were Line 1 followed by Tsi/Vee(S), ACSAD 925,

Sids 6 with relative reduction percentage of 32.844, 27.672, 22.398 and 18.313%, respectively. Whereas, the higher values of leaf chlorophyll content were recorded by Gemmeiza 5, ACSASD 939, Pr1(S) /Pew(S), ACSAD 903 and Sakha 94 with lower values of relative reduction percentage of 9.047, 8.475, 8.078, 5.188 and 1.185, respectively. Moreover, moderate values of leaf chlorophyll content were recoded in the rest genotypes.

Concerning flag leaf area, wheat genotypes under normal conditions varied significantly from 35.268(ACSAD 925) to 67.760 cm^2 (ACSAD 939), with grand mean of 49.886 cm^2. On the other hand under lead stress conditions, flag leaf area was decreased in response to lead treatment and ranged from 23.86 (ACSAD 925) to 62.566 cm^2 (ACSAD 939) with grand mean of 43.622 cm^2.The most affected wheat genotypes were the sensitive genotype ACSAD 925 followed by Sids 6 and Line1 with relative reduction of 32.347, 28.338 and 16.102%, respectively. Whereas, Toms (S)/Pew(S), Pr1(S)/Pew(S), Gemmeiza 5 and ACSAD 939 had the minimum reduction with values of 2.667, 3.052, 6.397 and 7.665%, respectively.

Based on, mean performance of wheat genotypes in respect to moro-physiological characters, Gemmeiza 5, ACSAD 903 and Pr1(S)/Pew(S) accumulated higher amounts of proline, leaf chlorophyll content with broader flag area under lead stress, also Sakha 94 exhibited higher values of proline and chlorophyll contents with smaller flag leaf area under lead stress. Whereas ACSAD 939 showed moderate values of leaf chlorophyll content with broader flag leaf area, but it exhibited relatively low value of proline content under lead stress treatment, these genotypes could be classified as tolerant ones to lead stress. Otherwise, Sids 6 and ACSAD 925 were the most affected genotypes by lead stress and might be considered as sensitive ones. Furthermore, Line 1, pronounced relatively high proline content with broader flag leaf area with low value of leaf chlorophyll content, it could be considered as moderate sensitive one. The significancy of interaction, demonstrate that wheat genotypes are affected by the studied treatments.

Heritability estimates in broad sense were high under normal conditions and moderate under lead stress one. The values of heritability under normal and stress conditions were 87.20 and 30.09% for proline content; 70.13 and 34.17% for leaf chlorophyll content as well as 76.80 and 36.50% for flag leaf area. The decline estimates of heritability from normal to stress could be due to the effect of lead stress. The moderate heritability estimates under stress conditions might be suggest that breeding for lead tolerance based on morpho-physiological characters is feasible (Mahgoub *et al.*, 1998 and Harada *et al.*, 2001).

A.2. Yield and its attributes:

Mean performance for all wheat genotypes in yield and its attributes (Table 4), except number of sterile spikelets/spike were generally, decreased from normal to lead treatment. Significant differences were observed among wheat genotypes for yield attributes under normal and lead stress treatment, suggesting the presence of great amount of genetic variability among wheat genotypes.

For spike characteristics, number of fertile spikelets/spike varied significantly under normal conditions from 18.83 (ACSAD 939) to 24.07 (Line l) with grand mean of 21.272, while under lead stress, it decreased and ranged from 16.07 (Sakha 94) to 20.93 (Pr1(S)/Pew(S)) with grand mean of 18.353. The highest reduction percentage (22.47%) in number of fertile spikelets/spike was detected in Gemmeize 5, whereas the minimum one of (8.27%) was recorded by ACSAD 925. Number of sterile spikelets / spike varied from 0.98 (Line1) to 2.0 (Pr1(S)/Pew(S)) under normal conditions, with grand mean of 1.463, while under lead stress it decreased significantly from 1.81 (ACSAD 903) to 2.87 (Pr1(S)/Pew(S)). The highest values of relative increase (99.16%) in number of sterile spikelets/spike as a result of lead hazared was recorded by Tom (S)/Pew(S) followed by Line1, ACSAD 925 and Tsi/Vee(S) with values of 96.938, 93.460 and 91.150, respectively, whereas ACSAD 903 was the lowest one (0.56%).

For grain yield (ard/fed.) and its components, it is clear that, under normal conditions, wheat genotypes varied significantly from 1.57 (ACSAD 10) to (3.67) (Line1) for number of spikes/plant; from 43.36 (ACSAD 939) to 72.0 (Line 1) for number of grains / spike; from 39.86 (Tow(S)/Pew(S)) to 54.86 gm (Pr1(S)/Pew(S)) for 1000-grain weight and from 10.066 (ACSAD 903) to 24.242 ard/fed. (Line1) for grain yield. Whereas, under lead stress conditions, yield and its components were reduced and varied from 1.33 (ACSAD 925) to 3.33 (Line1) for number of spikes / plant; 32.07 (Gemmeiza 5) to 69.0 (Line1) for number of grains / spike; 34.666 (ACSAD 925) to 54.10gm (Pr1(S)/Pew(S)) for 1000-grain weight as well as from 7.406 (ACSAD 925) to 19.689 ard/fed. (Pr1(S)/Pew(S)) for grain yield. Significant interaction was registered for grain yield and its attributes, indicating that wheat genotypes differed significantly in their response to the studied treatments.

Table (4): Genetic variation and heritability of ten bread wheat genotypes, for grain yield and its attributes under normal and lead stress conditions.

Character	No. of fertile spikelets/spike			No. of sterile spikelets/spike			No. of spikes/ plant			No. of grains/ spike			1000 grain weight (g.)			Grain yield/fed.		
Genotype	Control	Lead stress	RD%	Control	Lead stress	RI %	Control	Lead stress	RD %	Control	Lead stress	RD %	Control	Lead stress	RD %	Control	Lead stress	RD %
Sakha 94	18.93	16.07	15.11	1.53	2.00	30.72	2.73	2.85	4.39	60.00	42.87	28.550	43.666	40.160	8.031	17.821	15.512	12.956
Gemmeiza 5	22.87	17.73	22.47	1.25	2.07	65.60	2.63	2.67	1.52	62.66	32.70	48.819	53.310	48.200	9.585	18.948	15.601	17.664
Sids 6	22.75	19.00	16.48	1.80	2.33	29.44	2.00	1.50	25.00	71.62	64.00	10.639	51.850	41.950	19.090	19.405	12.932	33.357
ACSAD 903	19.67	17.67	10.17	1.80	1.81	0.56	2.67	2.40	10.11	43.40	39.00	10.138	42.460	40.660	4.239	10.066	9.504	5.583
ACSAD 925	20.20	18.53	8.27	1.07	2.07	93.46	1.57	1.33	15.29	48.00	35.00	27.080	49.916	34.666	30.551	12.512	7.406	40.808
ACSAD 939	18.83	16.00	15.03	1.87	1.93	3.21	3.33	2.88	20.12	43.36	41.93	3.290	45.325	41.700	7.998	13.802	11.996	13.085
Line 1	24.07	20.80	13.59	0.98	1.93	96.94	3.67	3.33	9.26	72.00	69.00	4.160	54.50	49.550	9.083	24.242	18.063	25.489
Tsi (S)/Vee(S)	20.80	18.00	13.46	1.13	2.16	91.15	3.10	2.66	14.19	52.26	46.07	11.840	41.076	35.366	13.901	16.811	12.424	26.096
Pr1 (S) /Pew(S)	23.00	20.93	9.00	2.00	2.87	43.50	2.67	2.33	12.73	68.53	68.00	0.770	54.860	54.100	1.385	23.376	19.689	15.773
Tow(S)/Pew(S)	21.60	18.80	12.96	1.20	2.39	99.16	2.33	2.30	1.29	62.50	43.60	30.240	39.860	37.750	5.294	13.531	11.174	17.419
Grand mean	21.272	18.353	13.722	1.463	2.156	47.368	2.568	2.425	5.568	58.433	48.154	17.591	47.682	42.410	11.056	17.051	13.430	21.236
L.S.D 0.05	2.139	2.928		0.340	0.728		1.354	1.255		8.118	6.39		5.28	3.36		4.04	3.07	
Tb%	61.71	59.92		74.74	54.39		47.35	38.54		61.00	42.18		84.6	53.5		28.23	21.75	
$GxT_{0.05}$	*			N.S			*			*			*			*		

* Significant at 0.05 level of probability.
Tb%: heritability in broad sense as percentage

The lowest and highest reduction in wheat grain yield and its components due to lead stress were 1.29 (Tow(S)/Pew(S)) to 25.0% (Sids 6) for number of productive tillers/plant; 0.77 (Pr1(S)/Pew(S)) to 48.819% (Gemmeiza 5) for number of grains/spike; 1.385 (Pr1 (S)/Pew(S)) to 30.55% (ACSAD 925) for 1000-grain weight as well as 5.583 (ACSAD 903) to 40.808% (ACSAD 925) for grain yield ard/fed. The decrease in grain yield characters due to lead stress could be attributed to its effects on pollination and fertilization processes and grain filling period, also reduction in photosynthesis and translocation of assimilates to grains (Gupta, 1997). Genetic variation among cereal genotypes in their reaction to lead stress has been registered for morph-physiological and biochemical characters by Kacabova and Natr, (1986), Harada *et. al* (2001) and Brkic *et al*, (2004).

Heritability estimates varied from condition to another and from character to another. Generally, it was moderately high for yield attributes under both normal and stress environments and valued 61.71 and 59.92% for number of fertile spikelets/spikes; 74.74 and 54.39% for number of sterile spikelets/spike; 47.35 and 38.54% for number of productive tillers/plant; 61.00 and 42.18% for number of grains/spike as well as 84.60 and 53.50% for 1000-grain weight. However, heritability estimate for grain yield/fed was low and valued 28.23 and 21.75% under normal and stress conditions, respectively. Clarke *et al.*, (1997) recorded high heritability estimates (78%) for grain cadmium concentration in five durum wheat crosses.

B. Lead tolerance measurements:

Data of lead tolerance measurements (Table 5) show that, wheat genotypes with highest values of relative performance (P) such as ACSAD 7, Sakha 94, ACSAD 939, Pr1(S)/Pew(S), Tow(S)/Pew(S) and Gemmeiza 5, yielded less different values **(**Tolerance index ToL) between yield stress (YS) and yield potential (YP) and coupled with lead sensitivity index (LSI), where higher values of LSI (>1) indicated a higher degree of sensitivity to lead stress for genotypes and vice versa (Bruckner and Frohberg, 1987).

The results of (P) and (ToL) are coupled with (LSI) and indicated that ACSAD 925 ranked in the first order (1.911) in sensitivity to lead stress followed by Sids 6 (1.565) and Tsi (S) (1.223), whereas Line1, had L.S.I of 1.194 and might be considered as relatively moderate sensitive to lead stress. The results of lead tolerance measurements coupled with morpho-physiological characters, so ACSAD 925, Sids 6 and Tsi (S) exhibited lower values of proline content, leaf chlorophyll content and flag leaf area, also Line1 attained lower values of leaf chlorophyll content and flag leaf area, but supported by moderately high values of proline content (3.75 µmoles/g.f.w) as moderate sensitive cultivar. These genotypes exhibited low to moderate values of yield contributing characters. The

remaining wheat genotypes ACSAD 903, Sakha 94, ACSAD 939, Pr1(S)/Pew(S), Tom (S)/Pew(S) and Gemmeiza 5 appeared to be more tolerant to lead stress as they recorded L.S.I less than unity, these genotypes exhibited in general high values of proline content, leaf chlorophyll content, flag leaf area along with higher values of yield attributes, except ACSAD 939 for proline content which exhibited low value (2.3 µmoles/g.f.w.) rather than the grand mean (3.283 µmoles/g.f.w.), but it was good performance for the remaining characters.

C. Correlation coefficient between lead sensitivity index with morpho-physiological and yield attributes characters:

The correlation coefficient between lead sensitivity index and the studied characters were computed under both normal and stress conditions to demonstrate the important characters for developing lead tolerance in wheat genotypes.Results given in Table (6) show that lead sensitivity index under normal conditions appears to be positively but did not reach the level of significance with each of; proline content, leaf chlorophyll content, number of fertile spikelets/spike, number of grains/spike, 1000-grain weight and grain yield/fed. Whereas, negative association only was observed between LSI with the remaining characters.

Table (5): Lead tolerance measurements of grain yield for ten bread wheat genotypes.

Character / Genotype	Tolerance index	Lead sensitivity index (LSI)	Relative performance (P)
Sakha 94	2.309	0.609	1.105
Gemmeiza 5	3.347	0.829	1.045
Sids 6	6.473	1.565	0.846
ACSAD 903	0.562	0.263	1.199
ACSAD 925	5.106	1.911	0.752
ACSAD 939	1.806	0.614	1.103
Line 1	6.179	1.194	0.946
Tsi (S)/Vee(S)	4.387	1.223	0.938
Pr1 (S)/Pew(S)	3.687	0.740	1.069
Tow(S)/Pew(S)	2.357	0.815	1.049

Table (6): Correlation coefficients between lead sensitivity index with the studied morpho-physiological and yield contributing characters under normal and lead stress treatments (Pooled data of the two seasons).

Character	Normal	Lead stress
Proline content	0.034	- 0.609*
Leaf chlorophyll content	0.074	- 0.734**
Flag leaf area	-0.399	- 0.581*
No. of fertile spikelets/spike	0.308	0.347
No. of sterile spikelets/spike	-0.485	0.121
No. of productive tillers/plant	-0.474	- 0.587*
No. of grains /spike	0.231	0. 167
1000-grain weight	0.354	- 0.291
Grain yield/fed.	0.161	- 0.239

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Whereas, under stress conditions, it is clear that, LSI was negatively and significantly associated with the morpho-physiological characters, proline content, leaf chlorophyll content, flag leaf area in addition to number of productive tillers/plant .In continuous, the association was negative but did not reach the level of significance between LSI and each of 1000-grain weight and grain yield/fed. The obtained results reveal that proline content, leaf chlorophyll content, flag leaf area could be used as selection criteria for improving lead stress tolerance in wheat breeding programs. Also, increasing number of productive tillers/plant may be supported wheat grain yield under stress conditions and compensate the reduction in grain weight under lead stress. In this respect, Mahgoub *et al.* (1998**)** showed that the level of total chlorophyll, cartinoids and proline can serve as a simple, reliable and early indicator of

environmental pollution and represents a good model for the assessment of heavy metal toxicity in higher plants.

D. Path coefficient analysis:

Data presented in Table (7) show direct and joint effects of the studied characters i.e.; proline content, leaf chlorophyll content, flag leaf area and number of productive tillers/plant under lead stress conditions. It is important to mention that, the maximum direct effect on lead sensitivity index of wheat grain yield was accounted for leaf chlorophyll content (22.268%) followed by flag leaf area (12.250%), proline content (5.697%) and then number of productive tillers/plant (1.397%). Hereby, direct selection for leaf chlorophyll content, flag leaf area and proline content may have resulted in improving lead tolerance.

Concerning the indirect effects, it is clear that, the highest indirect effects on lead sensitivity index of wheat grain yield variation were observed for proline content via leaf chlorophyll content (12.841%) followed by leaf chlorophyll content via flag leaf area (7.795%), flag leaf area via number of productive tillers/plant (5.253%), then leaf chlorophyll content via number of productive tillers/plant (4.127%). Therefore, simultaneous selection for the foregoing pair characters may have resulted in enhancing lead tolerance.

It is evident to mention that, the studied characters accounted for by 76.478% of the total lead sensitivity index of wheat grain yield variation, however the residual effect was 23.522%. In this regard, total chlorophyll and proline content could be used as biochemical markers for increasing lead and cadmium tolerance in higher plants (Mahgoub *et al.*, 1998).

Generally, on the basis of the total contribution, the studied characters could be arranged according to its relative importance to lead sensitivity index of wheat grain yield variation as follows; leaf chlorophyll content (34.649%), flag leaf area (20.345%), proline content (14.543%) and then number of productive tillers/plant (6.941%). Hereby, leaf chlorophyll content, flag leaf area and proline content were the most important characters of both direct and indirect effects, which enhance their importance to be used as selection criteria amining to improve environmental pollutants tolerance.

Table (7): Direct and joint effects of some morpho-physiological characters as percentage of lead sensitivity index variation of wheat grain yield (Pooled data of the two seasons).

Source of variation	C.D	RI%
Proline content	0.05697	5.697
Leaf chlorophyll content	0.22268	22.268
Flag leaf area	0.1225	12.250
No. of productive tillers/plant	0.01397	1.397
Proline content x leaf chlorophyll content	0.12841	12.841
Proline content x flag leaf area	0.03141	3.141
Proline content x No. of Productive tillers/plant	0.01709	1.709
Leaf chlorophyll content x flag leaf area	0.07795	7.795
Leaf chlorophyll content x No. of productive tillers/plant	0.04127	4.127
Flag leaf area x No. of productive tillers/plant	0.05253	5.253
R^2	0.76478	76.478
Residual	0.23522	23.522
Total	1.00000	100.000
Total contribution		
Proline content	0.14543	14.543
Leaf chlorophyll content	0.34649	34.649
Flag leaf area	0.20345	20.345
No. of productive tillers/plant	0.06941	6.941

C.D: coefficient of determination
RI%: relative importance

RAPD analysis

The total number of amplified fragments observed among the wheat genotypes based on RAPD analysis with all primer was 40 with an average of 8 easily detectable fragments per primer. The number of amplified fragments produced per primer ranged from 6 to 10 and size of the products ranged from 254 bp to 1930 bp. The total number of polymorphic fragments and the percentage of polymorphism were 33 and 82.5, respectively (Table

8). The primer OPB-05 presented the highest percentage of RAPD polymorphism (90 %). Some RAPD primers (OPA-11 and OPB-05) produced more bands (10) probably because genomic DNA sequences possess high frequency of annealing sites (Virk *et al*, 1995).Primer OPB-10 presented three unique bands (975 bp, 440 bp and 250bp) to Line 1, Gemmeiza 5 and Pr1(S)/Pew(S) genotypes, respectively (Figure 1) while, Primer OPB-18 presented one unique band (1540 bp) to Line 1.

The patterns obtained with primer OPB-10 for genotypes suggested that this primer has the ability to produce lead tolerant markers. Since four fragments of about (700,790, 1290 and 1650 bp) were visualized using this primer in the genomic DNA of the lead tolerant genotypes while were absent in the sensitive genotypes, they can be considered as positive lead tolerant markers (Figure 1). These bands can be considered as potential markers to identify lead tolerant genotypes or may even be more useful when converted into a simple-sequence PCR-based marker that can be used for large-scale lead tolerance screening of genotypes. In this respect, many investigators exploited DNA markers and detected some markers to abiotic stress. Pakniyat and Tavakol (2007) found markers related to drought tolerance in bread wheat genotypes using RAPD markers. Pakniyat *et al*., (2004) introduced markers linked to salt tolerance in cultivated and wild barley using these markers. Also, Nazari and Pakniyat (2008) found markers associated with drought tolerance in wild and cultivated barley genotypes using RAPD markers. Youssef *et al*., (2010) found molecular markers for new promising drought tolerant lines of rice under drought stress via RAPD-PCR and ISSR markers.

The similarity coefficients based on 40 amplified fragments are presented in Table (9) and ranged from 0.35 to 1.00. ACSAD 903 and ACSAD 939 genotypes showed the highest similarity index (1.00) and the lowest similarity index (0.35) showed between ACSAD 925 and Gemmeiza 5. Cluster analysis was performed based on the Jaccard's similarity coefficient matrices, calculated from RAPD markers to generate a dendrogram of wheat genotypes. The dendrogram separated 10 genotypes into three clusters. The first cluster included ACSAD 903, ACSAD 939, Tow(S)/Pew(S), Gemmeiza 5, Sakha 94 and Prl(S)/Pew(S) and the second cluster included Tsi/Vee(S), Sids 6 and ACSAD 925, while Line 1 formed the third cluster (Figure 2).

The development of DNA markers in wheat is somewhat problematic due to three features. Firstly, the size of the wheat genome (16×10^9 bp, compared to barley or maize with 5×10^9 bp), which makes the application of several marker techniques difficult. Secondly, the hexaploid nature of wheat adds complexity to many marker assays (Chao *et al.*, 1989). Three sets of bands usually appear (often in the same size range), which are difficult to manage and interpret. Thirdly, there is a generally low level of polymorphism in wheat relative to other cereal crops. This implies that a larger number of markers must be screened than in the case of rice, barley or maize (Chao *et al*., 1989 and Lui *et al.*, 1990).

Higher plants have been reported to produce varied responses to heavy metals in their environment and interfere with the genetic constitution of plants (De Wolf *et al*., 2004). Recently, the development of molecular marker technology has provided new tools for detection of genetic alteration in response to heavy metal tolerance by looking directly at the level of DNA sequence and structure. Random amplified polymorphic DNA (RAPD) is a PCR-based technique and extremely efficient for DNA analysis in complex genomes as it is relatively inexpensive and yields information on a large number of loci without having to obtain sequence data for primer design (DeWolf *et al*.,2004).

Table (8): Number of monomorphic, polymorphic bands and polymorphism percentage produced by each RAPD primer for 10 wheat genotypes.

PRIMERS	bP RANGED	Total No. of bands	Monomorphic bands	Polymorphic bands	Polymorphism %
OPA-11	266-1864	10	2	8	80.00
OPB-05	254-1566	10	1	9	90.00
OPB-10	311-1122	8	1	7	87.50
OPB-18	310-1543	6	2	4	66.66
OPC-20	343-1930	6	1	5	83.33
Total	254-1930	40	7	33	82.50
Average		8	1.2	6.6	

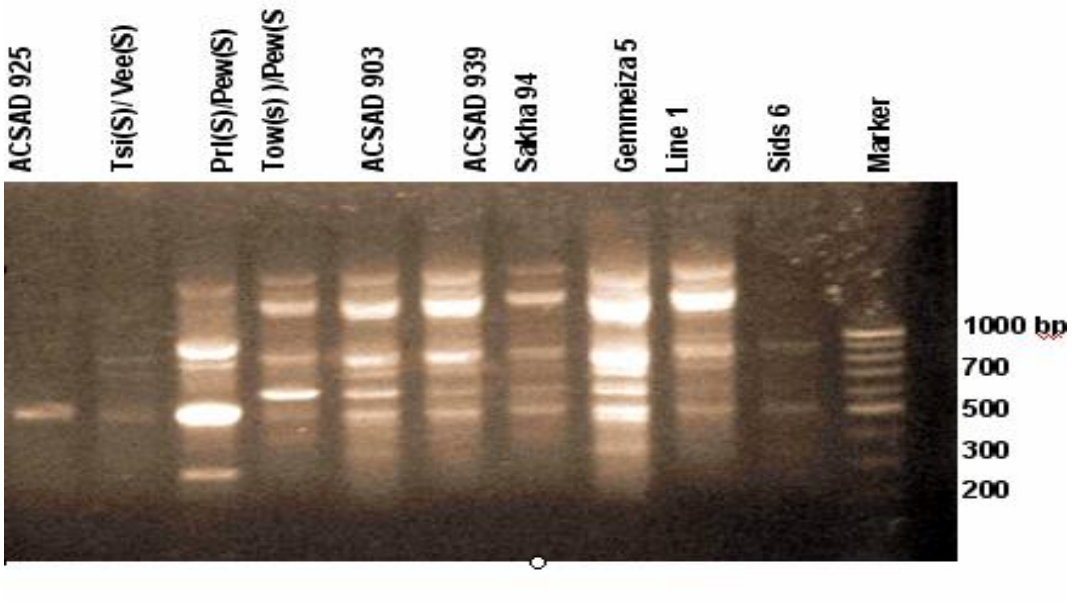

Fig. (1): Results of RAPD-PCR amplification based on the use of primer OPB-10 in the 10 wheat genotypes

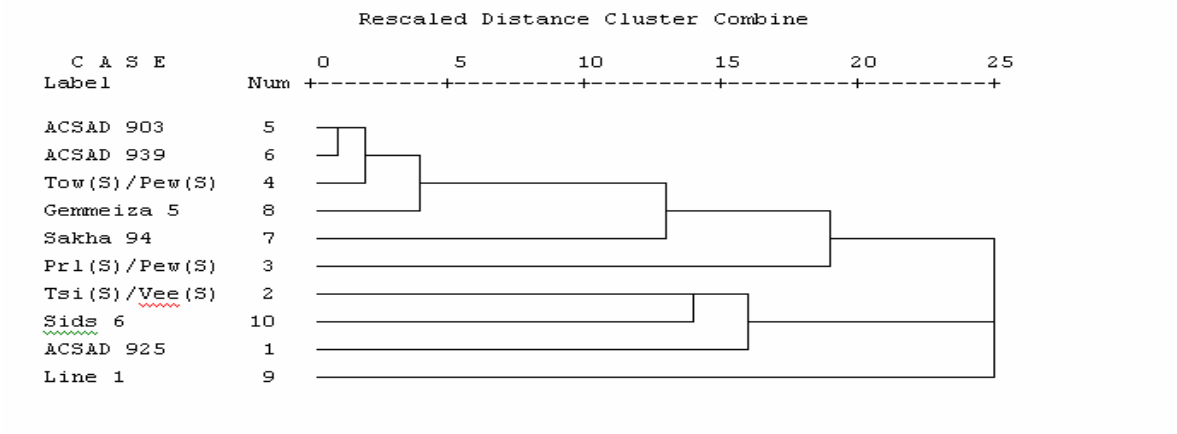

Fig. (2): The dendrogram of genetic distances among all tested genotypes based on band polymorphisms generated by RAPD-PCR after using the primers.

Table (9): The similarity coefficient values among 10 wheat genotypes based on band polymorphisms generated by RAPD-PCR after using the primers

Case	Tsi/Vee(S)	Prl(S)/Pew(S)	Tow(S)/Pew(S)	ACSAD 903	ACSAD 939	Sakha 94	Gemmeiza 5	Line 1	Sids 6
ACSAD 925	0.75	0.575	0.375	0.36	0.36	0.575	0.35	0.625	0.65
Tsi/Vee(S)		0.475	0.575	0.6	0.6	0.725	0.55	0.475	0.75
Prl(S)/Pew(S)			0.7	0.675	0.675	0.55	0.675	0.55	0.375
Tow(S)/Pew(S)				0.975	0.975	0.75	0.925	0.5	0.575
ACSAD 903					1	0.775	0.95	0.475	0.6
ACSAD 939						0.775	0.95	0.475	0.6
Sakha 94							0.725	0.65	0.625
Gemmeiza 5								0.475	0.55
Line 1									0.525

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4. References:

1. Abo- Elwafa, A. and Bakheit B. R. (1999). Performance, correlation and path coefficient analysis in faba bean. Assiut J. of Agric. Sci. 30: 77-91.
2. Bates, L. S., Waldren R. P. and Teare I. D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207.
3. Brkic, I., Simic D., Zdunic Z., Jambrovic A., Ledencan T., Kovacevic V. and Kadar I. (2004). Genotypic variability of micronutrient element concentrations in maize kernels. Cereal. Res. Communications 32 (1): 107-112.
4. Bruckner, P. L. and R. C. Frohberg (1987). Stress tolerance adaptation in spring wheat. Crop Scie. 27:31-36
5. Burzynski, M. (1987). The influence of lead and cadmium on the absorption and distribution of potassium, calcium, magnesium and iron in cucumber seedings. Acta physiologie Prantarum 9: 229-38.
6. Castelli, F.; Contillo R. and Miceli F. (1996). Non-destructive determination of leaf chlorophyll content in four crop species. J. Agron. and Crop Sci., 177: 275-283.
7. Chalmers, J. K., Campbell, A. W., Krestschmer, J., Karakousis, A., Henschke, P. H., Pierens, S., Harker, N., Pallotta, M., Cornish, G. B., Shariflou, M. R., Rampling, L. R., McLauchlan, A., Daggard, G., Sharp, P. J., Holton, T. A., Sutherland, M. W., Appels, R. and Langridge, P. (2001). Construction of three linkage maps in bread wheat, Triticum aestivium L. Australian Journal of Agriculture Research 52, 1089–1119.
8. Chao, S., Sharp,P. J., Worland, A. J., Warham, E. J., Koebner, R. M. D and Gale, M. D. (1989). RFLP-based genetic linkage maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet. 78:495-504.
9. Clarke, J. M.; Leisle D. and Kopytko G. L. (1997). Inheritance of cadmium concentration in five durum wheat crosses. Crop. Sci. 37: 1722-1726.
10. Demek, T., Lynch, D. R., Kawchuk, L. M., Kozub, G. C and Armstrong, J. D. (1996). Genetic diversity of potato determined by random amplified polymorphic DNA analysis. Plant Cell Reports 15, 662– 667.
11. De Wolf H, Blust R and Backeljiau T (2004). The use of RAPD in ecotoxicology: A review. *Mutation Research*, 566: 249– 262.
12. Doyle, J. J. and Doyle, J. L., (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Photochemistry Bulletin 19, 11– 15.
13. Fischer, R .A. and Maurer R. (1978). Drought resistance in spring wheat cultivars: I- Grain yield response. Aus. J. Agric. Res. 29: 897-912.
14. Gupta, U.S. (1997). Crop Improvement. Volume 2 Stress Tolerance. Science Publishers, Inc.
15. Hanson, C.H.; Robinson H. P. and Comstock R. E. (1956). Biometrical studies of yield in segregating populations of Korean Lespedeza. Agron. J. 48: 268-272.
16. Harada, E.;, Yongeui C., Tsuchisaka A., Obata H. and Sano H. (2001). Transgenic tobacco plants expressing a rice cysteine synthase gene are tolerate to toxic levels of cadmium. J. of Pl. Physiol. 158: 655-661.
17. Heckenberg M., Melchinger, A. E., Ziegle, J. S., Joe, L. K., Hauser, J. D., Hutton, M. and Bohn, M. (2002). Variation of DNA Fingerprints among accessions within maize inbred lines with regard to the identification of essentially derived genotypes. I. Genetic and technical sources of variation in SSR data. Mol. Breed. 10: 181–191.
18. Hernendez, P., Rosa, R., Rallo, L., Dorado, G. and Martin, A.(2001). Development of SCAR markers in Olive (Olea europaea) by direct sequencing of RAPD products: application in olive germplasm evaluation and mapping. Theoretical and Applied Genetics 103, 788– 791.
19. Kacabova, P. and Natr L. (1986). Effect of lead on growth characteristics and chlorophyll content in barley seedlings. Photossynthetica 20: 411-17.
20. Khabaz- Saberi, H.; Graham R. D., Pallotra M. A., Rathjen A. J. and Williams K. J. (2002). Genetic markers for manganese efficiency in durum wheat. Plant Breed. 121: 224-227.
21. Lagriffoul, A.; Mocquot B., Mench M. and Vangronsveld J. (1998). Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Article 200: 241-250.
22. Lin, H. T.; Wong S. S. and Li G. C. (2004). Heavy metal content of rice and shellfish in Taiwan. J. Food and Drug Analysis 12: 167-174.
23. Lui, Y. G., Mori, N. and Tsunewaki, K. (1990). Restriction fragment polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jap. J. Genet. 65:367-380.
24. Lynch M. (1990). The similarly index and DNA fingerprinting. Molecular Biology and Evolution, 7: 478-484.
25. Lynch M. (1991). Analysis of population genetic structure by DNA fingerprinting. In: DNA Fingerprinting Approaches and Applications (ed. by T. Burke, G. Dolf, A.J. Je‹reys & R.Wolf), pp.113-126. Springer Verlag, Basel.

26. Mahgoub, E. M. I., Abd El-Sayyed S. M. and Al-Refaey A. (1998). A genetical approach for assessing the environmental hazards of heavy metals in higher plants. Zagazig J. Agric. Res. 25 (3): 399-410.
27. Maric S., Bede M., Martincic J. and Guberac V. (1998). Variability of some winter wheat traits from breeding process. Sjemenarstvo 15, 421-433.
28. Meyers, M. W., Fricke F. L., Holmgren G. G., Kubota S. J. and Chaney R. L. (1982). Cadmium and lead in wheat grain and associated surface soils of major wheat production areas in the United States, P. 34. In Agronomy Abstracts. ASA, Madison, WI.
29. Nazari N., and Pakniyat, H. (2008). Genetic diversity of wild and cultivated barley genotypes under drought stress using RAPD markers. Biotechnology 7 (4): 745-750.
30. Pakniyat, H. and Tavakol, E. (2007). RAPD markers associated with drought tolerance in bread wheat (*Triticum aestivum* L.). Pak. Biol. Sci., 10: 3237-3239.
31. Pakniyat, H., Namayandeh A. and Forster B. P. (2004). Identification of RAPD markers linked to salt tolerance in cultivated and wild barley. Proceedings of the 9th International Barley Gentics Symposium,
32. Pallotta, M. A.; Graham R. D., Langride P., Sparrow D. H. B. and Barker S. J. (2000). RFLP mapping manganese efficiency in barley. Theor. Appl. Genet. 101: 1100-1108.
33. Penner, G. A.; Clarke J., Bezte L. J. and Leiste D. (1995). Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38: 543-547.
34. Poskuta, J. W. Parys; E. and Romanowska E. (1987). The effect of Pb on the gaseous exchange and photosynthetic carbon metabolism of pea seedlings. Acta Societats Botanicorum Polonial 56: 127-37.
35. Rosielle, A. A. and Hambling J. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci. 21: 943-946.
36. Rowland, P.; Evans G. and Walcott J. (1997). The Environmental and Food Quality. Bureau of Resources Sciences, Common wealth of Australia. Technical Paper Series.
37. Shutu, W.; Chenjen Y., Bojein K. and Fusheng T. (2000). Diallel analysis of cadmium tolerance in seedling rice. SABRAO. J. of Breed. and Genet. 32: 57-61.
38. Singh, B. D. (2004). Plant Breeding, Principles and Methods. Kalyani Publishers, New Delhi, India.
39. Sneath P. H. A. and Sokal R. R. (1973). Numerical Taxonomy.W.H. Freeman, San Francisco, USA.
40. Snedecor, G. W. and Cochran W. G. (1967). Statistical methods 6th edition. Iowa State Collage. Press, Ames Iowa. USA.
41. Sun, G., Bond, M., Nass, H., Martin, R. and Dong, Z.(2003). DNA polymorphisms in spring wheat cultivars and lines with different level of Fusarium resistance. Theoretical and Applied Genetics 106, 1059– 1067.
42. Svab, J. (1973). Biometriai modszerek a Kutatasban. Mezogazdasagi Kiado, Budapest.
43. Virk, P. S., Ford-Lloyd, B. V., Jackson, M. T. and Newbury, H. J., (1995). Use of RAPD for the study of diversity within plant germplasm collections. Heredity, 74, 170–179.
44. Welsh J. and McClelland M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18, 7213–7218.
45. Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18, 6531– 6535.
46. Wu, P.; Liao C. Y., Hu B., Yi K. K., Jin W. Z., Ni J. J. and He C. (2000). QTLs and epistasis for aluminum tolerance in rice (Oryza sativa L.) at different seedling stages. Theor and Appl. Genet. 100: 1295-1303.
47. Yang YoungYell, J. J. Young, S. WonYong, S. Haksoo and YoungSkook L. (2000). Identification of rice genotypes with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiology 124 (3): 1019-1026.
48. Youssef; M. A., Mansour A. and Solliman S. S (2010). Molecular markers for new promising drought tolerant lines of rice under drought stress via RAPD-PCR and ISSR markers. Journal of American Science; 6(12):355-363.

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